Environ Sci Pollut Res (2016) 23:25343–25355
DOI 10.1007/s11356-016-7758-y

RESEARCH ARTICLE

Glial markers and emotional memory in rats following acute cerebral radiofrequency exposures

Amélie Barthélémy[1,2] · **Amandine Mouchard**[1,3] · **Marc Bouji**[1,4] · **Kelly Blazy**[1,5] · **Renaud Puigsegur**[1,6] · **Anne-Sophie Villégier**[1,5]

Received: 15 June 2016 / Accepted: 21 September 2016 / Published online: 30 September 2016

Abstract The widespread mobile phone use raises concerns on the possible cerebral effects of radiofrequency electromagnetic fields (RF EMF). Reactive astrogliosis was reported in neuroanatomical structures of adaptive behaviors after a single RF EMF exposure at high specific absorption rate (SAR, 6 W/kg). Here, we aimed to assess if neuronal injury and functional impairments were related to high SAR-induced astrogliosis. In addition, the level of beta amyloid 1–40 (Aβ 1–40) peptide was explored as a possible toxicity marker. Sprague Dawley male rats were exposed for 15 min at 0, 1.5, or 6 W/kg or for 45 min at 6 W/kg. Memory, emotionality, and locomotion were tested in the fear conditioning, the elevated plus maze, and the open field. Glial fibrillary acidic protein (GFAP, total and cytosolic fractions), myelin basic protein (MBP), and Aβ1–40 were quantified in six brain areas using enzyme-linked immunosorbent assay. According to our data, total GFAP was increased in the striatum (+114 %) at 1.5 W/kg. Long-term memory was reduced, and cytosolic GFAP was increased in the hippocampus (+119 %) and in the olfactory bulb (+46 %) at 6 W/kg (15 min). No MBP or Aβ1–40 expression modification was shown. Our data corroborates previous studies indicating RF EMF-induced astrogliosis. This study suggests that RF EMF-induced astrogliosis had functional consequences on memory but did not demonstrate that it was secondary to neuronal damage.

Keywords Electromagnetic fields · Astrogliosis · Glial fibrillary acidic protein · Myelin basic protein · Beta amyloid 1–40 · Fear conditioning · Elevated plus maze · Open field

Responsible editor: Philippe Garrigues

Amélie Barthélémy and Amandine Mouchard have equally contributed

✉ Anne-Sophie Villégier
anne-sophie.villegier@ineris.fr

[1] Institut national de l'environnement industriel et des risques (INERIS), Unité de Toxicologie Expérimentale, Parc Technologique ALATA, BP no. 2, 60550 Verneuil-en-Halatte, France

[2] Institut des Neurosciences Cellulaires et Intégratives, CNRS UPR 3212, 5 rue Blaise Pascal, 67084 Strasbourg, France

[3] Institut des Maladies Neurodégénératives CNRS UMR5293 Université de Bordeaux, 146 rue Léo Saignat, 33076 Bordeaux, France

[4] Campus des sciences et technologies, Université Saint-Joseph, Dekwaneh, Mar Roukos, Lebanon

[5] Unité mixte PERITOX EA 4285-UM INERIS 01 Laboratoire Périnatalité et risques toxicologiques CHU Amiens-Picardie Hôpital, Sud Avenue Laënnec, 80 480 Salouël, France

[6] Sous-direction de la police technique et scientifique, 31 Avenue Franklin Roosevelt, 69130 Ecully, France

Introduction

With the widespread use of mobile phones, the possible neurobiological effects of radiofrequency electromagnetic fields (RF EMF) have become a crucial question.

Glial reactivity was reported after an acute RF EMF exposure to the rat brain at an averaged specific absorption rate (SAR) of 6 Watts/kg (W/kg). Mausset-Bonnefont et al. (2004) showed an increase in glial fibrillary acidic protein (GFAP) staining in the hippocampus, the striatum, and the cortex 3 days after exposure. This data was reproduced by Brillaud et al. (2007) who showed GFAP staining increase in the striatum and the frontal cortex 2 to 3 days after the same exposure. Whether RF EMF leads to gliosis is an important matter of debate as data from the literature are contradictory. Mausset et al. (2001), Mausset-Bonnefont et al. (2004), Brillaud et al. (2007), Ammari et al. (2008a, b), Maskey

et al. (2010, 2012), and Lu et al. (2014) looked at the link between SAR and GFAP increase using acute or repeated RF EMF exposures and reported positive effects. Fritze et al. (1997), Thorlin et al. (2006), Grafstrom et al. (2008), Watilliaux et al. (2011), Bouji et al. (2012), Court-Kowalski et al. (2015), Bouji et al. (2016), and Petitdant et al. (2016) did not show any positive effect. Astrocytes insure a variety of functions in the brain. Neurologic function could be altered in some important respects if RF EMF mobile phone exposures did cause astrocytic reaction. This question needs new insights.

Astrogliosis is a nonspecific consequence of many insults. It aims at protecting the brain against deleterious processes as excitotoxic glutamate neurotransmitter excess, neuron lesions, oxidative stress, neuroinflammation, or toxicity of the beta amyloid peptide (Aβ). Increased staining of GFAP, the main intermediate filament of astrocytes, is the hallmark of reactive astrogliosis (Eng 1985; Schiffer et al. 1986). Mechanisms of this response are rearrangements of the intermediate filament network, including breakdown of F-actin stress fibers, polymerization of upregulated GFAP, and vimentin and nestin overexpression. Increased GFAP staining is not always linked to significant rise in protein content, but may be correlated to cytoskeleton organization changes, usually detectable by variations in GFAP detergent solubility (Boran and Garcia 2007). The consequent cell morphology modifications, as processes hypertrophy, reflect metabolic activation, as shown by increased organelles number or size, and release of chemical factors (cytokines) in the case of mild to moderate astrogliosis (Schmidt-Kastner and Szymas 1990; Sofroniew 2009).

Astrogliosis can be secondary to demyelinating lesions. Progenitor cell differentiates into myelinating oligodendrocytes to reestablish myelination in the demyelinated zones (Nishiyama et al. 2009; Zhao et al. 2009). Oligodendrocytes selectively express myelin basic protein (MBP). The transitory decrease in brain MBP expression was shown after exposure to several environmental contaminants (chemicals, plants, medicines, or metals) (Coria et al. 1984; Schang et al. 2013; Bhasker et al., 2014; Ma et al. 2015). MBP increase was also shown within 2 to 3 days post-injury in toxicological conditions.

In healthy organisms, astrocytes are involved in neuronal electrical activity regulation through reciprocal communications in the tripartite synapses (Wenzel et al. 1991; Rapanelli et al. 2011). Astrocytes are in a symbiotic relationship with neurons to optimize neural information processing, to modulate synaptic transmission and neuronal activity (Hewett 2009). Neuronal electrical activity in the subcortical structures (as the striatum and the hippocampus) and in cortical regions participates to generate mental functions: memory, emotionality, and novelty perception. Astrocyte-released pro-inflammatory molecules may affect these functions and adaptative behaviors (Eddleston and Mucke 1993; Rothstein et al. 1996). Severe astrogliosis may induce collateral neurotoxicity on biomarkers and cognitive functions by exacerbating the deleterious processes (Sofroniew and Vinters 2010; Middeldorp and Hol 2011). Memory performances have been shown to be impaired by lipopolysaccharide-induced inflammatory (Lee et al. 2009; Spitzer et al. 2010). Some authors indicated that long-term RF EMF exposures impaired memory performances and modified the levels of Aβ (Arendash et al. 2010; Dragicevic et al. 2011). Amyloidogenesis has been the endpoint of recent studies in health assessments of environmental contaminants. Aβ synthesis was shown to be stimulated by several neurotoxic agents including medications, metal mixtures, hormones, or ozone (Blasko et al. 2008; Ma and Liu 2015; Ashok et al. 2015; Deng et al. 2015; Hernandez-Zimbron and Rivas-Arancibia 2015).

Because of the important neurological consequences if RF EMF mobile phone exposures did cause memory impairments, this question needs new insights. Up to date, there are no consensual conclusions on the possible effect of RF EMF exposure on memory. Both positive (Preece et al. 1999; Koivisto et al. 2000; Krause et al. 2000; Maier et al. 2004; Keetley et al. 2006; Nittby et al. 2008; Arendash et al. 2010) and negative reports (Sienkiewicz et al. 2000; Dubreuil et al. 2002; Haarala et al. 2007; Ammari et al. 2008a, b; Petitdant et al. 2016; Bouji et al. 2016) were published.

Here, we aimed to reproduce the neurotoxicity of an acute high SAR RF EMF exposure (Mausset-Bonnefont et al. 2004; Brillaud et al. 2007). Rats were exposed at 0 and 1.5 W/kg for 15 min, 6 W/kg for 15 min, or 6 W/kg for 45 min. Responses to a single exposure aimed to assess mechanisms for RF EMF and nervous tissue interaction, which are not necessarily detectable using intermittent exposures due to habituation or sensitization processes of the organism. The exposure durations of 15 and 45 min are compatible with the duration of daily calls in moderate and intensive mobile phone users (INTERPHONE group 2010; Hardell and Carlberg 2013). In order to test if biological responses would be in the direct ratio of the total absorbed energy, four rising levels were tested by increasing the brain averaged SAR (BASAR) and the exposure duration of the highest BASAR (to 45 min). We evaluated emotional memory, anxiety-like behavior, and locomotor activity. Tests were performed in the fear conditioning paradigm, the elevated plus maze (EPM), and the open field. Three days after RF EMF exposure, markers were assessed using enzyme-linked immunosorbent assay (ELISA). Previous studies indicated that the increase of GFAP and Aβ peptide as well as the decrease of MBP was rapidly observed, 2 to 3 days after a toxic stimulation (Morin-Richaud et al. 1998; Ma and Liu 2015). Two phenotypes of astrogliosis were quantified: the total GFAP expression and the (soluble monomeric GFAP)/(total GFAP content) ratio (Eng et al. 2000; Tseng et al. 2006; Maskey et al. 2010; Watilliaux et al. 2011; Lu et al. 2014), as well as MBP and Aβ 1–40. Six brain structures involved in

mental functions were exanimate: the cortex (anterior and posterior), the striatum, the hippocampus, the olfactory bulb, and the cerebellum).

Material and methods

Animals

Sixty-eight 6-week-old Sprague Dawley male rats (OFA Iffa Credo, France) were daily weighed and handled for 1 week before the start of experiments. We used male rats to be consistent with previous studies from our laboratory on dosimetry, behavioral paradigms, and restraint effect. The rats were housed two per cage in controlled environmental conditions (room temperature 22 °C; 12-h light/dark cycle) with food and tap water ad libitum. The cage environment was enriched with a plastic tube identical in size, shape, and appearance to RF EMF exposure rockets (Brillaud et al. 2005). The rats were free to explore, and move in and out of the tube from their arrival in the vivarium until sacrifice. INERIS Use Committee and French State Council guidelines were followed for the care and use of laboratory animals (Decree no. 87–849, October 19, 1987).

Experimental groups

The rats were randomly assigned to four independent groups (n = 14–20 rats/group) exposed to SAR doses: 0 W/kg for 45 min, 1.5 W/kg for 15 min, 6 W/kg for 15 min, and 6 W/kg for 45 min. Restraining in the exposure rocket lasted 45 min for all the groups. On the first day, the rats were subjected to fear conditioning training, immediately followed by an acute RF EMF exposure. The contextual memory test was performed 3 h after the end of the training session (and 3 h after the beginning of RF EMF exposure). On the second day, cue memory test was performed in the morning (21 h after the beginning of RF EMF exposure) and the 24 h contextual memory test in the afternoon (25 h after the beginning of RF EMF exposure). The 24-h contextual memory test was performed 24 h following the end of the 3-h contextual memory test during which there was a recall of the context/tone/electrical shock presentation. On the third day, the rats were tested in the open field in the morning (45 h after the beginning of RF EMF exposure) and in the elevated plus maze (EPM) in the afternoon (50 h after the beginning of RF EMF exposure). The open field and the EPM tests were applied at the same day as in previous study from Petitdant et al. (2016). The rats were sacrificed on the fourth day (72 h after the beginning of RF EMF exposure).

Exposure system

Exposure setup was previously described (Mausset et al. 2001; Dubreuil et al. 2002). A radio frequency power source (900–64 type, radio frequency power amplifier, France) emitting a 900 MHz RF EMF (1/8 duty factor) pulse modulated at 217 Hz was connected to a four-output divider. Each output was connected to a loop antenna allowing local exposure of four animal's head simultaneously in an anechoic chamber. During exposure, each animal was placed in a Plexiglas rocket capped with a truncated cone containing an individual loop antenna. The rocket body was lined with air holes to facilitate breathing and minimize the rise in body temperature. SAR calculations were made with homogeneous phantoms (Leveque et al. 2004).

Behavior

Fear conditioning

Fear conditioning is a Pavlovian conditioning, in which a neutral conditioned stimulus (a sound) is paired with an aversive unconditioned stimulus (a footshock). It occurred in two standard fear conditioning apparatus purchased from Bioseb (France). Two freezing boxes (25.5 × 27 × 26 cm) were each located inside a larger, insulated plastic chamber that provided protection from outside light and noise. The boxes were made with black methacrylate walls and a transparent front door. The floor consisted of 20 stainless steel rods through which a shock could be delivered. The rods were connected to a high-sensitivity weight transducer system that measured animal movements. The conditioning apparatus was controlled by the experimenter with specific software (Panlab, Barcelona). Freezing response was defined as complete immobilization of the rat, except for respiration (Blanchard and Blanchard 1972). It was measured in response to stimuli presentation (context and tone) by amplitude of movement of the animal and was scored as percentage of time spent freezing. At the end of each session, the animals were returned to their home cages, and the boxes were cleaned with non-alcohol disinfectant.

The training phase began with a 2-min habituation period (no auditory or aversive stimulus), followed by five training cycles (28 s of sound (2 kHz, 100 dB); 2 s of sound + electrical shock (0.25 mA); a 30-s exploration period (no auditory or aversive stimulus)). To test contextual memory, the animals were introduced for 3 min in the boxes used for the training. To avoid extinction, the 3-h contextual memory test ended with two training cycles. To perform the cue memory test, the animals were introduced in boxes with modified environment (different box size, wall color, and floor texture) for 2 min and were exposed to the sound for 3 min.

Open field

The open field test was performed according to previously described methods (Prut and Belzung 2003). Movements were monitored for 30 min using an automated apparatus consisting of four Plexiglas boxes (60 × 60 × 40 cm) equipped with 16-bottom and 8-top (for vertical activity evaluation) infrared beams located along two adjacent sides of one chamber (Imetronic, France). Activity was measured by the number of counts, i.e., the number of infrared beam interruptions. Anxiety-related behavior was assessed by the time spent in the central area of the apparatus.

Elevated plus maze

The EPM test was performed according to previously described methods (Daniels et al. 2004). The apparatus was composed of a plus shaped acryl maze with two opposite open arms and two opposite closed arms (50 cm in length, 10 cm in width, and 31 cm in height), extending out from a central platform (10 × 10 cm) and elevated 50 cm above the floor. The rats were placed in the center of the maze, the head facing an open arm, and were allowed to explore for 5 min. The maze was cleaned after each test to prevent influences of previously tested rat. Two paws had to be inside the entrance line to each arm, which signaled the start of the time spent in the specific arm, and then the end time was recorded when all four paws were outside the line again. Tests were recorded and the time spent in the different arms was analyzed using Smart software (Panlab). Data were presented as percentage of time spent in the open arm compared to the time spent in the four arms during the first 30 s of the test.

Brain sample preparation

Animals were anesthetized with isoflurane. Intra-cardiac perfusion was performed with 0.9 % NaCl solution. Following decapitation, the brains were immediately removed and placed on a cold plate. The brains were divided in two hemispheres. The striatum, the hippocampus, the cortex, the olfactory bulb, and the cerebellum were dissected from one hemisphere with curved forceps. The third ventricle was the landmark to dissect the anterior and the posterior cortex. Samples of brain tissue were snap-frozen on a bed of dry ice and then grounded into a powder. Tissue was stored at −80 °C.

Enzyme-linked immunosorbent assay

Total protein assay

Total protein was assayed by the method of Bradford. Bovine serum albumin (BSA, Sigma-Aldrich) was used as standard. Absorbance was read at 595 nm using a Molecular Devices UV Max microplate reader (Infinite M200 TECAN, Menlo Park, CA, USA) coupled to a Macintosh computer running a Soft Max (Molecular Devices, Menlo Park, CA, USA) program.

GFAP

The method was slightly modified from O'Callaghan (1991). Brain samples were weighed and homogenized using an homogenizing vessel in hot (90–95 °C) 1 % SDS in PBS supplemented with 1× protease inhibitor cocktail (1/25, Roche) to obtain the total cell fraction. Part of the total cell fraction was centrifuged (15 min 15,000*g*) to obtain the cytosolic fraction. Both total and cytosolic fractions were analyzed for their GFAP content. Standard curve samples were prepared with human GFAP (Merck) in sample buffer at dilutions 0.0075, 0.015, 0.03, 0.06, 0.1, 0.25, 0.5, and 1.0 μg/100 μl. Immulon-2 flat-bottom microtiter plates (Greiner Bio-One) were coated with the rabbit polyclonal anti-GFAP (Dako Cytomation) (1.0 μg/100 μl in PBS) for 1 h at 37 °C and then overnight at 4 °C. Blocking was performed with BSA 2 %. Standards and samples were incubated for 1 h in duplicates (100 μl/well). Monoclonal mouse anti GFAP (Abcam) (1:500 in BSA containing Triton ×100) was then added (for 1 h). Finally, rabbit polyclonal to mouse IgG horseradish peroxidase (HRP)-conjugated (Abcam) (1:3000 in BSA containing 0.5 % Triton ×100) was incubated for 30 min. Revelation was performed by adding 3,3′,5,5′-tetramethylbenzidine (TMB) (Interchim) and was stopped 10 min later with H_3PO_4 1 M (Uptima). Optical densities were read at 450 nm.

MBP

ELISA assay was performed using commercialized kits for rat MBP (USCN Life Science Inc.). Tissue samples were diluted in PBS 1× supplemented with protease inhibitor cocktail. MBP standard curve was prepared in standard diluent at 0.156, 0.312, 0.625, 1.25, 2.5, and 5 ng/ml. Blank, standard dilutions and samples were incubated for 2 h at 37 °C in duplicate in the pre-coated plate. Detection reagent containing the primary antibody anti-MBP was added (for 1 h at 37 °C). After washing, detection was performed using the reagent containing the HRP-conjugated secondary antibody (30 min at 37 °C). Finally, the substrate solution was added to each well (15–25 min at 37 °C) before stopping the reaction. Optical densities were read at 450 nm.

Amyloid β 1–40

Aβ 1–40 concentration was measured using commercialized kits allowing Aβ 1–40 detection with a 0.049 pmol/l sensitivity (Wako Chemicals, GmbH, Germany) and based on the sandwich ELISA method (Silverberg et al. 2010). Tissue

samples were prepared with tissue powder extracted by sonication with 70 % formic acid and centrifugation (100,000*g* for 1 h). The supernatant was removed and neutralized with 20 volumes of Tris Base (1 mol/l). Samples and standards (0, 1, 2.5, 5, 10, 25, 50, and 100 pmol/l) were diluted with the kit diluent and added in duplicate to the monoclonal antibody anti-Aβ11–28 (BNT77)-pre-coated 96-well microtiter plate (for 2 h at room temperature). Then, HRP-conjugated antibody solution was incubated for 2 h at 4 °C. Finally, TMB was added to each well, and reaction was terminated 30 min later using the kit stop solution. Total Aβ 1–40 concentrations (pg/mg total protein) were expressed as percentages of sham group. Optical densities were read at 450 nm.

Statistical analysis

Statistical analyses were performed using SPSS 17 software (Inc., Chicago, IL, USA). Values are given as mean ± standard error of mean per group. Control for normal distribution was performed using Levene's test. Analyses were performed using two-way analysis of variance (ANOVA) (for time × SAR level, time × exposure duration) and one-way ANOVA (for SAR level or exposure duration effect). SAR level effect was assessed by comparing the 0, 1.5, and 6 W/kg groups exposed for 15 min, while exposure duration effect was assessed by comparing the 0 and 6 W/kg 15 min, and 6 W/kg 45 min groups. Main effects were analyzed using Bonferroni's post hoc corrected *t* test. Time was analyzed as within-subject factors while exposure doses were analyzed as between-subject factors. Significant effects were found when $p < 0.05$. The *p* value was one-tailed for total GFAP increase, MBP decrease, and Aβ increase and two-tailed for behavioral tests and cytosolic GFAP. Linear models and correlations (Pearson) were performed using R statistical environment. The linear model aimed to explain long-term memory by Aβ in the hippocampus and in the striatum and by their interactions with RF EMF exposure.

Results

Effect of RF EMF on total GFAP

Figure 1 shows total GFAP in the six cerebral structures 3 days following RF EMF exposure. ANOVA performed on each structure showed significant SAR level effect in the striatum ($p = 0.03$), but no exposure duration effect. There was no exposure duration or SAR level effect in the other structures ($p > 0.05$). Bonferroni's post hoc corrected *t* test showed significant GFAP increase in the striatum in the 1.5 W/kg-exposed rats when compared to the sham-exposed group ($p < 0.05$).

Effect of RF EMF on cytosolic GFAP

Figure 2 shows cytosolic GFAP. ANOVA performed on the each structure showed a significant SAR level effect in the striatum ($p = 0.01$) and the hippocampus ($p = 0.02$). Both SAR level and exposure duration effects were obtained in the olfactory bulb ($p = 0.04$ and $p = 0.05$), while no significant SAR level or exposure duration effect was obtained in the other structures ($p > 0.05$). Post hoc Dunnett's-corrected *t* test showed that soluble GFAP was significantly decreased in the 1.5 W/kg-exposed group when compared to the sham group in the striatum ($p = 0.05$) and significantly increased in the 6 W/kg–15 min-exposed group when compared to both the sham and the 1.5 W/kg–15 min-exposed groups in the hippocampus ($p = 0.05$) and in the olfactory bulb when compared to the sham group ($p < 0.05$).

Effect of RF EMF on MBP

Figure 3 shows MBP in the six cerebral structures 3 days following RF EMF exposure. ANOVA performed on MBP for each structure did not show any SAR level effect ($p > 0.07$) or exposure duration effect ($p > 0.09$).

Effect of a post-conditioning RF EMF exposure on emotional memory

Figure 4a–c shows time spent freezing in response to the context 3 and 24 h after the conditioning, and to the tone, respectively. Figure 5d shows the comparison between the 3- and 24-h tests. ANOVA performed separately on the three tests (Fig. 4a–c, respectively) showed significant time effect ($F(35,2240) = 4.7$, $p < 0.001$; $F(35,2240) = 10$, $p < 0.001$; and $F(35,2240) = 15.9$, $p < 0.001$), but no SAR level effect ($F(2,45) = 0.6$, $p = 0.6$; $F(2,45) = 0.9$, $p = 0.4$; and $F(2,45) = 1$, $p = 0.4$), time × SAR level interaction ($F(70,1575) = 1.1$, $p = 0.4$; $F(70,1575) = 1.1$, $p = 0.3$; and $F(70,1575) = 0.8$, $p = 0.9$), exposure duration effect ($F(2,51) = 0.7$, $p = 0.5$; $F(2,51) = 0.4$, $p = 0.7$; and $F(2,51) = 0.4$, $p = 0.6$), and exposure duration × time interaction ($F(70,1785) = 0.8$, $p = 0.9$; $F(70,1785) = 0.9$, $p = 0.7$; and $F(70,1785) = 0.8$, $p = 0.8$). ANOVA performed to compare contextual memory at 3 and 24 h showed no significant test delay × SAR level interaction ($F(3,64) = 0.4$, $p = 0.7$) or SAR level effect ($F(3,64) = 0.9$, $p = 0.5$) but a significant test delay effect ($F(1,64) = 4.1$, $p = 0.04$). Bonferroni's post hoc corrected *t* test showed that contextual memory was significantly decreased at 24 h when compared to the test at 3 h in the 6 W/kg (15 min)-exposed group ($p = 0.03$) (Fig. 4d). Exploration of the data using a linear model indicated that long-term memory was

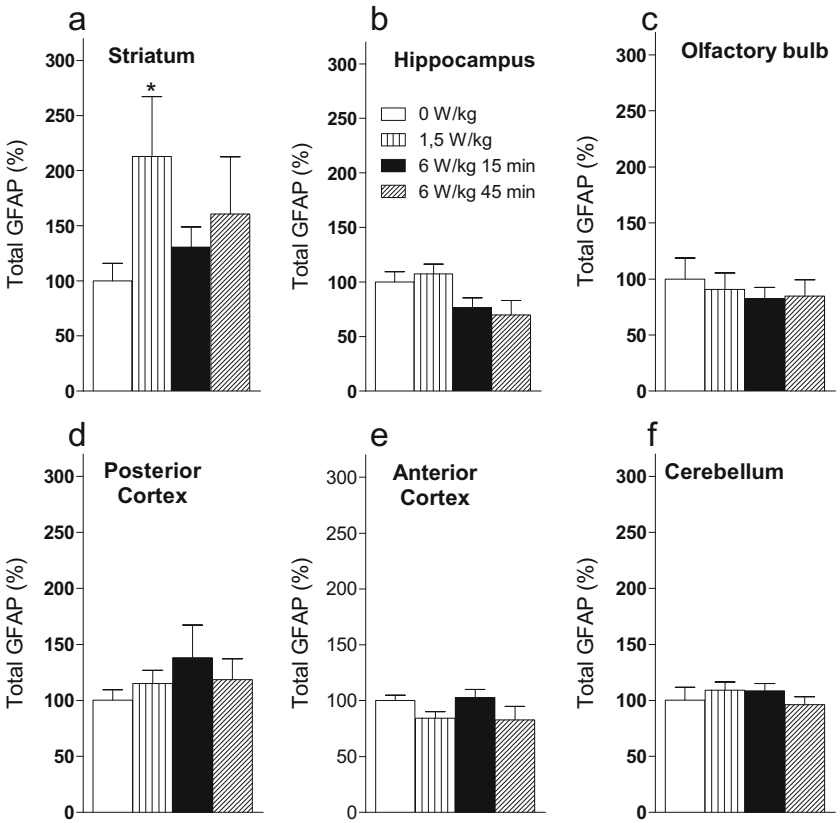

Fig. 1 Total GFAP in the striatum (**a**), the hippocampus (**b**), the olfactory bulb (**c**), the posterior cortex (**d**), the anterior cortex (**e**), and the cerebellum (**f**) in the sham- and RF EMF-exposed rats. Total striatal GFAP in the 1.5 W/kg-exposed rats was significantly increased compared to that in the sham-exposed group (*$p < 0.05$). $N = 8$/group

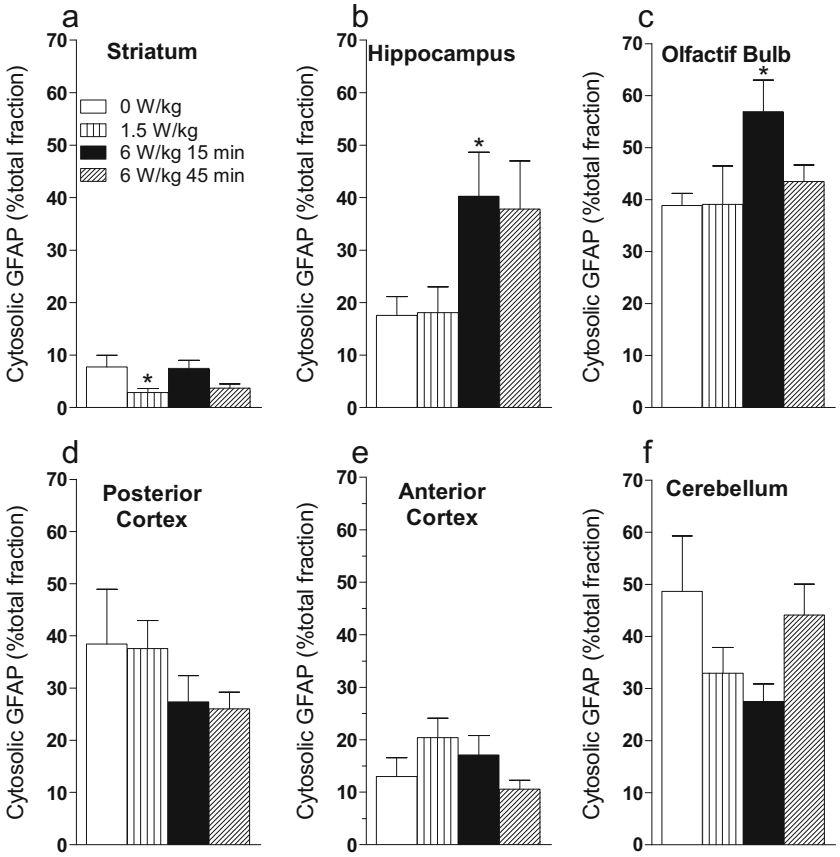

Fig. 2 Cytosolic GFAP in the striatum (**a**), the hippocampus (**b**), the olfactory bulb (**c**), the posterior cortex (**d**), the anterior cortex (**e**), and the cerebellum (**f**) in the sham- and RF EMF-exposed rats. Cytosolic striatal GFAP in the 1.5 W/kg-exposed rats was significantly decreased compared to that in the sham-exposed group (*$p < 0.05$). Cytosolic GFAP in the hippocampus and the olfactory bulb in the 6 W/kg (15 min)-exposed rats was significantly increased compared to that in the sham-exposed group (*$p < 0.05$). $N = 8$/group

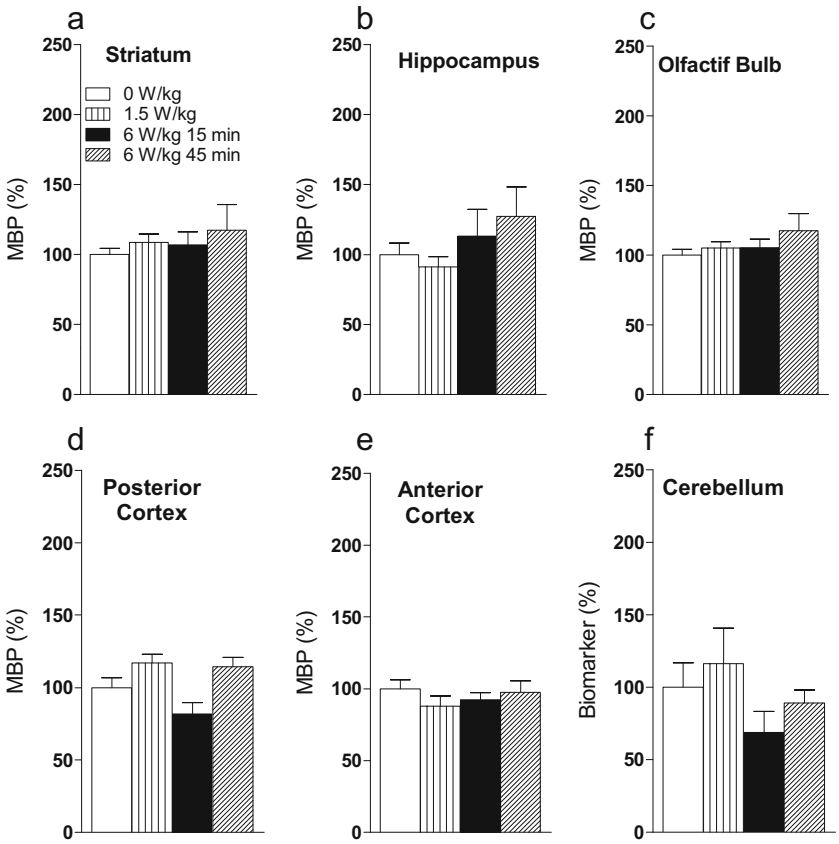

Fig. 3 MBP in the striatum (**a**), the hippocampus (**b**), the olfactory bulb (**c**), the posterior cortex (**d**), the anterior cortex (**e**), and the cerebellum (**f**) in the sham- and RF EMF-exposed rats. No RF EMF effect was reported. $N = 14$/group

explained by the level of GFAP in the striatum and by its interaction with RF EMF exposure (1.5 W/kg). The calculation of the Pearson coefficient indicated that long-term memory scores correlated with total striatal GFAP ($r = -0.38$, $p = 0.047$) and with short-term memory ($r = 0.38$, $p = 0.0013$).

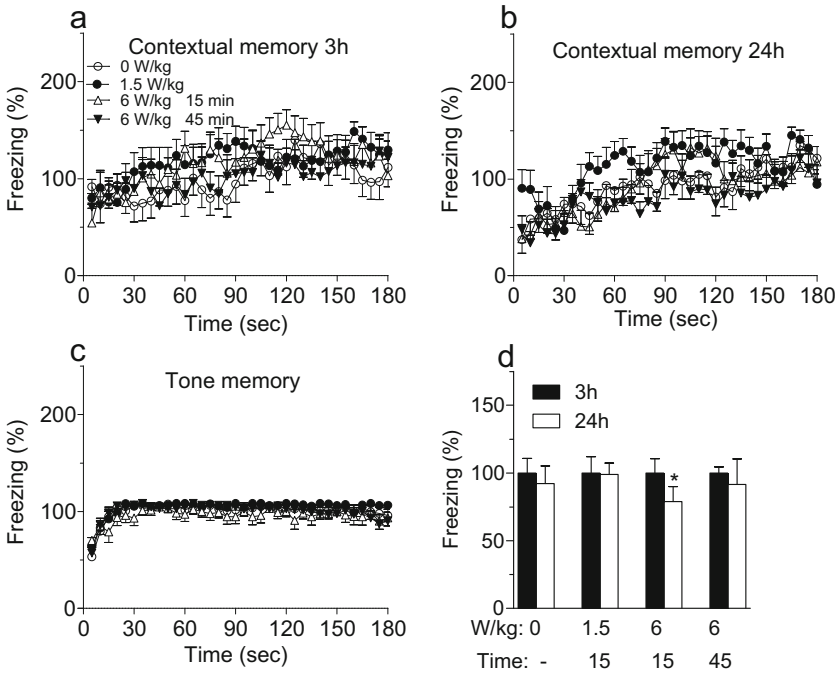

Fig. 4 Emotional memory in the sham- and RF EMF-exposed rats. There was no RF EMF effect on the time spent freezing to the context (after 3 h (**a**) and after 24 h (**b**)) and to the tone (**c**). There was a significant RF EMF effect when comparing the short- and long-term memories to the context (**d**). The 1.5 W/kg-exposed rats decreased significantly there contextual memory over time (*$p < 0.05$). $N = 17$/group

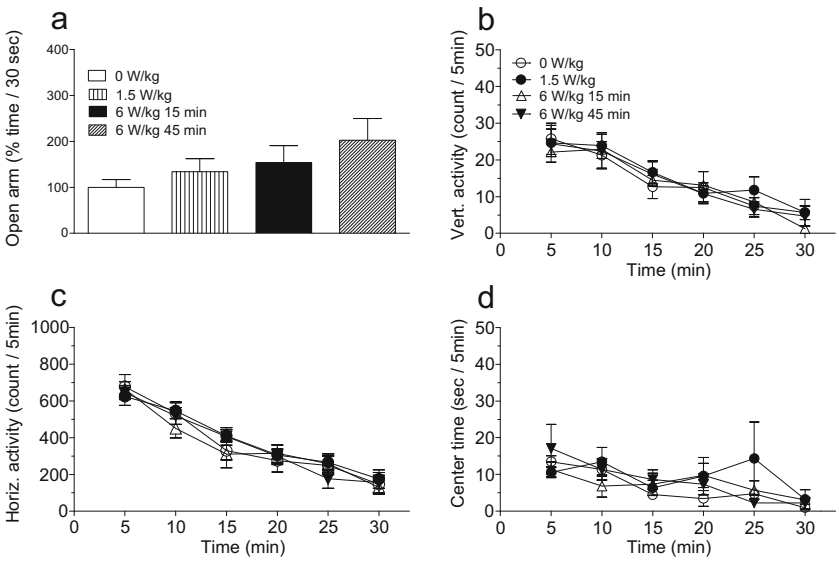

Fig. 5 Anxiety-like behavior (**a**) and locomotor activity (**b**, **c**, **d**) in the sham- and RF EMF-exposed groups. No RF EMF effect was reported in the percentage of time spent in the open arms of the EPM (**a**). There was no RF EMF effect on the vertical (**b**) and horizontal (**c**) activities measured in the open field ($N = 14$/group) and on the time spent in the central area (**d**) ($N = 8$/group)

Effect of RF EMF exposure on anxiety-like behavior and locomotor activity

Figure 5a shows the percentage of time spent in the open arm of the EPM. No effect was detected with ANOVA comparing SAR level ($F(2,43) = 0.8$, $p = 0.4$) or exposure duration ($F(2,49) = 1.6$, $p = 0.2$). Figure 5b, c shows vertical and horizontal locomotor activities measured in the open field. ANOVA performed on vertical activity showed significant time effect ($F(5,105) = 26.1$, $p < 0.001$) but no SAR level effect ($F(2,21) = 0.2$, $p = 0.8$), exposure duration effect ($F(2,21) = 0.01$, $p = 0.9$), time × SAR level interaction ($F(10,105) = 0.5$, $p = 0.9$), or time × exposure duration interaction ($F(10,105) = 0.4$, $p = 0.9$) (Fig. 5b).

ANOVA performed on horizontal activity, comparing SAR levels or exposure durations, showed significant time effect ($F(5105) = 85.7$, $p < 0.001$ and $F(5105) = 79.4$, $p < 0.001$, respectively) but no SAR level effect ($F(2,21) = 0.2$, $p = 0.8$), exposure duration effect ($F(2,21) = 0.04$, $p = 0.9$), time × SAR level interaction ($F(10,105) = 0.9$, $p = 0.5$), or time × exposure duration interaction ($F(10,105) = 1.0$, $p = 0.4$) (Fig. 5c). Similarly, there was a significant time effect ($p < 0.05$) but no SAR level effect, exposure duration effect, time × SAR level interaction, or time × exposure duration interaction ($p > 0.05$) for the time spent in the central area of the open field (Fig. 5d).

Effect of RF EMF on Aβ 1–40

Figure 6 shows Aβ 1–40 quantities in the six cerebral structures 3 days following RF EMF exposure. ANOVA performed on Aβ 1–40 for each structure did not show any SAR level or exposure duration effect ($p > 0.2$). Exploration of the data using a linear model indicated that long-term memory was explained by the level of Aβ 1–40 in the striatum and by its interaction with RF EMF (1.5 W/kg). Long-term memory was explained by the interactions of RF EMF exposure with Aβ (in the striatum, $p = 0.022$ and in the hippocampus, $p = 0.047$). Long-term memory was explained by Aβ in the striatum (estimate −0.74, standard error 0.31, $p = 0.02$), by its interactions with RF EMF exposure (at 1.5 W/kg, estimate 1.38, standard error 0.64, $p = 0.04$ and at 6 W/kg 15 min, estimate 1.38, standard error 0.64, $p = 0.04$), and by the interaction Aβ in the hippocampus × RF EMF exposure (at 6 W/kg 15 min, estimate −1.66, standard error 0.64, $p = 0.02$). Multiple R-squared was 0.38 and adjusted R-squared was 0.18 ($p = 0.10$).

Discussion

This study aimed to investigate the neurobiological effect of an acute RF EMF exposure at high SARs. Results showed a +114 % GFAP increase in the striatum following RF EMF exposure at 1.5 W/kg. At 6 W/kg (15 min), GFAP cytosolic fraction was increased in the hippocampus (+119 %) and the olfactory bulb (+46 %), and long-term contextual memory was decreased (−21 %). No MBP or Aβ 1–40 expression modification was shown.

Astrocytes insure a variety of functions in the brain. Thus, neurologic function could be altered in some important respects if RF EMF mobile phone exposures did cause astrocytic reaction. Here, we afforded new insights to this question, which is still an important matter of debate. Several previous investigations using acute and repeated RF EMF exposures looked at the link between SAR and GFAP increase. Mausset et al. (2001), Mausset-Bonnefont et al. (2004), Brillaud et al. (2007), Ammari et al. (2008a, b), Maskey et al. (2010, 2012), and Lu et al. (2014) showed positive effects. Fritze et al.

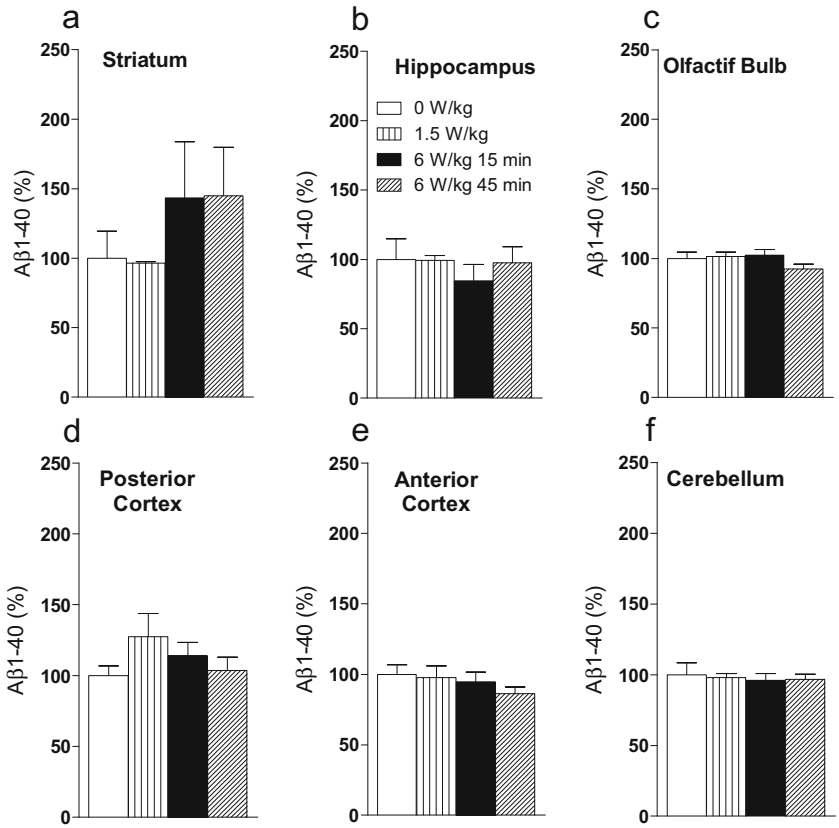

Fig. 6 Aβ 1–40 in the striatum (**a**), the hippocampus (**b**), the olfactory bulb (**c**), the posterior cortex (**d**), the anterior cortex (**e**), and the cerebellum (**f**) in the sham- and RF EMF-exposed rats. No RF EMF effect was reported. $N = 8–17$/group

(1997), Thorlin et al. (2006), Grafstrom et al. (2008), Watilliaux et al. (2011), Bouji et al. (2012), Court-Kowalski et al. (2015), Bouji et al. (2016), and Petitdant et al. (2016) did not show any positive effect. There is no clear explanation for these discrepancies. Contradictory data were shown with similar experimental designs: local (to the brain) acute exposure (Brillaud et al. 2007; Bouji et al. 2012) or intermittent (Ammari et al. 2008a, b; Petitdant et al. 2016), whole body continuous exposure (Grafstrom et al. 2008; Fragopoulou et al. 2012), high SAR (Brillaud et al. 2007; Bouji et al. 2012), environmental SAR (Watilliaux et al. 2011; Fragopoulou et al. 2012), mice (Maskey et al. 2010; Kim et al. 2008), or rats (Brillaud et al. 2007; Bouji et al. 2012). Similar data were obtained with Sprague Dawley and Wistar male rats (Bouji et al. 2012, 2016). The method of immunohistochemistry or ELISA may detect differently cytoskeletal protein redistribution and GFAP overexpression (Boran and Garcia 2007). Variability may also be caused by contention used to perform local brain exposures. However, there was no behavioral effect of restraint stress (Petitdant et al. 2016).

Here, total GFAP quantity was increased in the striatum of the 1.5 W/kg-exposed group, and cytosolic fraction of GFAP was increased in the hippocampus and the olfactory bulb in the 6 W/kg (15 min)-exposed group. Total GFAP levels did not increase in the direct ratio of the total absorbed energy. We may hypothesize that GFAP response was modified by activation of thermoregulatory processes or occurred with a different kinetic in the 6 W/kg-exposed group compared to the 1.5 W/kg-exposed group. In the 1.5 W/kg-exposed group, the decrease of the ratio cytosolic/total fraction of GFAP in the striatum is explained by the increase of the total GFAP, while cytosolic fraction remained unchanged. In the hippocampus and the olfactory bulb, the increased ratio cytosolic/total fraction of GFAP suggested either increased synthesis of soluble monomeric GFAP or reduction of its conversion to insoluble GFAP filament. There was no effect of exposure duration or proximity to the RF EMF source. Effect may have occurred in a SAR window in the more vulnerable cerebral structures.

The present study is the first to assess the effect of RF EMF exposure on MBP. There was no impact of RF EMF on MBP quantities in the six brain regions. In addition, there was no correlation between MBP and total or cytosolic GFAP levels. This data suggests that RF EMF-induced GFAP variation in the striatum, the hippocampus, and the olfactory bulb may not have been secondary to oligodendrocytes reactivity and/or myelin injury.

Aβ was largely studied because of its central role in Alzheimer disease neurobiochemical impairment (Silverberg et al. 2010). However, its physiological role state is not clear. In healthy young animals, Aβ peptide seems implicated in activity-dependent regulation of synaptic vesicle release, and in learning and memory (Abramov et al. 2009; Morley et al.

2010). Here, our exploratory linear model suggests that the level of Aβ 1–40 in the striatum explains long-term memory. This exploratory data corroborates a possible role of Aβ 1–40 in memory processes.

The present study is the first quantification of endogenous Aβ 1–40 following acute RF EMF exposure. Our data did not indicate any RF EMF effect on cerebral Aβ 1–40 3 days following an acute exposure. In addition to the absence of RF EMF biological effect on MBP and Aβ 1–40 in the present experimental design, further time points and neurotoxicity markers should be assessed to prove the absence of RF EMF-induced injury. In another study, neuronal damage and cell loss in the hippocampus (CA area) were shown after long-term and repeated RF EMF exposures at 900 MHz (Bas et al. 2009).

Meanwhile, an exploratory linear model indicated that the interaction between the levels of Aβ 1–40 in the striatum and RF EMF (1.5 W/kg) explained long-term memory. This exploratory data should be tested in a separated experiment. One can expect Aβ 1–40 changes to be more likely to occur following long-term exposures.

Up to date, there is no consensus regarding the possible effect of RF EMF on memory. Both positive (Preece et al. 1999; Koivisto et al. 2000; Krause et al. 2000; Maier et al. 2004; Keetley et al. 2006; Nittby et al. 2008; Arendash et al. 2010) and negative (Sienkiewicz et al. 2000; Dubreuil et al. 2002; Haarala et al. 2003; Haarala et al. 2007; Ammari et al. 2008a, b) reports were published. RF EMF exposures in freely moving rodents also gave contradictory behavioral effects. Nittby et al. (2008) indicated deleterious effect on episodic like memory with no effect on the open field. Lai (2004) showed alteration of spatial learning and memory. Cassel et al. (2004) and Cosquer et al. (2005) showed no alteration of spatial working memory in free-moving rats. Here, we brought new insights to this question by assessing the effect of RF EMF on retrograde memory using post-training RF EMF exposure in the fear conditioning paradigm. We studied only one type of memory, which was not shown from the literature to be basically affected by RF EMF comparing to other types of memory (spatial, recognition). The asset of this paradigm is to produce rapid, robust, and enduring (for months) learning to assess hippocampal and amygdala functions (Quinn et al. 2008). The 6 W/kg (15 min)-exposed group showed decreased long-term memory compared to short-term contextual memory. In a previous study, 6 W/kg (15 min)-exposed adult rats displayed lower and slower freezing response to context compared to middle age rats (Bouji et al. 2012). RF EMF may be perceived as an external cue (a distracter), which may disturb memory. Perception of RF EMF as a cue was also suggested in previous studies showing that EMF improved cognition and modified glutamatergic and electrophysiological responses (Lai et al. 1990; Eulitz et al. 1998; Lee et al. 2003; Krause et al. 2004). In addition, the correlation between striatal GFAP and long-term memory may suggest that impaired long-term emotional memory was secondary to astrogliosis in the striatum. Moreover, striatal GFAP and its interaction with RF EMF exposure were shown to explain long-term memory in a linear model. Conversely, memory decline was not correlated with the increase of cytosolic fraction of GFAP in the hippocampus or in the olfactory bulb.

Our experimental data indicates that striatal markers (GFAP and Aβ 1–40) as well as the interactions RF EMF (1.5 W/kg) × striatal GFAP and RF EMF (1.5 W/kg) × striatal Aβ 1–40 explain long-term environmental emotional memory. This data support the involvement of striatum in neuroanatomy of emotional memory and the particular sensitivity of the striatum to RF EMF exposure at high SARs. Previous studies showed that contextual fear conditioning was impaired after the selective ablation of striatal neurons and in striatum-specific dopaminergic receptor D1R knockout mice (Ikegami et al. 2014). Moreover, a previous study reported strong RF EMF effects in the striatum on the expression of N-mehyl d-aspartate receptors subunits at the synaptic plasma membrane and on GFAP levels (Mausset-Bonnefont et al. 2004). This structure may be of particular sensitivity due to RF EMF-induced cellular stress through the synthesis of heat shock proteins or molecular pathways of excitotoxic events.

Our data suggests no effect of RF EMF exposure on anxiety and locomotor activity. It confirms Mausset-Bonnefont's study showing no impairment immediately and 24 h after a single RF EMF exposure. Overall, there was no correlation between GFAP levels in the six structures and locomotion or anxiety-related behavior.

Conclusion

The transposition of our results to human is not straightforward as the SARs (1.5 and 6 W/kg averaged on the whole brain) are higher than in the brain of mobile phone users (0.9 W/kg averaged on 10 g of tissues) and as controlled exposures with loop antennas in rodents differ from cell phone use in human. Our experimental design aimed at explaining biological effects of high SARs. Overall, our data corroborates previous studies indicating RF EMF-induced astrogliosis. This study suggests that RF EMF-induced astrogliosis had functional consequences on memory but did not demonstrate that it was secondary to neuronal damage.

Acknowledgments This work was funded by the Pr 190 of French Ministry of Ecology.

References

Abramov E, Dolev I, Fogel H, Ciccotosto GD, Ruff E, Slutsky I (2009) Amyloid-beta as a positive endogenous regulator of release probability at hippocampal synapses. Nat Neurosci 12:1567–1576

Ammari M, Brillaud E, Gamez C, Lecomte A, Sakly M, Abdelmelek H, de Seze R (2008a) Effect of a chronic GSM 900 MHz exposure on glia in the rat brain. Biomed Pharmacother 62:273–281

Ammari M, Jacquet A, Lecomte A, Sakly M, Abdelmelek H, de Seze R (2008b) Effect of head-only sub-chronic and chronic exposure to 900-MHz GSM electromagnetic fields on spatial memory in rats. Brain Inj 22:1021–1029

Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C (2010) Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis 19:191–210

Ashok A, Rai NK, Tripathi S, Bandyopadhyay S (2015) Exposure to As-, Cd-, and Pb-mixture induces Abeta, amyloidogenic APP processing and cognitive impairments via oxidative stress-dependent neuroinflammation in young rats. Toxicol Sci 143:64–80

Bas O, Odaci E, Mollaoglu H, Ucok K, Kaplan S (2009) Chronic prenatal exposure to the 900 megahertz electromagnetic field induces pyramidal cell loss in the hippocampus of newborn rats. Toxicol Ind Health 25:377–384

Bhasker AS, Sant B, Yadav P, Agrawal M, Lakshmana Rao PV (2014) Plant toxin abrin induced oxidative stress mediated neurodegenerative changes in mice. Neurotoxicology 44:194–203

Blanchard DC, Blanchard RJ (1972) Innate and conditioned reactions to threat in rats with amygdaloid lesions. J Comp Physiol Psychol 81: 281–290

Blasko I, Jungwirth S, Jellinger K, Kemmler G, Krampla W, Weissgram S, Wichart I, Tragl KH, Hinterhuber H, Fischer P (2008) Effects of medications on plasma amyloid beta (Abeta) 42: longitudinal data from the VITA cohort. J Psychiatr Res 42:946–955

Boran MS, Garcia A (2007) The cyclic GMP-protein kinase G pathway regulates cytoskeleton dynamics and motility in astrocytes. J Neurochem 102:216–230

Bouji M, Lecomte A, Hode Y, de Seze R, Villegier AS (2012) Effects of 900 MHz radiofrequency on corticosterone, emotional memory and neuroinflammation in middle-aged rats. Exp Gerontol 47:444–451

Bouji M, Lecomte A, Gamez C, Blazy K, Villegier AS (2016) Neurobiological effects of repeated radiofrequency exposures in male senescent rats. Biogerontology

Brillaud E, Morillion D, de Seze R (2005) Modest environmental enrichment: effect on a radial maze validation and well being of rats. Brain Res 1054:174–182

Brillaud E, Piotrowski A, de Seze R (2007) Effect of an acute 900 MHz GSM exposure on glia in the rat brain: a time-dependent study. Toxicology 238:23–33

Cassel JC, Cosquer B, Galani R, Kuster N (2004) Whole-body exposure to 2.45 GHz electromagnetic fields does not alter radial-maze performance in rats. Behav Brain Res 155:37–43

Coria F, Berciano MT, Berciano J, Lafarga M (1984) Axon membrane remodeling in the lead-induced demyelinating neuropathy of the rat. Brain Res 291:369–372

Cosquer B, Vasconcelos AP, Frohlich J, Cassel JC (2005) Blood-brain barrier and electromagnetic fields: effects of scopolamine methylbromide on working memory after whole-body

Court-Kowalski S, Finnie JW, Manavis J, Blumbergs PC, Helps SC, Vink R (2015) Effect of long-term (2 years) exposure of mouse brains to global system for mobile communication (GSM) radiofrequency fields on astrocytic immunoreactivity. Bioelectromagnetics 36: 245–250

Daniels WM, Pietersen CY, Carstens ME, Stein DJ (2004) Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor. Metab Brain Dis 19:3–14

Deng Z, Fu H, Xiao Y, Zhang B, Sun G, Wei Q, Ai B, Hu Q (2015) Effects of selenium on lead-induced alterations in Abeta production and Bcl-2 family proteins. Environ Toxicol Pharmacol 39:221–228

Dragicevic N, Bradshaw PC, Mamcarz M, Lin X, Wang L, Cao C, Arendash GW (2011) Long-term electromagnetic field treatment enhances brain mitochondrial function of both Alzheimer's transgenic mice and normal mice: a mechanism for electromagnetic field-induced cognitive benefit? Neuroscience 185:135–149

Dubreuil D, Jay T, Edeline JM (2002) Does head-only exposure to GSM-900 electromagnetic fields affect the performance of rats in spatial learning tasks? Behav Brain Res 129:203–210

Eddleston M, Mucke L (1993) Molecular profile of reactive astrocytes—implications for their role in neurologic disease. Neuroscience 54:15–36

Eng LF (1985) Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. J Neuroimmunol 8:203–214

Eng LF, Ghirnikar RS, Lee YL (2000) Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000. Neurochem Res 25:1439–1451

Eulitz C, Ullsperger P, Freude G, Elbert T (1998) Mobile phones modulate response patterns of human brain activity. Neuroreport 9:3229–3232

Fragopoulou AF, Samara A, Antonelou MH, Xanthopoulou A, Papadopoulou A, Vougas K, Koutsogiannopoulou E, Anastasiadou E, Stravopodis DJ, Tsangaris GT, Margaritis LH (2012) Brain proteome response following whole body exposure of mice to mobile phone or wireless DECT base radiation. Electromagn Biol Med 31: 250–274

Fritze K, Wiessner C, Kuster N, Sommer C, Gass P, Hermann DM, Kiessling M, Hossmann KA (1997) Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain. Neuroscience 81:627–639

Grafstrom G, Nittby H, Brun A, Malmgren L, Persson BR, Salford LG, Eberhardt J (2008) Histopathological examinations of rat brains after long-term exposure to GSM-900 mobile phone radiation. Brain Res Bull 77:257–263

Haarala C, Aalto S, Hautzel H, Julkunen L, Rinne JO, Laine M, Krause B, Hamalainen H (2003) Effects of a 902 MHz mobile phone on cerebral blood flow in humans: a PET study. Neuroreport 14:2019–2023

Haarala C, Takio F, Rintee T, Laine M, Koivisto M, Revonsuo A, Hamalainen H (2007) Pulsed and continuous wave mobile phone exposure over left versus right hemisphere: effects on human cognitive function. Bioelectromagnetics 28:289–295

Hardell L, Carlberg M (2013) Use of mobile and cordless phones and survival of patients with glioma. Neuroepidemiology 40:101–108

Hernandez-Zimbron LF, Rivas-Arancibia S (2015) Oxidative stress caused by ozone exposure induces beta-amyloid 1-42 overproduction and mitochondrial accumulation by activating the amyloidogenic pathway. Neuroscience 304:340–348

Hewett JA (2009) Determinants of regional and local diversity within the astroglial lineage of the normal central nervous system. J Neurochem 110:1717–1736

Ikegami M, Uemura T, Kishioka A, Sakimura K, Mishina M (2014) Striatal dopamine D1 receptor is essential for contextual fear conditioning. Sci Rep 4:3976

INTERPHONE group (2010) Brain tumour risk in relation to mobile telephone use: results of the INTERPHONE international case-control study. Int J Epidemiol 39:675–694

Keetley V, Wood AW, Spong J, Stough C (2006) Neuropsychological sequelae of digital mobile phone exposure in humans. Neuropsychologia 44:1843–1848

Kim TH, Huang TQ, Jang JJ, Kim MH, Kim HJ, Lee JS, Pack JK, Seo JS, Park WY (2008) Local exposure of 849 MHz and 1763 MHz radiofrequency radiation to mouse heads does not induce cell death or cell proliferation in brain. Exp Mol Med 40:294–303

Koivisto M, Krause CM, Revonsuo A, Laine M, Hamalainen H (2000) The effects of electromagnetic field emitted by GSM phones on working memory. Neuroreport 11:1641–1643

Krause CM, Sillanmaki L, Koivisto M, Haggqvist A, Saarela C, Revonsuo A, Laine M, Hamalainen H (2000) Effects of electromagnetic field emitted by cellular phones on the EEG during a memory task. Neuroreport 11:761–764

Krause CM, Haarala C, Sillanmaki L, Koivisto M, Alanko K, Revonsuo A, Laine M, Hamalainen H (2004) Effects of electromagnetic field emitted by cellular phones on the EEG during an auditory memory task: a double blind replication study. Bioelectromagnetics 25:33–40

Lai H (2004) Interaction of microwaves and a temporally incoherent magnetic field on spatial learning in the rat. Physiol Behav 82: 785–789

Lai H, Carino MA, Horita A, Guy AW (1990) Corticotropin-releasing factor antagonist blocks microwave-induced decreases in high-affinity choline uptake in the rat brain. Brain Res Bull 25:609–612

Lee TM, Lam PK, Yee LT, Chan CC (2003) The effect of the duration of exposure to the electromagnetic field emitted by mobile phones on human attention. Neuroreport 14:1361–1364

Lee YK, Yuk DY, Lee JW, Lee SY, Ha TY, KW O, Yun YP, Hong JT (2009) (−)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. Brain Res 1250:164–174

Leveque P, Dale C, Veyret B, Wiart J (2004) Dosimetric analysis of a 900-MHz rat head exposure system. IEEE Trans Microwave Theory Tech:2076–2083

Lu Y, He M, Zhang Y, Xu S, Zhang L, He Y, Chen C, Liu C, Pi H, Yu Z, Zhou Z (2014) Differential pro-inflammatory responses of astrocytes and microglia involve STAT3 activation in response to 1800 MHz radiofrequency fields. PLoS One 9:e108318

Ma F, Liu D (2015) 17beta-trenbolone, an anabolic-androgenic steroid as well as an environmental hormone, contributes to neurodegeneration. Toxicol Appl Pharmacol 282:68–76

Ma T, Wu X, Cai Q, Wang Y, Xiao L, Tian Y, Li H (2015) Lead poisoning disturbs oligodendrocytes differentiation involved in decreased expression of NCX3 inducing intracellular calcium overload. Int J Mol Sci 16:19096–19110

Maier R, Greter SE, Maier N (2004) Effects of pulsed electromagnetic fields on cognitive processes—a pilot study on pulsed field interference with cognitive regeneration. Acta Neurol Scand 110:46–52

Maskey D, Pradhan J, Aryal B, Lee CM, Choi IY, Park KS, Kim SB, Kim HG, Kim MJ (2010) Chronic 835-MHz radiofrequency exposure to mice hippocampus alters the distribution of calbindin and GFAP immunoreactivity. Brain Res 1346:237–246

Maskey D, Kim HJ, Kim HG, Kim MJ (2012) Calcium-binding proteins and GFAP immunoreactivity alterations in murine hippocampus after 1 month of exposure to 835 MHz radiofrequency at SAR values of 1.6 and 4.0 W/kg. Neurosci Lett 506:292–296

Mausset AL, de Seze R, Montpeyroux F, Privat A (2001) Effects of radiofrequency exposure on the GABAergic system in the rat cerebellum: clues from semi-quantitative immunohistochemistry. Brain Res 912:33–46

Mausset-Bonnefont AL, Hirbec H, Bonnefont X, Privat A, Vignon J, de Seze R (2004) Acute exposure to GSM 900-MHz electromagnetic fields induces glial reactivity and biochemical modifications in the rat brain. Neurobiol Dis 17:445–454

Middeldorp J, Hol EM (2011) GFAP in health and disease. Prog Neurobiol 93:421–443

Morin-Richaud C, Feldblum S, Privat A (1998) Astrocytes and oligodendrocytes reactions after a total section of the rat spinal cord. Brain Res 783:85–101

Morley JE, Farr SA, Banks WA, Johnson SN, Yamada KA, Xu L (2010) A physiological role for amyloid-beta protein:enhancement of learning and memory. J Alzheimers Dis 19:441–449

Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci 10:9–22

Nittby H, Grafstrom G, Tian DP, Malmgren L, Brun A, Persson BR, Salford LG, Eberhardt J (2008) Cognitive impairment in rats after long-term exposure to GSM-900 mobile phone radiation. Bioelectromagnetics 29:219–232

O'Callaghan JP (1991) Quantification of glial fibrillary acidic protein: comparison of slot-immunobinding assays with a novel sandwich ELISA. Neurotoxicol Teratol 13:275–281

Petitdant N, Lecomte A, Robidel F, Gamez C, Blazy K, Villégier A (2016) Cerebral radiofrequency exposures during adolescence: impact on astrocytes and brain functions in healthy and pathologic rat models. Bioelectromagnetics

Preece AW, Iwi G, Davies-Smith A, Wesnes K, Butler S, Lim E, Varey A (1999) Effect of a 915-MHz simulated mobile phone signal on cognitive function in man. Int J Radiat Biol 75:447–456

Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463:3–33

Quinn JJ, Ma QD, Tinsley MR, Koch C, Fanselow MS (2008) Inverse temporal contributions of the dorsal hippocampus and medial prefrontal cortex to the expression of long-term fear memories. Learn Mem 15:368–372

Rapanelli M, Frick LR, Zanutto BS (2011) Learning an operant conditioning task differentially induces gliogenesis in the medial prefrontal cortex and neurogenesis in the hippocampus. PLoS One 6: e14713

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 16:675–686

Schang AL, Van Steenwinckel J, Chevenne D, Alkmark M, Hagberg H, Gressens P, Fleiss B (2014) Failure of thyroid hormone treatment to prevent inflammation-induced white matter injury in the immature brain. Brain Behav Immun 37:95–102

Schiffer D, Giordana MT, Migheli A, Giaccone G, Pezzotta S, Mauro A (1986) Glial fibrillary acidic protein and vimentin in the experimental glial reaction of the rat brain. Brain Res 374: 110–118

Schmidt-Kastner R, Szymas J (1990) Immunohistochemistry of glial fibrillary acidic protein, vimentin and S-100 protein for study of astrocytes in hippocampus of rat. J Chem Neuroanat 3:179–192

Sienkiewicz ZJ, Blackwell RP, Haylock RG, Saunders RD, Cobb BL (2000) Low-level exposure to pulsed 900 MHz microwave radiation does not cause deficits in the performance of a spatial learning task in mice. Bioelectromagnetics 21:151–158

Silverberg GD, Miller MC, Messier AA, Majmudar S, Machan JT, Donahue JE, Stopa EG, Johanson CE (2010) Amyloid deposition and influx transporter expression at the blood-brain barrier increase in normal aging. J Neuropathol Exp Neurol 69:98–108

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638–647

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7–35

Spitzer P, Herrmann M, Klafki HW, Smirnov A, Lewczuk P, Kornhuber J, Wiltfang J, Maler JM (2010) Phagocytosis and LPS alter the maturation state of beta-amyloid precursor protein and induce different Abeta peptide release signatures in human mononuclear phagocytes. J Neuroinflammation 7:59

Thorlin T, Rouquette JM, Hamnerius Y, Hansson E, Persson M, Bjorklund U, Rosengren L, Ronnback L, Persson M (2006)

Exposure of cultured astroglial and microglial brain cells to 900 MHz microwave radiation. Radiat Res 166:409–421

Tseng WC, Lu KS, Lee WC, Chien CL (2006) Redistribution of GFAP and alphaB-crystallin after thermal stress in C6 glioma cell line. J Biomed Sci 13:681–694

Watilliaux A, Edeline JM, Leveque P, Jay TM, Mallat M (2011) Effect of exposure to 1,800 MHz electromagnetic fields on heat shock proteins and glial cells in the brain of developing rats. Neurotox Res 20(2):109–119

Wenzel J, Lammert G, Meyer U, Krug M (1991) The influence of long-term potentiation on the spatial relationship between astrocyte processes and potentiated synapses in the dentate gyrus neuropil of rat brain. Brain Res 560:122–131

Zhao JW, Raha-Chowdhury R, Fawcett JW, Watts C (2009) Astrocytes and oligodendrocytes can be generated from NG2+ progenitors after acute brain injury: intracellular localization of oligodendrocyte transcription factor 2 is associated with their fate choice. Eur J Neurosci 29:1853–1869