Int. J. Radiat. Biol., Vol. 86, No. 5, May 2010, pp. 367–375

informa
healthcare

GFAP expression in the rat brain following sub-chronic exposure to a 900 MHz electromagnetic field signal

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(Received 25 May 2009; Revised 26 November 2009; Accepted 11 December 2009)

Abstract

Purpose: The rapid development and expansion of mobile communications contributes to the general debate on the effects of electromagnetic fields emitted by mobile phones on the nervous system. This study aims at measuring the glial fibrillary acidic protein (GFAP) expression in 48 rat brains to evaluate reactive astrocytosis, three and 10 days after long-term head-only sub-chronic exposure to a 900 MHz electromagnetic field (EMF) signal, in male rats.

Methods: Sprague-Dawley rats were exposed for 45 min/day at a brain-averaged specific absorption rate (SAR) = 1.5 W/kg or 15 min/day at a SAR = 6 W/kg for five days per week during an eight-week period. GFAP expression was measured by the immunocytochemistry method in the following rat brain areas: Prefrontal cortex, cerebellar cortex, dentate gyrus of the hippocampus, lateral globus pallidus of the striatum, and the caudate putamen.

Results: Compared to the sham-treated rats, those exposed to the sub-chronic GSM (Global System for mobile communications) signal at 1.5 or 6 W/kg showed an increase in GFAP levels in the different brain areas, three and ten days after treatment.

Conclusion: Our results show that sub-chronic exposures to a 900 MHz EMF signal for two months could adversely affect rat brain (sign of a potential gliosis).

Keywords: *astrocyte, Glial fibrillary acidic protein (GFAP), electromagnetic field, rat, brain*

Abbreviations: CNS, central nervous system; GSM, Global System for Mobile communications; SAR, Specific Absorption Rate; RF, Radio frequency; RSF, radio frequency power source; RFPA, Radio Frequency Power Amplifier; RFR, radiofrequency radiation; GFAP, Glial fibrillary acidic protein; EMF, electromagnetic field; mRNA, messenger ribonucleic acid; PBS, phosphate buffer saline; PfCx, prefrontal cortex; DG, dentate gyrus; CPu, caudate putamen; LGP, lateral globus pallidus; CCx, cerebellar cortex; CCD, Charge Coupled Device; OD, optical density; RF, radio frequency; MDA, malonaldehyde; E6, exposed animals (15 min, SAR = 6 W/kg); E1.5, exposed animals (45 min, SAR = 1.5 W/kg); S, Sham control group; CC, cage control group; SEM, standard deviation of mean; INERIS, National Institute of Industrial Environment and Risk; ELISA, Enzyme-linked immunosorbent assay; IL, interleukin; ANOVA, analysis of variance

Introduction

Glial fibrillary acidic protein (GFAP) was identified as an intracellular intermediate filament (Schiffer et al. 1986) protein that is highly specific to the cells in the astroglial lineage (Eng 1988). GFAP is expressed in the astrocyte cells in the central nervous system. Astrocytes are the principal glial cell type and are involved in many cellular processes, including cell structure and movement, cell communication, and maintenance of the blood-brain barrier (Eddleston and Mucke 1993; Ridet and Privat 1995; Suzuki et al. 2003).

Activated astrocytes are involved in reactive gliosis. This process occurs as a result of tissue brain damage and is characterised by the hypertrophy and hyperplasy of astrocytes (Eng and DeArmond 1981; Eng 1982, 1988; Kimelberg 1992; Norton et al. 1992;

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ISSN 0955-3002 print/ISSN 1362-3095 online © 2010 Informa UK Ltd.
DOI: 10.3109/09553000903567946

O'Callaghan 1993; Eng and Ghirnikar 1994; Norenberg 1994; Eng et al. 2000; Little and O'Callaghan 2001; Panickar and Norenberg 2005), as well as by the proliferation of microglial cells (Norton et al. 1992). Astrogliosis occurs in response to all types of central nervous system (CNS) injuries, regardless of the regional, cellular or molecular basis of a given insult. The GFAP protein level increases at the site of damage and can appear from 24 h to many days post injury (Schiffer et al. 1986; Hatten et al. 1991; Norenberg 1994). The hallmark of reactive astrogliosis is an increase in the GFAP level (Eng 1985, 1988).

Mobile phones are increasingly being used worldwide. The potential health risks of the radiofrequency electromagnetic fields (EMF) emitted by mobile phones are therefore of considerable public interest (Sienkiewicz and Kowalczuk 2006). The close proximity of a mobile phone to a user's head enhances the absorption of part of the microwave energy from mobile phones into the head and the brain (Schonborn et al. 1998). An increasing number of studies indicate that EMF emitted by mobile phones could affect brain activity (Schonborn et al. 1998; Lebedeva et al. 2001; Maby et al. 2006; Xu et al. 2006; Ammari et al. 2008b; Lopez-Martin et al. 2009). GSM microwaves modify the electrophysiological brain activity of mobile phone users (Hermann and Hossmann 1997; Eulitz et al. 1998; Borbely et al. 1999; Krause et al. 2004; Hinrikus et al. 2008) and influence the neurotransmitter systems (Merritt et al. 1977; Modak et al. 1981; Millar et al. 1984; Lai et al. 1987, 1989, 1990; Inaba et al. 1992; Mausset-Bonnefont et al. 2001).

Fritze et al. (1997) showed that neither the whole-body exposure of rats to a GSM signal at specific absorption rates (SAR) of 0.3 or 1.5 W/kg, nor a continuous wave signal at 7.5 W/kg directed at 0.05 g of brain tissue induced changes in the GFAP mRNA (messenger ribonucleic acid) levels of the brain. This held true, irrespective of whether the mRNA levels were analysed immediately or after two or seven days following the GSM signal exposure. Mausset-Bonnefont et al. (2004) found that a 15-min exposure to GSM-type radiation specifically to the head at an SAR of 6 W/kg could activate astroglia 72 h later in the striatum, hippocampus, and cortex, based on the immunohistochemical analysis of the GFAP protein. This study was confirmed by Brillaud et al. (2007). Nevertheless, this astroglial activation is a transient effect, as it disappears within six or 10 days after exposure. In an earlier study, 10 days after chronic exposure (six months) to a 900 MHz EMF signal (SAR = 6 W/kg) GFAP staining in the rat brain (Ammari et al. 2008a) increased.

Our experiment was aimed at determining whether exposure to a sub-chronic electromagnetic field signal to the head alone, over a two-month period induces persistent astroglial activation in the brain, a potential sign of gliosis. Rats were exposed every day to a 900 MHz signal for 45 min at a SAR = 1.5 W/kg or for 15 min at 6 W/kg. They were then sacrificed three or 10 days after the end of the sub-chronic exposure. Five brain areas were analysed: The prefrontal cortex (FCx), the cortex of the cerebellum (CCx), the dentate gyrus of the hippocampus (DG), the caudate putamen of the striatum (Cpu), and the lateral globus pallidus of the pallidum (LGP). The GFAP immunodetection protocol was used on the sagittal slices of rat brains, where GFAP staining was detected by the image analysis of each brain structure.

Materials and methods

Animals

Forty-eight male Sprague-Dawley rats (OFA Iffa Credo, France; six weeks old) were acclimatised to the laboratory for five days before experimental use. The rats were kept under controlled environmental conditions (ambient temperature, 22°C; 12 h light/dark cycle) and given food and tap water, *ad libitum*. They were housed in groups of two animals per 'breeding-enriched' cage. The enrichment included a supply of corn chips and a plastic tube to the cage, the diameter and length of which were equal to GSM exposure rockets (Brillaud et al. 2005). The cylinder familiarises the rats with the appearance of the rocket used for GSM exposure. French State Council guidelines were followed for the care and use of laboratory animals (Decree no. 87–849, October 19, 1987), and the protocol was approved by the Institutional Animal Care and Use Committee at the INERIS.

Exposure system

The exposure set-up has been described earlier (Mausset-Bonnefont et al. 2001; Dubreuil et al. 2002; Brillaud et al. 2007; Ammari et al. 2008a, 2008b). A radio frequency power source (RFS 900–64 type, Radio Frequency Power Amplifier (RFPA), France) emitting a 900 MHz electromagnetic field (1/8 duty factor) pulse modulated at 217 Hz was connected to a four-output divider, thus simultaneously exposing four animals in an anechoic chamber. Each output was connected to a loop antenna allowing local exposure of the animal's head. During exposure, the animal was placed in a Plexiglas rocket capped with a truncated cone containing an individual loop antenna; the rat's head was inserted into the cone, the end of which was kept open. The rocket body was lined with airholes to

facilitate breathing and minimise the rise in body temperature. A Plexiglas disk was placed at the back to minimise animal movements, and particularly to prevent the rat from backing out of the rocket. The SAR calculations were made with homogeneous and non-homogeneous phantoms (Leveque et al. 2004).

Experimental groups

Forty-eight male Sprague-Dawley rats were randomly assigned to eight different groups and exposed to a 900 MHz electromagnetic signal five days per week for eight weeks: 24 rats were sacrificed three days after the sub-chronic exposure, and 24 were sacrificed 10 days after exposure. Within each group of 24 rats, the following regimes of sub-chronic exposure administered to the rats: Six rats were exposed for 15 min at SAR = 6 W/kg (E6), six rats were exposed for 45 min at SAR = 1.5 W/g (E1.5), six rats served as sham-treated controls (three for 15 min and three for 45 min, SAR = 0 W/kg) (S), and six rats were given no treatment or manipulation (cage control) (CC).

Brain preparation

At either three or 10 days after completing the sub-chronic exposure, the animals were anaesthetised with pentobarbital (i.p, 50 mg/kg) (Centravet, France) until brain death was achieved. Intra-cardiac perfusion was performed first with 0.9% NaCl solution, followed by a 4% paraformaldehyde solution (Sigma-Aldrich, France) in 0.1 M phosphate buffer (pH = 7.6) (Sigma-Aldrich, France). The brain was removed and cryoprotected by submersion in a 30% sucrose/4% paraformaldehyde solution for 48 h at 4°C. Sagittal brain slices (50 μm) were prepared using a cryostat microtome, and five sections of each brain area were processed as free-floating sections for GFAP immunodetection.

GFAP expression

Brain slices were washed (3 × 20 min) in phosphate-buffered saline (PBS) (pH = 7.4) and in H_2O_2 solution (Sigma-Aldrich, France) (30 min, H_2O_2 at 30%), then they were incubated in blocking solution at room temperature (20 min, Vector Elite kit in PBS). The sections were incubated overnight at 4°C with the primary anti-GFAP antibody (rabbit antibody, DAKO Z 0334, diluted 1/5000 in PBS + 1% goat serum + 0.3% Triton). On the second day, slices were washed with PBS (3 × 5 min) and incubated (30 min) with the secondary antibody (anti-rabbit, Vector Elite kit in PBS). Sections were washed in PBS (3 × 5 min) and then incubated with the avidine-biotine solution (ABC, Vector Elite kit, 30 min) and washed again in PBS (3 × 5 min). GFAP immunostaining was performed by treating sections with 3,3′-diaminobenzidine (DAB, Sigma Tabs, 1/5 ml deionised water) and H_2O_2 (Sigma Tabs, one tablet per 5 ml of deionised water) for 10 min. To stop the reaction, sections were washed with PBS. The slices were mounted on slides with agar, and subsequently analysed.

GFAP immunoreactivity was semiquantified by image analysis using an optical microscope (Zeiss) coupled with a Colour Camera 3 Charge Coupled Device (CCD) (Sony) and the Visilog 6.2 (NOESIS society, Les Ulis, France) analyser system (Brillaud et al. 2007; Ammari et al. 2008a)

The analysis occurs in three steps: First, the coloured image is transformed into three black and white images according to the three pixel colours: red, green and blue. Black and white images were divided into 256 grey levels (0–255, 0 corresponding to black). Analyses were then performed on the black and white image obtained using red (in accordance of colour staining). Secondly, for each region of interest, a specific area called the 'mask' was chosen corresponding to the cell layers without artefact. Thirdly, for each section, an optical density (O.D.) threshold was determined. This threshold was chosen as the O.D. value above which the entire glial cells were detectable without background noise. A positive stained area appeared in regions with O.D. values higher than the defined threshold. The percentage was automatically calculated as the positive stained area/mask area. Significantly, the threshold value for each section is readjusted, an important step in obtaining the optimal conditions to detect glial cells and discard some artefacts that could introduce false results. To avoid bias, analyses were made using blind protocol. Furthermore, this parameter is statistically analysed to identify differences, if any, between the groups. Areas of immunoreactive astrocytes were semiautomatically determined in the prefrontal cortex (PfCx), dentate gyrus (DG), caudate putamen (Cpu), lateral globus pallidus (LGP), and cerebellar cortex (CCx) in slices from exposed, sham-treated, and cage control rats.

Statistical analysis

All statistical analyses were performed using the SPSS 15 software (Inc., Chicago, IL, USA). All values are given as mean ± standard deviation of mean (SEM) per group. After control for normal distribution by the Levene's test, for each region tested (FCx, CCx, DG, Cpu, and LGP), the parameter measured (percentage of staining) was tested using the one-way analysis of variance (ANOVA), followed by a Bonferroni's post hoc test

for multiple comparisons between the experimental groups. The accepted probability of rejecting H0 (equality of variances) was $P < 0.05$.

Results

Reactive astrocytes three days following exposure

One-way ANOVA (four groups) revealed a qualitatively more GFAP immunostaining in prefrontal cortex (PfCx) ($P = 0.001$). The Bonferroni post-hoc test showed a significantly greater surface area of reactive astrocytes in the exposed groups (E6 and E1.5) than in the sham-treated animals (Figures 1 and 2). Specifically, the increase of reactive astrocytes in PfCx was observed between the E6 and S groups (+110%, $P = 0.001$) and between the E1.5 and S groups (+73%, $P = 0.038$), although no difference was seen between the CC and S groups ($P > 0.05$). In the dentate gyrus (DG), a significant increase in GFAP immunostaining was observed between the E1.5 and S groups (+45%, $P = 0.016$) and between the E6 and S groups (+25%, $P = 0.03$) (Figures 1 and 2). In the caudate putamen (Cpu), all the exposed groups showed significantly higher levels of reactive astrocytes than the sham-treated group showed ($P < 0.001$). As evident in Figure 1, an increase was observed at day 3 between the E1.5 and S groups (+57%, $P = 0.001$) and between the E6 and S groups (+60%, $P = 0.001$). In the lateral globus pallidus (LGP), all exposed groups showed significantly higher GFAP immunostaining levels than that of the sham-treated group ($P = 0.027$) (Figures 1 and 2). An increase in the stained area was observed at day 3 between the E1.5 and S groups (+129%, $P = 0.01$) and between the E6 and S groups (+70%, $P = 0.009$). Finally, in the cerebellar cortex (CCx), all the exposed groups showed significantly higher GFAP levels than seen in the sham-treated group ($P = 0.01$) (Figures 1 and 2). The surface area of the reactive astrocytes was observed to increase between the E1.5 and S groups (+60%, $P = 0.01$) and between the E6 and S groups (+50%, $P = 0.01$).

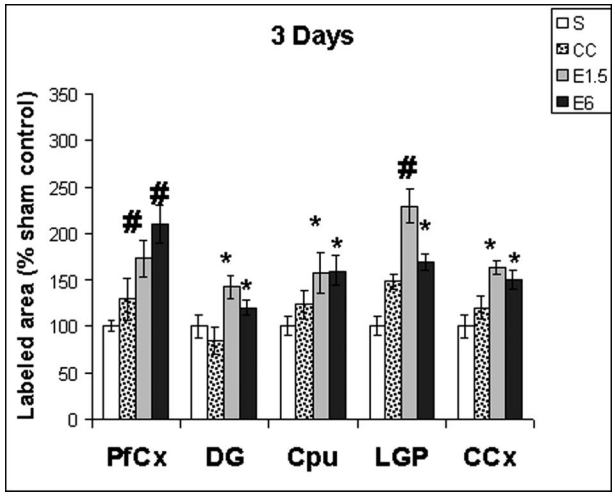

Figure 1. Mean (± SEM) of percentage of GFAP staining in the various brain regions of adult Sprague-Dawley rats three days after sub-chronic exposure (8 weeks) to a GSM signal at 900 MHz. Values of cage control and exposed animals are expressed as a percentage of the sham-treated controls. (S) Sham control group, (CC) cage control group, (E1.5) exposed animals (45 min, 1.5 W/kg), and (E6) exposed animals (15 min, 6 W/kg). Brain regions shown are as follows: PfCx, prefrontal cortex; DG, dentate gyrus; Cpu, caudate putamen; LGP, lateral globus pallidus; CCx, cerebellar cortex. $^{\star}P < 0.05$; $^{\#}P < 0.001$.

Reactive astrocytes 10 days after sub-chronic exposure

Figure 3 reveals that most brain areas show a greater increase in GFAP staining on day 10 than on day 3. Statistical analysis (one-way ANOVA test) revealed greater GFAP immunostaining qualitatively in the PfCx ($P = 0.037$). The Bonferroni post-hoc test showed a significant increase in reactive astrocyte surface area in the E6 rats when compared with the sham-treated group (+138%, $P = 0.008$); this test also showed a more dramatic increase in staining in the PfCx on day 10 than on day 3 (Figures 3 and 4). However, a tendency for stain increase was noted in the PfCx of E1.5 rats than in the PfCx of the sham-treated animals ($P = 0.056$). In the DG, a significant increase in reactivity was observed between the E6 and S groups (+148%, $P = 0.01$), and a similar tendency was observed between the E1.5 and S groups (+97%, $P = 0.08$). In the Cpu, no significant differences in GFAP staining were seen between the exposed and sham-treated groups ($P > 0.05$). By contrast, a significant increase in GFAP immunostaining was observed in the LGP ($P = 0.03$). This effect is the result of increased astrocyte reactivity in the LGP of E6 rats when compared with sham animals (+91%, $P = 0.01$), and E1.5 rats compared with sham animals (+83%, $P = 0.02$). Finally, no significant difference in reactive astrocytes was observed in the CCx of E6 and E1.5 rats when compared with sham-treated rats ($P > 0.05$).

Discussion

The present study shows that sub-chronic exposure to GSM microwaves for 45 min/day at SAR = 1.5 W/kg or for 15 min/day at SAR = 6 W/kg induces an increase in GFAP expression in the rat brain three days following the exposure. In several rat brain regions, this effect persisted for 10 days after the exposure period, suggesting that sub-chronic exposure to this signal could be associated with underlying neuronal damage.

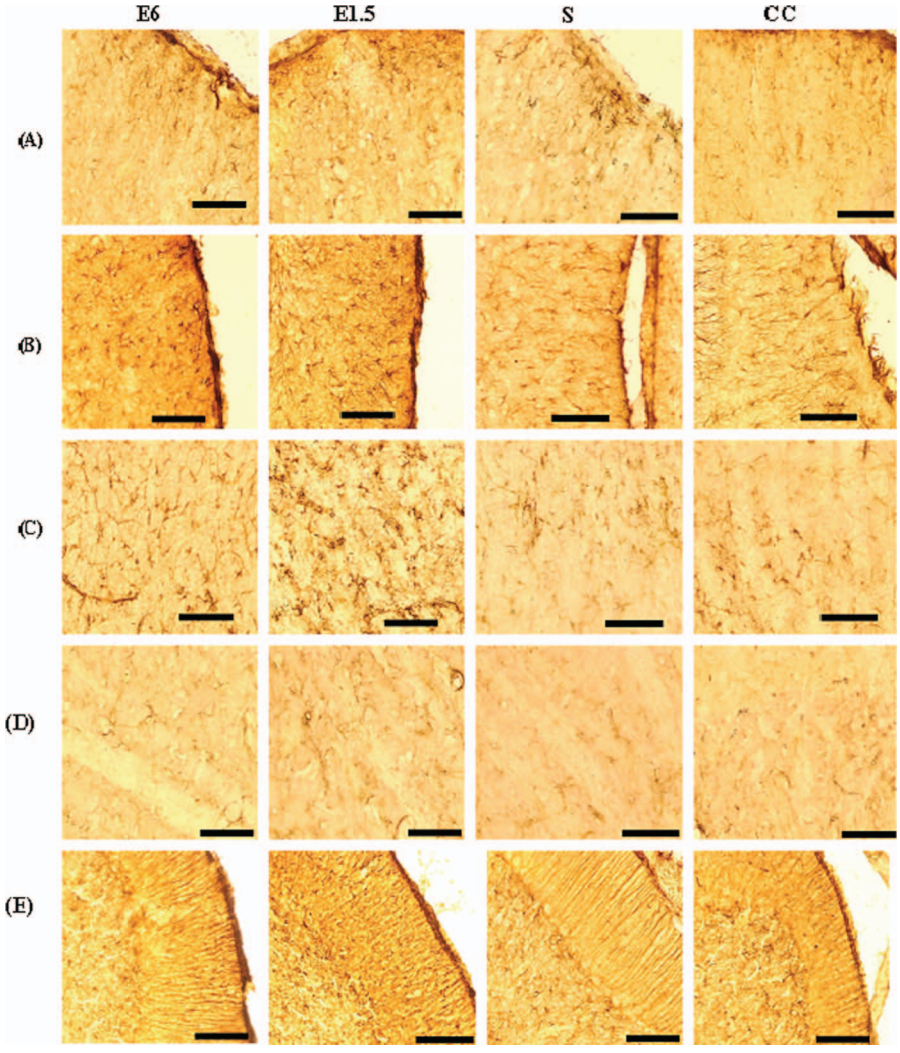

Figure 2. Enlargements (objective, 10 ×; ocular, 4 ×; scale bar, 50 μm) of GFAP immunostaining in the prefrontal cortex (A), dentate gyrus (B), lateral globus pallidus (C), caudate putamen (D), and cerebellar cortex (E) of exposed animals E6 (15 min, 6 W/kg), exposed animals E1.5 (45 min, 1.5 W/kg), sham (S) and cage control (CC) groups, three days after sub-chronic exposure (8 weeks) to a GSM signal at 900 MHz.

Specifically, our study shows significant differences between the GFAP levels of exposed (E6 and E1.5) and sham-treated animals three days post sub-chronic exposure, indicating that reactive astrocytes are histologically characterised by cytoplasmic hypertrophy of the glial cells. This hypertrophy indicates inflammation, although not necessarily a permanent injury such as neuronal death (Norenberg 1994; Jensen et al. 1997; Ridet et al. 1999).

At a SAR of 1.5 W/kg, the amount of GFAP expression increased significantly (75%) at three days after sub-chronic exposure to GSM microwaves in the five brain areas examined: PfCx, DG, Cpu, LGP, and CCx. Approximately 10 days after exposure, this increase rose to around 90%, which although notable, was not statistically significant, except in the LGP. This large increase appears to be due to extreme values; in fact, the highest GFAP staining values for each structure were not found in the same animal. Thus, the large SEM for these data reflects a non-homogeneous cell population. The statistical power for the analysis of these values (GFAP staining) is less than 80%, in the order of 40%, indicating that a larger number of animals would probably make these differences significant. Although the difference is not significant, the data suggest that the elevated expression of GFAP persists at least 10 days after electromagnetic field signal exposure, implying that gliosis has occurred. Studies with larger sample sizes are needed to verify this conclusion.

At a SAR of 6 W/kg, the exposure induced a significant rise in GFAP staining in the PfCx (110%), Cpu (60%), LGP (70%), DG (+25%), and CCx (50%) three days following exposure. Significantly, this effect is pronounced 10 days after exposure in the PfCx (130%), DG (148%), and LGP (90%). Our results show that two months

of sub-chronic exposure to a 900 MHz GSM signal at a SAR of 6 W/kg induces astrogliosis in rat brain. Further, this astrogliosis persists three and ten days after the end of the exposure. This contrasts with a previous study that showed that acute exposure to a GSM signal at a SAR = 6 W/kg induced glial reactivity after three days, but not after 10 days (Brillaud et al. 2007). This previous result suggests inflammation in the brain.

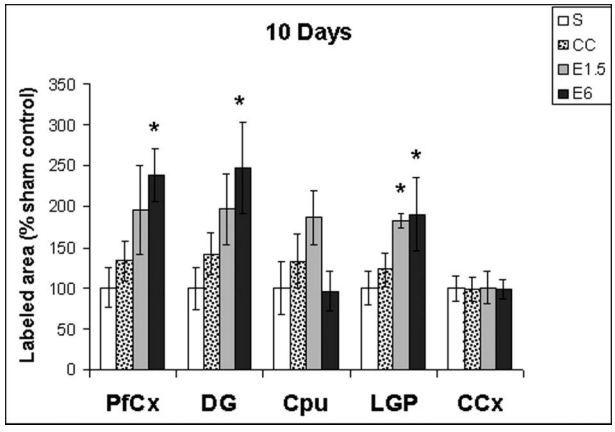

Figure 3. Mean (± SEM) of percentage of GFAP staining in the various brain regions 10 days after sub-chronic exposure (8 weeks) to a GSM signal at 900 MHz. Values of cage control and exposed animals are expressed as a percentage of the sham-treated controls. (S) Sham-treated control group, (CC) cage control group, (E1.5) exposed animals (45 min, 1.5 W/kg), and (E6) exposed animals (15 min, 6 W/kg). Brain regions shown are as follows: PfCx, prefrontal cortex; DG, dentate gyrus; Cpu, caudate putamen; LGP, lateral globus pallidus; CCx, cerebellar cortex. $*P < 0.05$.

Different factors can be proposed to explain the gliosis observed in rat brain. A thermal cause was ruled out, as the study of Brillaud et al. (2007) found that exposing animals to a GSM at 6 W/kg for 15 min did not increase skin temperature. Also, D'Andrea et al. (2003) showed that exposure to lower SAR levels did not noticeably affect body temperature, and the slight changes observed were mainly reversible. It therefore, seems unlikely that radio waves induce astrogliosis through a generic heating effect. In this study, exposing rats to an SAR of 1.5 W/kg is well below the International Commission on Non-Ionising Radiation Protection (ICNIRP 1998) limit of 2 W/kg for thermal effects, in the light of RF exposure.

Other studies show that cytokines can be effectors of gliosis (Schultz et al. 2004; Woiciechowsky et al. 2004). Pro-inflammatory cytokines such as interleukin (IL)- 1 and IL-6 have been known to boost glial scar formation (John et al. 2004; Okada et al. 2004; Woiciechowsky et al. 2004). Kyrkanides et al. (1999) suggested that ionising radiation-induced injuries to the central nervous system activate the glia and lead to the production of pro-inflammatory cytokines, usually associated with radiation-induced gliosis. Hwang et al. (2006) observed that irradiated microglia release prostaglandin E_2 (PGE_2), which induces astrocytes to further differentiate and show a

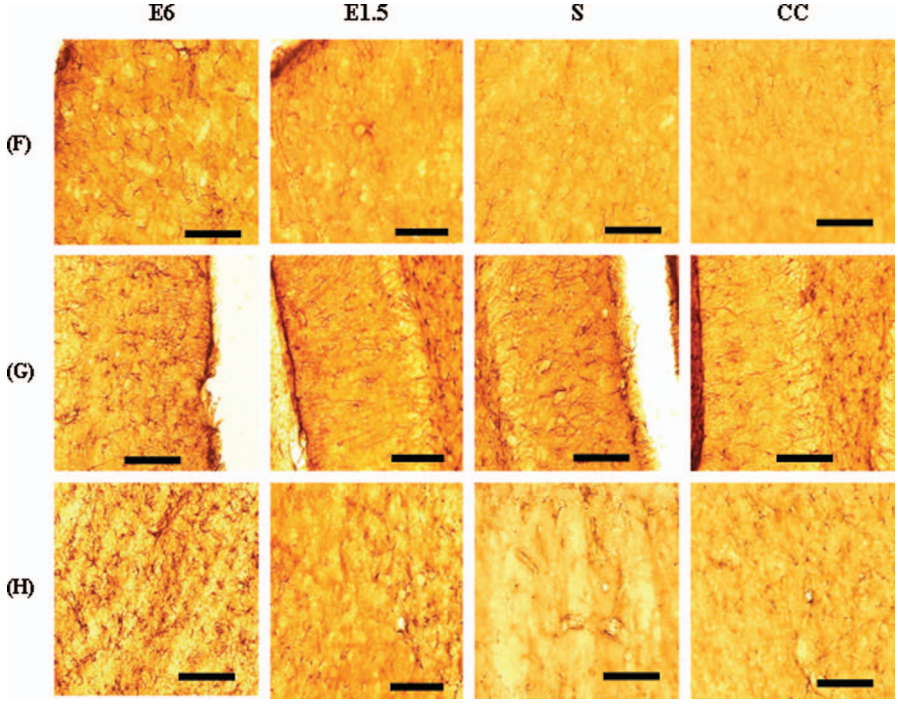

Figure 4. Enlargements (objective, 10 ×; ocular, 4 ×; scale bar, 50 μm) of GFAP immunostaining in the prefrontal cortex (F), dentate gyrus (G), and lateral globus pallidus (H) of E6-exposed animals (15 min, 6 W/kg), E1.5 exposed animals (45 min, 1.5 W/kg), sham-treated (S) and cage control (CC) groups, 10 days after sub-chronic exposure to a GSM signal at 900 MHz.

higher degree of GFAP immunostaining, both of which are typical astrogliosis symptoms.

Martinez et al. (2008) showed that the astrocytes are the targets of oxidative damage, whereas the GFAP is a target of lipoxidation. Other studies have shown a relationship between RF emission by mobile phones and oxidative stress. Ilhan et al. (2004) exposed rats to RF radiation at 900 MHz for 1 h per day at a specific absorption rate (SAR) in the brain of 2 W/kg for seven days. They found increased malonaldehyde (MDA) and nitric oxide (NO) levels in brain tissue. Dasdag et al. (2004) found that increased MDA levels in rat brains exposed to RFR from a 900 MHz source at SAR of 0.52 W/kg for 20 min a day, 7 days a week, for 1 month. In this study, oxidative stress is suspected to affect astrocytes (increased GFAP expression) in the rat brain after exposure to a 900 MHz electromagnetic field signal.

Prior studies showed that acute exposure to GSM microwaves induced reactive astrocytes. Mausset-Bonnefont et al. (2004) observed an increase in the reactive surface area three days following acute exposure to a GSM signal at 6 W/kg in the cortex, hippocampus, and the striatum. The authors concluded that exposure had induced astrocyte hypertrophy and/or hyperplasy. Consistent with the effect of microwaves on astrocytes, Brillaud et al. (2007) observed hypertrophy of the glial cells after a single exposure to GSM at a high SAR (6 W/kg). The chronic (6 months) exposure to a 900 MHz electromagnetic field signal (SAR = 6 W/kg) induced increased GFAP staining in the rat brain areas (prefrontal cortex, caudate putamen, lateral globus pallidus of striatum, dentate gyrus of hippocampus) (Ammari et al. 2008a).

In contrast to the latter studies, Grafström et al. (2008) reported that exposure of rats to GSM 900 MHz radiation (whole-body SAR-values of 0.6 and 60 mW/kg) for a long-term period of 55 weeks, did not induce alteration of histopathological parameters (glial reactions, the amount of albumin leakage, the occurrence of dark neurons, lipofuscin aggregation). Also, Thorlin et al. (2006) showed that exposure of cultured astroglial brain cells to 900 MHz microwave radiation for 4 h at a SAR = 27 W/kg or for 24 h at an SAR = 54 kg did not induce an increase in GFAP expression.

Finally, our results demonstrate that sub-chronic exposure to SARs of 1.5 W/kg and 6 W/kg can induce gliosis in the rat brain three and 10 days after the end of exposure. Thus microwave radiation affects reactive astrocytes not only in the cortex (superficial part of the brain) but also in deeper structures (striatum, hippocampus).

Future studies are warranted to obtain precise knowledge of astrogliosis, its type and pattern and the underlying signalling mechanisms for the neurotoxicity due to sub-chronic exposure to an electromagnetic field signal of 900 MHz. GFAP levels could be quantified by ELISA to evaluate the extent and time course of radiofrequency-induced gliosis.

Our study and previous works link GSM exposure to astrogliosis that can lead to neuronal damage, including cell death, synapse loss, axonal damage, and myelin damage. Application of both traditional and specialised staining procedures would therefore be highly useful to reveal the resultant histopathological damage. These could be performed according to the methods of Chen et al. (2002), Fields and Stevens-Graham (2002), Benkovic et al. (2004), Kurosaki et al. (2004), Milenkovic et al. (2005), and Ho et al. (2007), which include Nissl, hematoxylin, cupric-silver, Fluoro-Jade B (FJB), and lectin stains, to evaluate the histopathological alterations that can be induced by exposure to electromagnetic field.

Acknowledgements

The authors would like to acknowledge Maryse Marliere and Cray Valley (INERIS) for their technical assistance with the dose analysis and determination of SAR values. This work was funded by Project RFMemat, The Health and Radiofrequencies Foundation (2006–05), Pr 189 and RF chroTox DRC 02 – AP 07 of French Ministry of Ecology (MEDDAD).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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