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A study of neurotoxic biomarkers, c-fos and GFAP after acute exposure to GSM radiation at 900 MHz in the picrotoxin model of rat brains

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ABSTRACT

The acute effects of microwave exposure from the Global System for Mobile Communication (GSM) were studied in rats, using 900 MHz radiation at an intensity similar to mobile phone emissions. Acute subconvulsive doses of picrotoxin were then administered to the rats and an experimental model of seizure-proneness was created from the data. Seventy-two adult male Sprague-Dawley rats underwent immunochemical testing of relevant anatomical areas to measure induction of the c-fos neuronal marker after 90 min and 24 h, and of the glial fibrillary acidic protein (GFAP) 72 h after acute exposure to a 900 MHz electromagnetic field (EMF). The experimental set-up facilitated measurement of absorbed power, from which the average specific absorption rate was calculated using the finite-difference time-domain (FDTD) 2 h after exposure to EMF radiation at 1.45 W/kg in picrotoxin-treated rats and 1.38 W/kg in untreated rats.

Ninety minutes after radiation high levels of c-fos expression were recorded in the neocortex and paleocortex along with low hippocampus activation in picrotoxin treated animals. Most brain areas, except the limbic cortical region, showed important increases in neuronal activation 24 h after picrotoxin and radiation. Three days after picrotoxin treatment, radiation effects were still apparent in the neocortex, dentate gyrus and CA3, but a significant decrease in activity was noted in the piriform and entorhinal cortex. During this time, glial reactivity increased with every seizure in irradiated, picrotoxin-treated brain regions. Our results reveal that c-fos and glial markers were triggered by the combined stress of non-thermal irradiation and the toxic effect of picrotoxin on cerebral tissues.

1. Introduction

In recent years, brain exposure to continuous pulsed microwaves has been reported to affect electrophysiological activity in humans (Huber et al., 2003) and in vitro (Zhao et al., 2007), and to influence both neurotransmission (Paulraj and Behari, 2004) and signal transduction pathways (Belyaev et al., 2006).

In neurobiological applications, c-fos is the most widely used functional anatomical marker of activated neurons within the central nervous system (Kovács, 2008). c-fos induction is often correlated with increased electrical and metabolic cell activity and is involved in neuronal plasticity phenomena (Morgan and Curran, 1991b). When this marker was used to detect neural stress (Martinez et al., 2002) after full gestational exposure to mobile telephone-type radio frequency fields, no stress response was detected in the fetal mouse brain (Finnie et al., 2006). Selective expression of c-fos in rat brain has sometimes been observed following microwave-induced hyperthermia (Mickley et al., 1994).

Brain tissue is especially sensitive to electromagnetic phenomena. The rat model for seizure-proneness by injection of a subconvulsive dose of the GABA antagonist picrotoxin indicates that mobile phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation (López-Martín et al., 2006, 2008, 2009). This radiofrequency can also alter electrophysiological activity in an epileptic human (Relova et al., 2005). However, in studies based on Auditory Evoked Potentials (AEPs) in epileptic patients with exposure to RF, it was difficult to deduce the effect on human health (Maby et al., 2005, 2004). In vitro cortical neurons were also studied to detect alterations in the expression pattern of cytoskeleton regulating factors in early genes such as c-fos (Del Vecchio et al., 2009) when exposed to a modulated dose of 1 W/kg GSM at 900 MHz.

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Table 1
Description of the experimental groups.

Group 1	Picrotoxin treated[a] (PT) + irradiated (IR)[b], slaughtered and studied after 90 min
Group 2	Picrotoxin treated (PT) + non-irradiated (NIR), slaughtered and studied after 90 min
Group 3	Non-picrotoxin treated (NPT) + irradiated (IR), slaughtered and studied after 90 min
Group 4	Non-picrotoxin treated (NPT) + non-irradiated (NIR), slaughtered and studied after 90 min
Group 5	Picrotoxin treated (PT) + irradiated (IR), slaughtered and studied after 24 h
Group 6	Picrotoxin treated (PT) + non-irradiated (NIR), slaughtered and studied after 24 h
Group 7	Non-picrotoxin treated (NPT) + irradiated (IR), slaughtered and studied after 24 h
Group 8	Non-picrotoxin treated (NPT) + non-irradiated (NIR), slaughtered and studied after 24 h
Group 9	Picrotoxin treated (PT) + irradiated (IR), slaughtered and studied after 3 days
Group 10	Picrotoxin treated (PT) + non-irradiated (NIR), slaughtered and studied after 3 days
Group 11	Non-picrotoxin treated (NPT) + irradiated (IR), slaughtered and studied after 3 days
Group 12	Non-picrotoxin treated (NPT) + non-irradiated (NIR), slaughtered and studied after 3 days

[a] Intraperitoneal administration of 2 mg/kg of picrotoxin (Sigma).
[b] Radiated for 2 h with 900 MHz pulse-modulated GSM ±1000 MW.

The central nervous system responds to diverse neurological injuries with a vigorous activation of astrocytes. In vivo, this increase in glial fibrillary acidic protein-positive cells reflects mainly the phenotypic changes of resident astroglia rather than the migration or proliferation of such cells. The reactive astrocytes may benefit the injured nervous system by participating in diverse biological processes (Eddleston and Mucke, 1993). Possible neural damage from acute microwave exposure was addressed at the cellular level by Mausset-Bonnefont et al. (2004), who investigated the occurrence of glial activity. Acute, sub-chronic exposure to GSM 900 MHz microwaves may induce persistent astroglia activation in rat brain, which is a sign of potential gliosis (Brillaud et al., 2007; Ammari et al., 2010). Other studies showed that acute or longer duration exposure in vivo or in vitro to mobile telephone RF fields did not cause in brain cells any astroglial or microglial activation that was detectable by immunostaining (Thorlin et al., 2006; Finne et al., 2010).

Our simple model was obtained by administering picrotoxin to rats in the laboratory and was used to determine the timing of any morphological evidence of neurotoxicity in the brains of these small mammals. This fact may have a direct impact on the risk which can result from RF to the health of patients with epilepsy (López-Martín et al., 2006, 2008, 2009).

In this experiment we looked for signs of neural stress on cerebral activity after GSM-modulated 900 MHz radiation in a picrotoxin rat model, using positive immunochemical testing for neuronal (c-fos) and glial (glial fibrillary acidic protein) cells. We tested the chronological response cascade at 90 min, 24 h and 3 days, for the participation of c-fos protein expression, provoked by different noxas such as ischemia (Neumann-Haefelin et al., 1994), epileptic seizures (Kiessling and Gass, 1993) or cerebral trauma (Hayes et al., 1995), or glial fibrillar acidic adenosine protein-GFAP reactivity in brain tissues. The aim of the present study was to elaborate a time-course description after acute microwave exposure in rat brain by measuring (1) neuronal activation using the c-fos expression biomarker and (2) glial reactivity as an indicator of parallel neuronal damage in seizure-related anatomical circuits.

2. Materials and methods

2.1. Animals and picrotoxin treatment

The animals used in the study were adult male Sprague-Dawley rats weighing 230–250 g, kept in individual cages at 22 °C under a 12:12 h light/dark regime, with free access to food and water. Five minutes before immobilization, the treated rats received intraperitoneal administration of 2 mg/kg (1 ml intraperitoneal injection of a 0.5 mg/ml solution) of the convulsive agent picrotoxin (Sigma, St. Louis) dissolved in saline. Earlier experiments have shown this dose of picrotoxin to be subconvulsive (Nutt et al., 1982; López-Martín et al., 2006, 2008).

2.2. Experimental design

Rats were assigned to one of the following groups (Table 1):

Groups 1, 5, and 9, PT + R: rats were injected i.p. with picrotoxin (PT) and then immobilized in methacrylate tubes for 2 h with exposure to 900-MHz pulse-modulated GSM radiation (IR). The same treatment applies to groups 5 and 9.
Groups 2, 6, and 10, PT + NR: rats were injected i.p. with picrotoxin (PT) and then immobilized in methacrylate tubes for 2 h with no exposure to radiation (NR).
Groups 3, 7, and 11, NPT + R: rats were injected i.p. with vehicle only, not picrotoxin (NPT), and then immobilized in methacrylate tubes for 2 h with exposure to 900-MHz pulse-modulated GSM radiation (R).
Groups 4, 8, and 12, NPT + NR: rats were injected i.p. with vehicle only, not picrotoxin (NPT), and then immobilized in methacrylate tubes for 2 h without exposure to radiation (NIR).
Groups 1, 2, 3, and 4 were studied 90 min after they had been immobilized for 2 h in methacrylate tubes and either irradiated or non-irradiated.
Groups 5, 6, 7, and 8 were studied 24 h after they had been immobilized in methacrylate tubes for 2 h and were either irradiated or non irradiated.
Groups 9, 10, 11, and 12 were studied 3 days after they had been immobilized in methacrylate tubes for 2 h and were either irradiated or non irradiated. These experimental groups had the same treatments as groups 1–8.

2.3. Exposure system

Five minutes after i.p. administration of 2 mg/kg of picrotoxin (Sigma) – with the dose adjusted to individual animal weight (groups 1, 2, 5, 6, 9, and 10), or of vehicle only (groups 3, 4, 7, 8, 11, and 12), the animals were immobilized in methacrylate tubes and placed in a 150 cm × 46 cm × 70 cm radiation cage (with a commercial transmitting antenna incorporated) that had previously been calibrated to enable measurement of the radiation absorbed by the animals (López-Martín et al., 2006, 2008; Trastoy-Rios et al., 2006). Animals in groups 1, 3, 5, 7, 9 and 11 were then radiated for 2 h with 900 MHz pulse-modulated GSM ± 1000 MW. The power level chosen was somewhat lower than the average power radiated by a GSM mobile phone. During radiation, the animals were videotaped to record clinical signs of seizure. The 2 h time period has been widely used in other studies (Fritze et al., 1997), and was used in this study to facilitate comparison. The animals were then removed from the cage and sedated with pentobarbital for transfer to the histochemistry

laboratory. Using this exposure system, we estimated the average specific absorption rate (SAR) and the 1-g average peak SAR for each tissue, or the peak specific absorbed radiation in a volume of tissue weighing 1 g. These indices were estimated with the aid of SEMCAD software (Schmid & Partner Engineering, 2009) by numerical simulation of absorption by the brain of a 198-g "phantom" rat and extrapolation of the results to the rats used in the experiments (López-Martín et al., 2006, 2008; Trastoy-Rios et al., 2006). The estimated specific absorption rate, SAR_E, for the animals used in the experiment was obtained by adjusting the simulated values to the actual weights and the actual absorbed power of the individual rats (López-Martín et al., 2006, 2008, 2009; Trastoy-Rios et al., 2006).

Since the software used for the simulation (Schmid & Partner Engineering, 2009) does not take into account transmission of antenna signals other than pure sinusoids, the numerical results had to be adapted by dividing the average power impinging on the transmitting antenna by the crest factor corresponding to GSM modulation, for a result of 8.3 (Huber et al., 2003).

2.4. Behavioural and clinical signs

All animals were continuously observed and videotaped for 2 h after intraperitoneal injection of picrotoxin. Behavioural changes, myoclonic jerks and the occurrence or latency of convulsive seizures were recorded. Myoclonic jerks are commonly associated with isolated spiking and were not considered as seizures. Convulsive seizures were characterized by clonic jerks of the limbs and the body, associated in varying degrees with tonic flexion or extension. Full recovery occurred in all cases.

2.5. Processing of brain tissue and immunohistochemistry

Ninety minutes, twenty-four hours and three days after the corresponding groups of rats were radiated they were given an overdose of pentobarbital and prefixed by transcardial perfusion with physiological saline followed by phosphate buffered formaldehyde (pH 7.4). The animals were slaughtered and the brains immediately removed from the skull, placed in fresh fixative solution for 4 h at 4 °C, and then transferred to phosphate-buffered saline (PBS) for 12 h at the same temperature. After that, 40-μm transverse sections were cut with a vibrotome, then collected free-floating in PBS and processed by pre-treating for 1 h with normal rabbit serum and Triton X-100 (10% and 0.25%, respectively, in 0.02% potassium phosphate-buffered saline, KPBS) and then exposed overnight at room temperature to polyclonal sheep anti-fos antibody (1:1000 in KPBS, Cambridge Research Biochemicals, Billingham), and anti-GFAP polyclonal rabbit antibody (1:1000 in KPBS, DAKO). The samples were then rinsed with KPBS, incubated for 1 h with biotinylated rabbit anti-sheep antibody or goat anti-rabbit antibody (1:500 in KPBS, Vector, Burlingame, CA, USA); or rinsed three times with KPBS and incubated for 30 min with an avidin–biotin–peroxidase complex prepared according to manufacturer instructions (DAKO, Glostrup, Denmark). Finally, the samples were labelled with 3.3-diaminobenzidine (DAB) in imidazole–HCl-buffered H_2O_2 solution (DAKO).

2.6. Regions of interest

fos immunoreactivity was observed in cell nuclei in several cortical (Willoughby et al., 1995) and subcortical (Paxinos and Watson, 1986; Willoughby et al., 1995) functional regions that are related to convulsive seizures. Specific regions within each major structure were selected for detailed analysis. In the cerebral region we looked at: (a) somatosensory areas, specifically the frontal motor cortex and the parietal somatosensory cortex, grouped together as the "neocortex", and (b) olfactory areas, specifically the piriform and the primary sensory and integrative entorhinal cortex, grouped together as the "paleocortex". We also examined cerebral subcortical hippocampal structures, specifically the dentate gyrus, CA1 and CA3. Picrotoxin-induced neuronal activation (Willoughby et al., 1995) has been described in some areas of these cortical and subcortical structures.

2.7. Quantification and statistical analysis

Nuclear c-fos expression was measured at the 9.2-mm level (interaval coordinates) in cortical areas (frontal cortex, parietal cortex, piriform cortex), at the 5.7-mm level in hippocampal areas (dentate gyrus, CA1, and CA3) and at the 4.2-mm level in the entorhinal cortex (López-Martín et al., 2009; Paxinos and Watson, 1986). The c-fos positive cell counts were taken by researchers who were blind to exposure conditions. Three or four brain sections from each rat were examined, from the cortical areas (frontal motor, parietal motor, piriform olfactory cortex and entorhinal olfactory cortex) and hippocampal structures (dentate gyrus, CA1 and CA3). To examine c-fos expression in localized regions of each area, c-fos-positive cells were counted in a 0.32 mm × 0.24 mm field magnified 20× in a Nikon Eclipse E200 microscope connected to a computer with morphometric software (Kappa, Monrovia, CA, USA). Counts per field in each of the areas were expressed as the averages of individual animals or experiments ± SEM per group. The statistical significance of between-group differences in c-fos positive cell counts was assessed by using the multi-way analysis of variance (ANOVA) technique: (a) considering all brain regions together, using treatment (picrotoxin, or no picrotoxin), radiation (no radiation, radiation) and time (90 min, 24 h, 3 days) factors; (b) considering each brain region separately according to treatment, radiation, region, area within a region and time factors; and (c) considering each specific brain area separately, using treatment/non-treatment, radiation and time factors. Similar studies were carried out for GFAP positive cells, but only after 3 days. Natural logarithm transformations were applied to data to obtain normality and homoscedasticity. Differences between regions and areas were considered significant at $p < 0.05$. The Tukey HSD test was used in *a posteriori* comparisons.

3. Results

3.1. Power absorption

The average power absorbed by rats in the six irradiated groups was estimated with the method explained in previous publications (see López-Martín et al., 2006, 2008, 2009) and is shown in Table 2, along with the average weight, average SAR in brain and body, and peak SAR averaged for 1 g of body and brain. All of the SAR values are below the European Union legislation limits (Royal Decree 1066/28-9-2001) for thermal values in the brain (Table 2).

3.2. Clinical behaviour

NPT + NIR rats in groups 4, 8 and 12 showed initial stress attributable to immobilization but did not exhibit any abnormal activity or signs of seizure.

NPT + IR rats in groups 3, 7 and 11 showed initial stress but did not exhibit any abnormal activity or signs of myoclonic jerk.

PT + NIR rats in groups 2, 6 and 10 displayed bursts of motor activity lasting between 5 and 10 min, after which the rats remained immobile but alert.

PT + IR rats in groups 1, 5 and 9 began to exhibit myoclonic jerks of the head and body within 10 min of picrotoxin administration. The myoclonic jerks persisted for long periods, but seizures were observed in only three animals, which underwent intermittent generalized convulsions for 20–30 min.

Table 2
Mean absorbed power and SAR values for the six experimental groups.

Group	Type	Mean absorbed power (mW)	Weight (g)	Mean SAR in brain (W/kg)	Peak SAR averaged in 1 g of brain (W/kg)	Mean SAR in body (W/kg)	Peak SAR averaged in 1 g of body (W/kg)
1	PT + IR/90 min p.e*	178.37	209.95	1.32	1.48	0.74	4.09
3	NPT + IR/90 min p.e	189.41	211.83	1.38	1.55	0.78	4.28
5	PT + IR/24 h p.e	192.67	225	1.32	1.49	0.74	4.11
7	NPT + IR/24 h p.e	202.33	228	1.37	1.54	0.77	4.25
9	PT + IR/3 days p.e	186.17	199.98	1.44	1.62	0.81	4.47
11	NPT + IR/3 days p.e	201.60	230.66	1.35	1.52	0.76	4.19

p.e*: post-irradiation time.

3.3. c-fos expression

3.3.1. c-fos expression throughout the entire brain

The proportion of c-fos-immunopositive neurons (c-fos expression) throughout the entire brain at the three times studied (90 min, 24 h and 3 days) and a multi-way ANOVA with radiation, treatment and time as factors, indicated a significant effect of the three factors ($p < 0.001$ in all cases). The effects of the time × radiation or time × treatment interaction were both significant ($p < 0.001$), as were the effects of the radiation × treatment interaction ($p < 0.001$). However, the radiation × treatment × time interaction had no significant effect ($p = 0.255$). See Table 3.

3.3.2. c-fos expression in various brain regions

All brain areas showed c-fos expression after picrotoxin treatment, with or without subsequent irradiation. In rats treated with picrotoxin, c-fos-positive neurons were observed systematically in the cortical areas and the hippocampus. A more or less scattered expression was also observed in the amygdala, the putamen–caudate complex, and/or the hypothalamus and the pontomesencephalic nuclei of some animals. This was seen in all cortical areas. Ninety minutes after radiation the mean c-fos expression in PT + IR rats was higher than in PT + NIR rats, showing twofold increases in some areas. However, in two of the three hippocampal areas studied this relation was inverted: PT + NIR rats showed higher mean c-fos expression levels than PT + IR rats. After 24 h most brain areas showed an important increase in the mean c-fos expression of picrotoxin treated rats, with similar values for PT + IR or PT + NIR rats. Finally, after three days average c-fos expression in the dentate gyrus, CA1, neocortex and piriform cortex was similar in PT + IR and PT + NIR rats. Conversely, after three days there was an important decrease in mean c-fos expression in the entorhinal cortex of picrotoxin treated rats.

3.3.2.1. Neocortex. In the initial analysis of the neocortex (frontal and parietal cortices), c-fos expression was significantly affected by time, treatment, radiation, and by the interactions of time with both radiation and treatment (see Table 4). However, no significant effects were found from the radiation × treatment × time interaction (see Table 4). There were significant effects on c-fos expression in the neocortex 90 min vs. 24 h vs. 3 days ($p < 0.001$) (see Table 8).

Separate analyses carried out in the frontal and parietal areas indicated that in both the effects of treatment, time, and treatment × time and radiation × time interactions all had signifi-

Table 3
Results of multi-way ANOVA (including Time × TT × Rad interaction) for c-fos in the entire brain.[a]

Effects	*F*	DF	*p*-Value
Entire brain			
Time	197.364	2	<0.001
TT[b]	263.590	1	<0.001
Rad	12.929	1	<0.001
Time × TT	41.864	2	<0.001
Time × Rad	45.569	2	<0.001
TT × Rad	11.685	1	0.001
Time × TT × Rad	1.368	2	0.255

[a] *F*: Fisher–Snedecor *F* statistic; DF: degrees of freedom; *p*-value: level of significance.
[b] Treatment (picrotoxin, or no picrotoxin).

Table 4
Results of multi-way ANOVA (including Area × time × TT × Rad interaction) for c-fos in the neocortex brain region.[a]

Effects	*F*	DF	*p*-Value
Neocortex region			
Area	56.553	1	<0.001
Time	120.715	2	<0.001
TT	571.803	1	<0.001
Rad	10.044	1	0.002
Area × time	19.195	2	<0.001
Area × TT	23.963	1	<0.001
Area × Rad	1.429	1	0.232
Time × TT	15.173	2	<0.001
Time × Rad	53.504	2	<0.001
TT × Rad	8.507	1	0.004
Area × time × TT	22.795	2	<0.001
Area × time × Rad	2.795	2	0.062
Area × TT × Rad	1.357	1	0.245
Time × TT × Rad	1.760	2	0.173
Area × time × TT × Rad	0.691	2	0.502

[a] *F*: Fisher–Snedecor *F* statistic; DF: degrees of freedom; *p*-value: level of significance.

Table 5
Results of multi-way ANOVA (including Area × time × TT × Rad interaction) for c-fos the paleocortex brain region.[a]

Effects	*F*	DF	*p*-Value
Paleocortex region			
Area	40.400	1	<0.001
Time	10.303	2	<0.001
TT	1009.443	1	<0.001
Rad	43.821	1	<0.001
Area × time	28.980	2	<0.001
Area × TT	19.135	1	<0.001
Area × Rad	0.280	1	0.597
Time × TT	33.953	2	<0.001
Time × Rad	54.101	2	<0.001
TT × Rad	26.324	1	<0.001
Area × time × TT	9.569	2	<0.001
Area × time × Rad	10.197	2	<0.001
Area × TT × Rad	4.951	1	0.027
Time × TT × Rad	15.636	2	<0.001
Area × time × TT × Rad	10.305	2	<0.001

[a] *F*: Fisher–Snedecor *F* statistic; DF: degrees of freedom; *p*-value: level of significance.

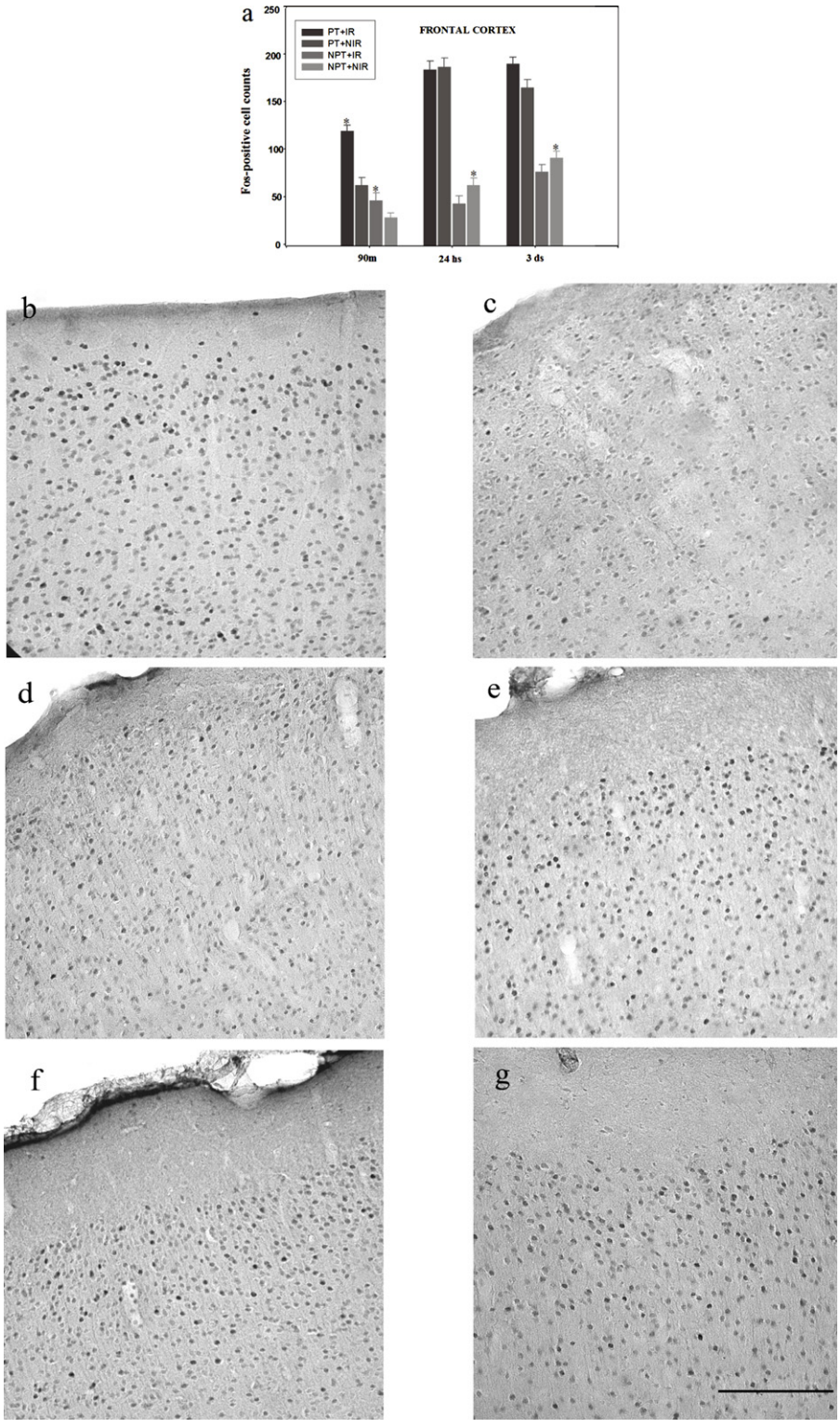

Fig. 1. Histograms and microphotographs showing the time course evolution of c-fos-positive cell counts in the neocortex (average of seven or eight rat per group); (a) histogram of the frontal cortex. *Average significant differences ($p < 0.05$) between PT + IR and NPT + IR, PT + NIR and NPT + NIR. The photographs show the c-fos immunomarkers for PT + IR (b, d, and f) and PT + NIR (c, e, and g) at 90 min (b and c), 24 h (d and e) and 3 days (f and g) in the frontal cortex. Scale bar = 60 μm.

cant effects on c-fos expression ($p < 0.001$ in all cases; see Table 4). The frontal area showed significant c-fos effects at 90 min vs. 24 h and 3 days ($p < 0.001$) (see Table 8). However in the parietal area there are significant c-fos differences at each time studied: 90 min vs. 24 h vs. 3 days ($p < 0.001$) (see Table 8).

After 90 min c-fos expression was significantly affected by radiation or treatment in frontal or parietal cortical areas ($p < 0.001$), but not by the interaction of these two factors ($p = 0.249$). After 90 min in PT + IR rats, radiation had significantly affected c-fos expression in the frontal or parietal areas when compared to NPT + IR rats ($p < 0.001$) (see Table 8) In both areas the mean c-fos expression in PT + IR rats 90 min after radiation was higher than in PT + NIR rats ($p < 0.001$) (see Microphotographs 1 and 2b, c and Table 8). In the frontal and parietal areas, c-fos

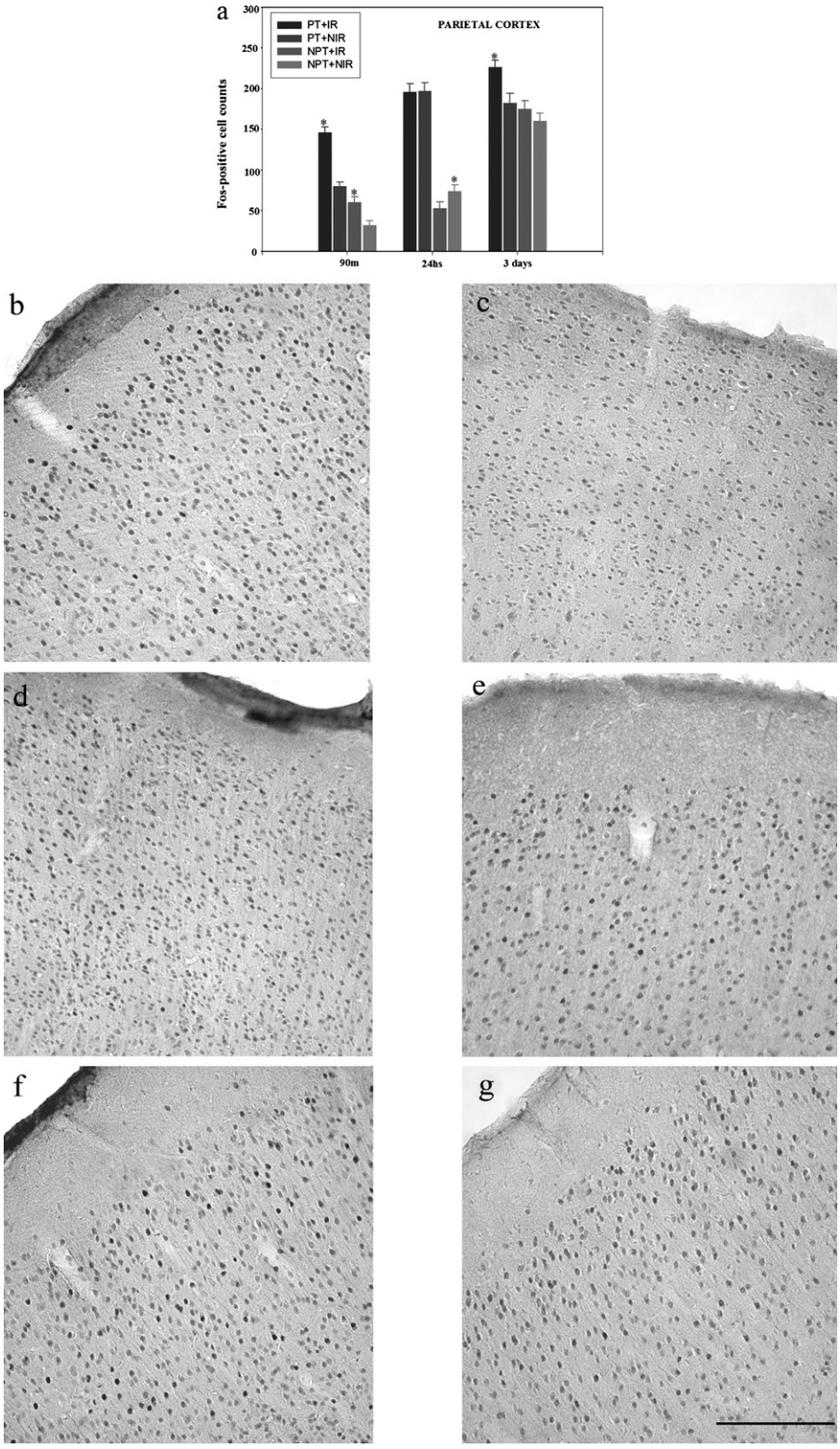

Fig. 2. Histograms and microphotographs showing the time course evolution of c-fos-positive cell counts in the neocortex (average of seven or eight rat groups); (a) histogram of the parietal cortex. *Average significant differences ($p < 0.05$) between PT + IR and NPT + IR, PT + NIR and NPT + NIR. The photographs show the c-fos immunomarkers for PT + IR (b, d, and f) and PT + NIR (c, e, and g) at 90 min (b and c), 24 h (d and e) and 3 days (f and g) in the parietal cortex. Scale bar = 60 μm.

expression in the NPT + IR group differed significantly from that of the NPT + NIR group ($p < 0.001$ in both) (see histograms in Figs. 1a and 2a, and Table 8).

Radiation had not significantly affected c-fos expression in the frontal or parietal areas of PT + IR rats when compared with PT + NIR rats ($p = 0.577$ or 0.674) (see Table 8) 24 h after radiation (Photographs 1 and 2d, e). NPT + IR rats were significantly affected when compared with NPT + NIR rats ($p = 0.023$ and $p = 0.006$) (see Table 8) but c-fos counts were greater in NIR animals. However, treatment significantly affected c-fos expression in the frontal or parietal areas of PT + IR rats with respect to NPT + IR rats ($p < 0.001$) (see Table 8).

Table 6
Results of multi-way ANOVA (including Area × time × TT × Rad interaction) for c-fos in the hippocampal brain region.[a]

Effects	*F*	DF	*p*-Value
Hippocampal areas			
Area	23.598	2	<0.001
Time	397.333	2	<0.001
TT	31.641	1	<0.001
Rad	28.525	1	<0.001
Area × time	8.564	4	<0.001
Area × TT	4.790	2	0.009
Area × Rad	0.086	2	0.917
Time × TT	92.697	2	<0.001
Time × Rad	24.079	2	<0.001
TT × Rad	29.168	1	<0.001
Area × time × TT	2.831	4	0.024
Area × time × Rad	8.213	3	<0.001
Area × TT × Rad	7.058	2	0.001
Time × TT × Rad	2.214	2	0.110
Area × time × TT × Rad	1.492	3	0.215

[a] *F*: Fisher–Snedecor *F* statistic; DF: degrees of freedom; *p*-value: level of significance.

Three days after radiation, there were significant differences in c-fos expression between PT + IR and NPT + IR rats ($p < 0.001$, $p < 0.010$) in the frontal and parietal areas. At first, in the frontal area, c-fos expression in the PT + IR rats did not differ significantly from PT + NIR rats ($p < 0.578$), while in the parietal cortex there were significant differences between PT + IR and PT + NIR rats ($p < 0.039$). However, these differences changed after 3 days, appearing in the frontal area and disappearing in the parietal area of NPT + IR and NPT + NIR rats ($p = 0.023$ and $p = 0.379$, respectively).

3.3.2.2. Paleocortex. Initial analysis of c-fos expression in the paleocortex (piriform and entorhinal cortex) indicated that it was significantly affected by time, radiation or treatment (see Table 5), by the interaction of radiation with treatment or time and the interaction of treatment and time (see Table 5). c-fos expression was also significantly affected by the radiation × treatment × time interaction (see Table 5).There were significant effects on c-fos expression in the paleocortex region at 90 min and 24 h vs. 3 days ($p < 0.001$) (see Table 8).

Analyses carried out separately in the piriform and entorhinal areas indicated in both cases that radiation and treatment, the interaction between these two factors, and the radiation × time interaction all had significant effects on c-fos expression in the entorhinal area, but not in the piriform area ($p = 0.200$). In the piriform area there were significant effects on c-fos at 90 min and 24 h vs. 3 days ($p < 0.001$), but in the entorhinal area there were no significant differences after 3 days ($p = 0.846$). See Histograms 3a and 4a and Table 8.

After 90 min c-fos expression in the piriform and entorhinal cortical areas was significantly affected by radiation or treatment ($p < 0.001$) or the interaction of both factors ($p = 0.044$ and $p < 0.001$, respectively). Radiation had significantly affected c-fos expression in the piriform and entorhinal areas of PT + IR rats with respect to NPT + IR rats ($p < 0.001$) (see Table 8). In both areas, the mean c-fos expression in PT + IR or NPT + IR rats 90 min after radiation was higher than in PT + NIR or NPT + NIR rats ($p < 0.001$) (see histograms in Figs. 3a and 4a; Photographs 3b, c and 4b, c and Table 8).

After 24 h significant effects from radiation were still seen in c-fos expression in PT + IR rats when compared with NPT + IR rats in both the piriform and the entorhinal cortex ($p < 0.001$ and $p = 0.001$). c-fos expression in the piriform and entorhinal cortex of treated rats was affected by the significant variations in cell counts between PT + NIR rats and NPT + NIR rats ($p < 0.001$in both cases) (see histograms, Figs. 3a and 4a; Photographs 3d, e and 4d, e, and Table 8).

Three days after radiation, c-fos expression in the piriform and the entorhinal cortex became inverted, showing an important decrease. There were no significant differences in c-fos expression in the entorhinal and piriform cortices for PT + IR rats with respect to PT + NIR rats ($p = 0.294$ and $p = 0.577$, respectively) (see photographs in Figs. 3 and 4f, g, and Table 8). In the piriform and entorhinal cortices of PT + IR rats c-fos expression was significantly affected when compared with NPT + IR rats ($p < 0.001$). Non-picrotoxin treated rats showed significant differences between irradiated and non-irradiated animals in the entorhinal cortex ($p < 0.001$) (see histograms, Figs. 3a and 4a, and Table 8).

3.3.2.3. Hippocampal areas. Initial analysis of c-fos expression in the hippocampal areas (dentate gyrus, CA1, and CA3) indicated that it was significantly affected by radiation, treatment and time, and by the radiation × treatment, radiation × time or treatment × time interactions ($p < 0.001$). However, c-fos expression in the hippocampal areas was not significantly affected by the radiation × treatment × time interaction ($p = 0.110$) (see Table 6). There were significant effects on c-fos expression at 90 min vs. 24 h vs. 3 days ($p < 0.001$) (see Table 8 and Histogram 5a–c, Histograms and Photographs d–f and DG g–i, amplified in Fig. 5).

Results obtained separately in the dentate gyrus, CA1 and CA3 areas indicated that treatment, time, and the radiation × time or treatment × time interactions had significant effects on c-fos expression ($p < 0.001$ and $p = 0.017$ in gyrus treatment and $p = 0.021$ in CA3 treatment, see Table 6). In CA1 and CA3 the interaction of the three factors, radiation × treatment × time, had significant effects on c-fos expression ($p = 0.002$), but not in the dentate gyrus ($p = 0.796$). In the DG and CA1 areas there were significant effects on c-fos expression at 90 min vs. 24 h vs. 3 days

Table 7
Results of multi-way ANOVA (including Area × TT × Rad interaction) for glia in the whole brain and for different brain regions.[a]

Effects	*F*	DF	*p*-Value
Whole brain			
TT	531.770	1	<0.001
Rad	4.616	1	0.032
TT × Rad	11.089	1	0.001
Brain regions			
Neocortex			
Area	0.691	1	0.407
TT	147.140	1	<0.001
Rad	19.688	1	<0.001
Area × TT	0.462	1	0.498
Area × Rad	3.279	1	0.072
TT × Rad	0.031	1	0.859
Area × TT × Rad	3.433	1	0.065
Paleocortex			
Area	11.636	1	0.001
TT	207.506	1	<0.001
Rad	3.742	1	0.055
Area × TT	0.948	1	0.331
Area × Rad	0.311	1	0.577
TT × Rad	30.712	1	<0.001
Area × TT × Rad	0.618	1	0.433
Hippocampal areas			
Area	0.198	1	0.820
TT	234.633	1	<0.001
Rad	5.189	1	0.024
Area × TT	0.655	1	0.520
Area × Rad	1.175	1	0.310
TT × Rad	0.870	1	0.352
Area × TT × Rad	1.729	1	0.179

[a] *F*: Fisher–Snedecor *F* statistic; DF: degrees of freedom; *p*-value: level of significance.

Table 8
Mean values ± SEM of the cell counts in the entire brain and in brain areas and for the different treatments (with and without radiation and with and without picrotoxin).

	90 min	24 h	3 days	
	c-fos	c-fos	c-fos	GFAP
Neocortex[a]				
FR[b]				
PT + NIR	64.31 ± 9.89[#]	188.69 ± 7.86	188.69 ± 7.86	27.50 ± 1.46[#]
PT + IR	132.40 ± 8.75[*,#]	185.44 ± 13.91[*]	185.44 ± 13.91[*]	32.13 ± 1.57[*,#]
NPT + NIR	30.16 ± 3.13[#]	64.36 ± 5.85[#]	64.36 ± 5.85[#]	15.83 ± 1.02[#]
NPT + IR	51.75 ± 3.69[*,#]	44.95 ± 6.59[*,#]	44.95 ± 6.59[*,#]	20.96 ± 0.73[*,#]
PAR[a]				
PT + NIR	79.83 ± 6.40[#]	197.38 ± 10.29	182.88 ± 8.85[#]	27.21 ± 1.11[#]
PT + IR	146.21 ± 10.54[#,*]	196.06 ± 16.93[*]	226.67 ± 12.27[*,#]	32.25 ± 1.52[*,#]
NPT + NIR	32.00 ± 4.32[#]	73.56 ± 4.41[#]	160.00 ± 6.83	18.96 ± 0.99[#]
NPT + IR	60.48 ± 3.33[*,#]	52.67 ± 7.53[*,#]	175.95 ± 10.45[*]	20.04 ± 1.18[*,#]
Paleocortex[c]				
PIR[c]				
PT + NIR	109.62 ± 9.79[#]	206.00 ± 6.24[*]	166.50 ± 15.68	23.71 ± 1.00[#]
PT + IR	196.05 ± 12.58[*,#]	185.44 ± 11.32[*]	152.13 ± 7.66[*]	30.46 ± 1.83[*,#]
NPT + NIR	23.58 ± 3.82[#]	44.59 ± 3.71[*]	95.61 ± 5.18	17.42 ± 0.77
NPT + IR	56.90 ± 4.78[*,#]	42.32 ± 4.01[*]	110.64 ± 5.67[*]	15.50 ± 0.79[*]
ENT				
PT + NIR	137.29 ± 5.92[#]	193.38 ± 10.55[*]	129.65 ± 7.64	26.38 ± 0.97[#]
PT + IR	187.32 ± 8.86[*,#]	159.81 ± 10.02[*]	117.19 ± 7.24[*]	36.21 ± 1.20[*,#]
NPT + NIR	20.06 ± 3.85[#]	49.64 ± 3.66[*]	20.13 ± 4.10[#]	19.25 ± 0.94
NPT + IR	65.10 ± 5.10[*,#]	33.00 ± 6.68[*]	65.10 ± 5.10[*,#]	17.13 ± 1.09[*]
Hippocampal areas[a]				
DG[a]				
PT + NIR	28.70 ± 10.48[#]	121.00 ± 14.17[#]	108.81 ± 6.83	29.25 ± 0.89
PT + IR	15.30 ± 1.58[*,#]	87.96 ± 2.90[#]	111.79 ± 9.82[*]	26.75 ± 1.01[*]
NPT + NIR	8.50 ± 0.52[#]	32.63 ± 3.12	91.45 ± 7.16	19.57 ± 1.08
NPT + IR	35.08 ± 3.03[*,#]	28.11 ± 4.40	84.76 ± 9.94[*]	19.25 ± 1.04[*]
CA1[a]				
PT + NIR	29.17 ± 10.48	91.75 ± 5.79[#]	90.19 ± 4.25	27.37 ± 0.92
PT + IR	14.08 ± 1.87[*]	73.96 ± 3.41[#]	78.38 ± 4.95	28.04 ± 0.98[*]
NPT + NIR	7.81 ± 0.79[#]	43.38 ± 3.04[#]	64.90 ± 4.45[#]	19.54 ± 0.89
NPT + IR	26.74 ± 3.07[*,#]	28.63 ± 5.15[#]	84.90 ± 2.41[#]	18.83 ± 1.04[*]
CA3[b]				
PT + NIR	10.44 ± 0.97	56.44 ± 5.86[#]	54.94 ± 6.11	29.67 ± 0.94
PT + IR	11.92 ± 1.34[*]	38.65 ± 2.07[#]	42.13 ± 3.66[*]	28.76 ± 1.08[*]
NPT + NIR	10.32 ± 0.80[#]	7.81 ± 0.79[#]	55.75 ± 2.42	20.67 ± 0.85[#]
NPT + IR	25.00 ± 2.10[*,#]	32.11 ± 4.53[#]	62.20 ± 4.44[*]	17.46 ± 1.11[*,#]

Multi-way ANOVA (treatment × radiation and treatment × radiation × area) for GFAP and cFos in the entire brain and for different brain regions. All analyses were carried out at each time separately.
[a] Significant differences between 90 min, 24 hours and 3 days.
[b] Significant differences between 90 min and 24 hours, and between 90 min and 3 days.
[c] Significant differences between 90 min and 3 days, and between 24 hours and 3 days.
[*] Significant differences in picrotoxin-treated vs. non treated animals for irradiated or non-irradiated animals.
[#] Significant differences in irradiated vs. non-irradiated animals for picrotoxin treated or non treated animals.

and in the CA3 area there were also different effects at 90 min vs. 24 h and 3 days ($p < 0.001$) (see Histogram 5a–c and Table 8).

After 90 min the radiation × treatment interaction showed an inverted and non-homogeneous effect on fos expression in the dentate gyrus, CA1 and CA3. In three areas the count in NPT + IR animals was higher than in PT + IR animals ($p < 0.001$). In the dentate gyrus 90 min after radiation the mean c-fos expression in PT + NIR rats was higher than in PT + IR rats ($p = 0.039$). c-fos expression in the PT + IR group did not differ significantly from the PT + NIR group in CA1 and CA3 ($p = 0.580$ and $p = 0.672$, respectively). In the three hippocampal areas, c-fos expression in the NPT + IR group differed significantly from the NPT + NIR group ($p < 0.001$) (see Histogram a–c in Fig. 5 and Table 8).

After 24 h, c-fos expression showed significant effects in the dentate gyrus, CA1 and CA3 of PT + IR rats when compared with PT + NIR rats ($p = 0.0180$, $p = 0.010$ and $p = 0.002$, respectively). In non-picrotoxin-treated rats the differences were significant in CA1 and CA3 ($p = 0.006$ and $p < 0.001$, respectively) (see Histogram a–c in Fig. 5, and Table 8).

After three days there were positive effects on c-fos expression, but not significant effects in PT + IR rats with respect to PT + NIR rats, and no significant differences in the dentate gyrus, CA1 and CA3 ($p = 0.89$, $p = 0.073$, and $p = 0.135$, respectively). c-fos expression in the PT + IR group after 3 days differed significantly from the NPT + IR group in the dentate gyrus and CA3 ($p < 0.027$ and $p < 0.022$). However, these differences disappeared for PT + IR rats in CA1 with respect to NPT + IR rats ($p = 0.136$). There were significant differences between NPT + IR and NPT + NIR rats in CA1 ($p = 0.001$) (see Histogram a–c in Fig. 5, and Table 8).

3.4. GFAP expression in different brain regions

3.4.1. GFAP expression throughout the entire brain

Results from applying two-way ANOVA with radiation and treatment as factors indicated that there were significant effects by both factors and their interaction ($p = 0.032$, $p < 0.001$, and $p < 0.001$, respectively) on the proportion of GFAP-immunopositive neurons (GFAP expression) throughout the entire brain after three days (Table 7).

3.4.2. GFAP expression in different brain regions

All brain areas showed GFAP expression after picrotoxin treatment, with or without subsequent irradiation. In rats treated

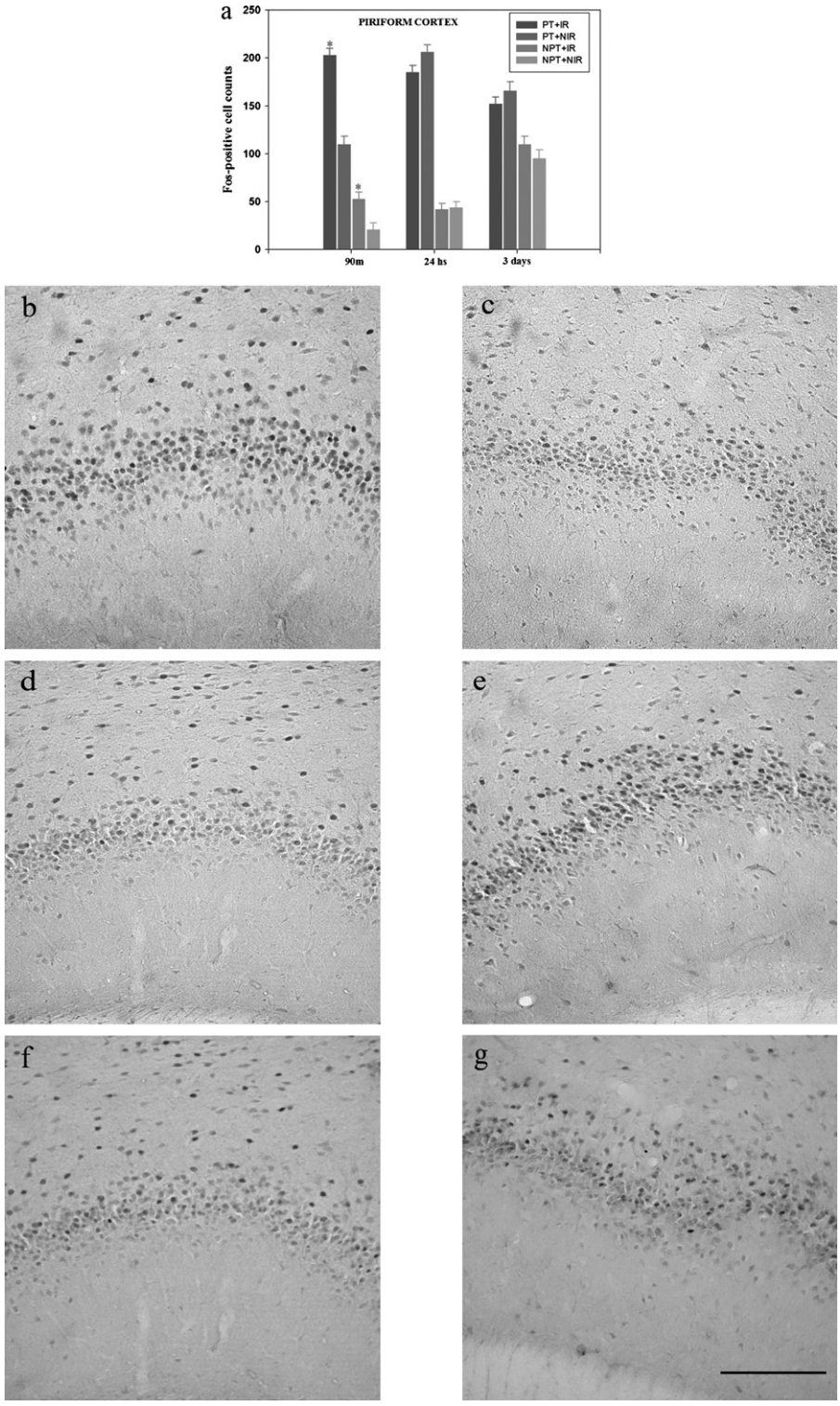

Fig. 3. Histograms and microphotographs showing the time course evolution of c-fos-positive cell counts in the paleocortex (average of seven or eight rat groups); (a) histogram of piriform cortex. *Average significant differences ($p < 0.05$) between PT + IR and NPT + IR, PT + NIR and NPT + NIR. The photographs show the c-fos immunomarkers for PT + IR (b, d, and f) and PT + NIR (c, e, and g) at 90 min (b and c), 24 h (d and e) and 3 days (f and g) in the piriform cortex. Scale bar = 60 μm.

with picrotoxin, GFAP-positive glia were observed systematically in the cortical areas and the hippocampus, and in some animals more or less scattered expression was also observed in the putamen–caudate complex, the hypothalamus and the pontomesencephalic nuclei. Three days after radiation the mean GFAP expression in PT + IR rats was higher than in PT + NIR rats in both the neocortex and the paleocortex. However, in the three hippocampus areas, PT + IR and PT + NIR rats showed similar mean GFAP expression levels (Table 7).

Initial analysis of GFAP expression in the neocortex showed it to be significantly affected by radiation and treatment ($p < 0.001$ in both cases) but not by the radiation × treatment interaction ($p = 0.859$) and area × radiation × treatment interaction ($p = 0.065$). No significant effects were found by area ($p = 0.407$) (see Table 7).

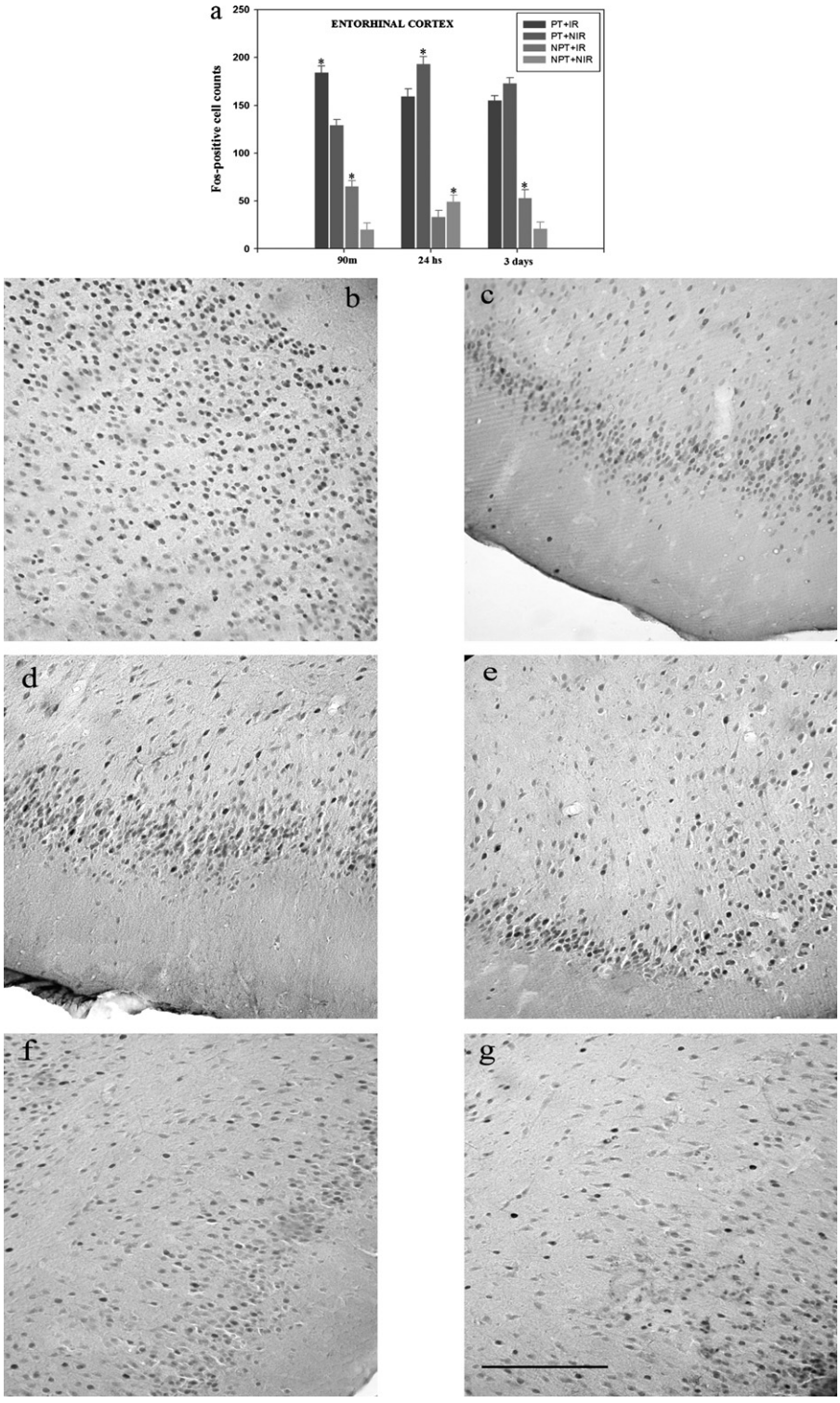

Fig. 4. Histograms and microphotographs showing the time course evolution of c-fos-positive cell counts in the paleocortex (average of seven or eight rat groups); (a) histogram of the entorhinal cortex. *Average significant differences ($p < 0.05$) between PT + IR and NPT + IR, PT + NIR and NPT + NIR. The photographs show the c-fos immunomarkers for PT + IR (b, d, and f) and PT + NIR (c, e, and g) at 90 min (b and c), 24 h (d and e) and 3 days (f and g) in the entorhinal cortex. Scale bar = 60 μm.

The ANOVA technique applied separately in the frontal and parietal cortices indicated that treatment had significant effects on GFAP expression ($p < 0.001$ in both areas; see Table 7). GFAP expression in the frontal cortex was significantly affected by radiation ($p < 0.001$). Neither area was significantly affected by the radiation × treatment interaction ($p = 0.151$ and $p = 0.243$). After 3 days, GFAP expression in the PT + IR group differed significantly from the NPT + IR group ($p < 0.001$ in both areas) (see Table 8).

GFAP expression in the frontal and parietal cortices was significantly greater in the PT + IR group than in the PT + NIR group ($p < 0.040$ and $p < 0.013$, respectively) (see Table 8 and Photographs 7c, e). The cell count in the frontal cortex was significantly higher in the NPT + IR rats than in the NPT + NIR rats ($p < 0.001$) (see Histogram 6a and Table 8).

Initial analysis of GFAP expression in the paleocortex showed that it was significantly affected by area and treatment ($p < 0.001$

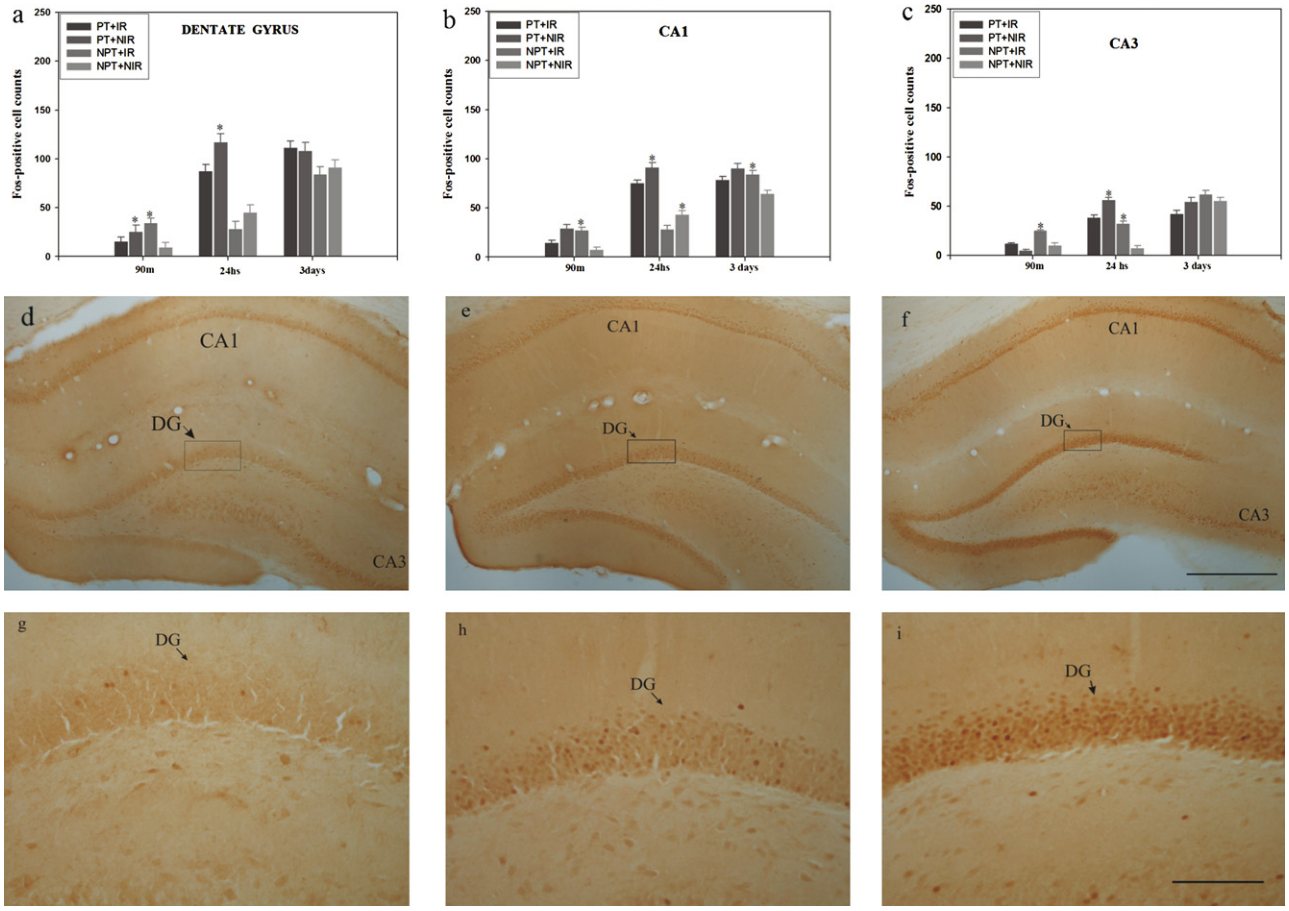

Fig. 5. Histograms and microphotographs showing the time course evolution of c-fos-positive cell counts in the hippocampal areas (average of seven or eight rat groups); (a) dentate gyrus (DG), (b) CA1, and (c) CA3. *Average significant differences ($p < 0.05$) between PT + IR and NPT + IR, PT + NIR and NPT + NIR. The photographs show the c-fos immunomarkers for PT + IR at 90 min (d and g); 24 h (e and h) and 3 days (f and i), in the hippocampal areas. The rectangle shows a portion of DG granular cell in the photographs at the time of study. Scale bar (d–f) = 500 μm; scale bar (g–i) = 60 μm.

in both cases), as well as by the radiation × treatment interaction ($p < 0.001$). Neither area was significantly affected by radiation, area × treatment, area × radiation or area × radiation × treatment interactions ($p = 0.055$, $p = 0.331$, $p = 0.577$, and $p = 0.433$, respectively) (see Table 7).

Separate analyses carried out in the piriform and entorhinal cortices indicated that picrotoxin treatment had significant effects on GFAP expression in both areas ($p < 0.001$; see Table 7), but neither area was significantly affected by radiation ($p = 0.350$ and $p = 0.070$). However the treatment-radiation interaction had significant effects on GFAP expression in both areas ($p = 0.002$ and $p < 0.001$). After 3 days GFAP expression in the PT + IR group differed significantly from the NPT + IR group ($p < 0.001$ in both areas; see Histogram 7b). GFAP expression in the piriform and entorhinal cortices was significantly greater in PT + IR rats than in PT + NIR rats ($p = 0.006$ vs. $p < 0.001$) (see Histogram 7b and Photographs 7d, f, and Table 8).

Initial analysis of GFAP expression in the hippocampal areas revealed it to be significantly affected by picrotoxin treatment ($p < 0.001$) or radiation ($p = 0.024$), but not by the radiation × treatment interaction nor by the area × radiation × treatment interaction ($p = 0.352$ and $p = 0.179$) (see Table 7).

Analyses carried out separately in the dentate gyrus, CA1 and CA3 indicated that picrotoxin treatment had significant effects on GFAP expression in all three areas ($p < 0.001$ in all cases; see Table 7), but the radiation × treatment interaction had no significant effect on any area ($p = 0.377$ in the dentate gyrus, $p = 0.401$ in CA1, $p = 0.095$ in CA3).

After 3 days, GFAP expression in the PT + IR group differed significantly from the NPT + IR group ($p < 0.001$ in the three hippocampal areas, Histogram 7a) (see Table 8).

The effects of radiation on GFAP expression in the dentate gyrus, CA1 and CA3 were not significant between PT + IR and PT + NIR rats in these hippocampal areas ($p = 0.050$, $p = 0.640$, and $p = 0.477$, respectively; see Photographs 7b–g). However, in CA3 the count was significantly lower in NPT + IR rats than in NPT + NIR rats ($p = 0.018$) (see Histogram 7a and Table 8).

4. Discussion

We have no knowledge of any prior work that evaluates the effects of radiation similar to GSM radiation at 900 MHz in rat brains using a sub-convulsive picrotoxin neurological model. There are studies (López-Martín et al., 2006, 2008, 2009) using this same animal model to determine immunomarking of the c-fos protein, but at lower power levels (0.225 W, producing an average SAR of less than 0.43 W/kg in the brain). In this study we used an emitted power of approximately 1 W, which produces a brain SAR below 1.45 W/kg, which is under the 2 W/kg limit established by the EU for a frequency of 900 MHz on the head and neck (Royal Decree 1066/28-9-2001). This power level was chosen because although it is above the average emitted power of mobile telephones (0.25 W), they can occasionally spike up to 2 W (Dimbylow and Mann, 1994; Burkhardt et al., 1997; Bernardi et al., 2003) (Fig. 8).

In this study both exposed and unexposed rats were immobilized during every experiment. Forced immobility can

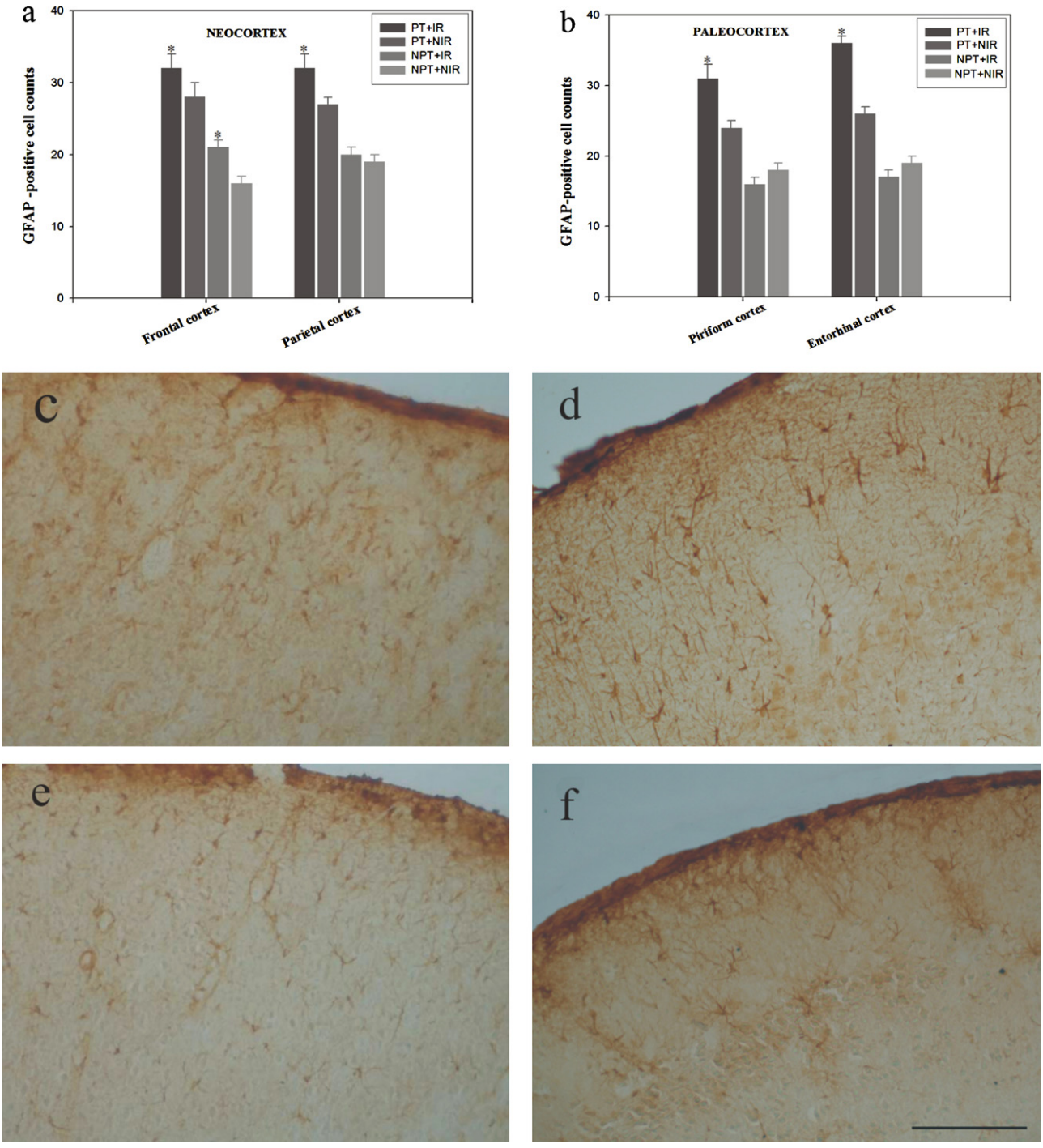

Fig. 6. Histograms and microphotographs showing the time course evolution of GFAP-positive cell counts in the neocortex and paleocortex (average of seven or eight rat groups); histograms in (a) neocortex and (b) paleocortex. *Average significant differences ($p < 0.05$) between PT + IR and NPT + IR, PT + NIR and NPT + NIR. The photographs show the GFAP immunomarkers for PT + IR (c and d) and PT + NIR (e and f) in the parietal and entorhinal cortices. Scale bar = 60 μm.

represent a significant stressor in rodents, as some authors report in studies of the effect of acute exposure to GSM radiation on immediate early genes (c-fos). In those studies no significant differences were reported when animals immobilized during GSM exposure were compared to immobilized, non-exposed samples. However, in our work significant differences were observed between both exposed and unexposed immobilized animals. So we did not consider necessary a group control of unexposed samples kept unrestrained in standard cages. Had there been no significant differences between irradiated and nonirradiated animals, such a control would have been indicated.

4.1. The influence and temporal evolution of picrotoxin treatment on c-fos expression

The c-fos early expression gene, by means of mRNA or fos protein levels, is a marker that is sensitive to neuronal activation (Morgan and Curran, 1991b). The effects of administering picrotoxin 'per se' on fosprotein expression, independently of irradiation, are generally significant in the three brain regions studied -the neocortex, the paleocortex and the hippocampus- and in all areas except the dentate gyrus ($p = 0.166$, see Histograms in Figs. 1–5a). c-fos expression is less active in restricted crises in the

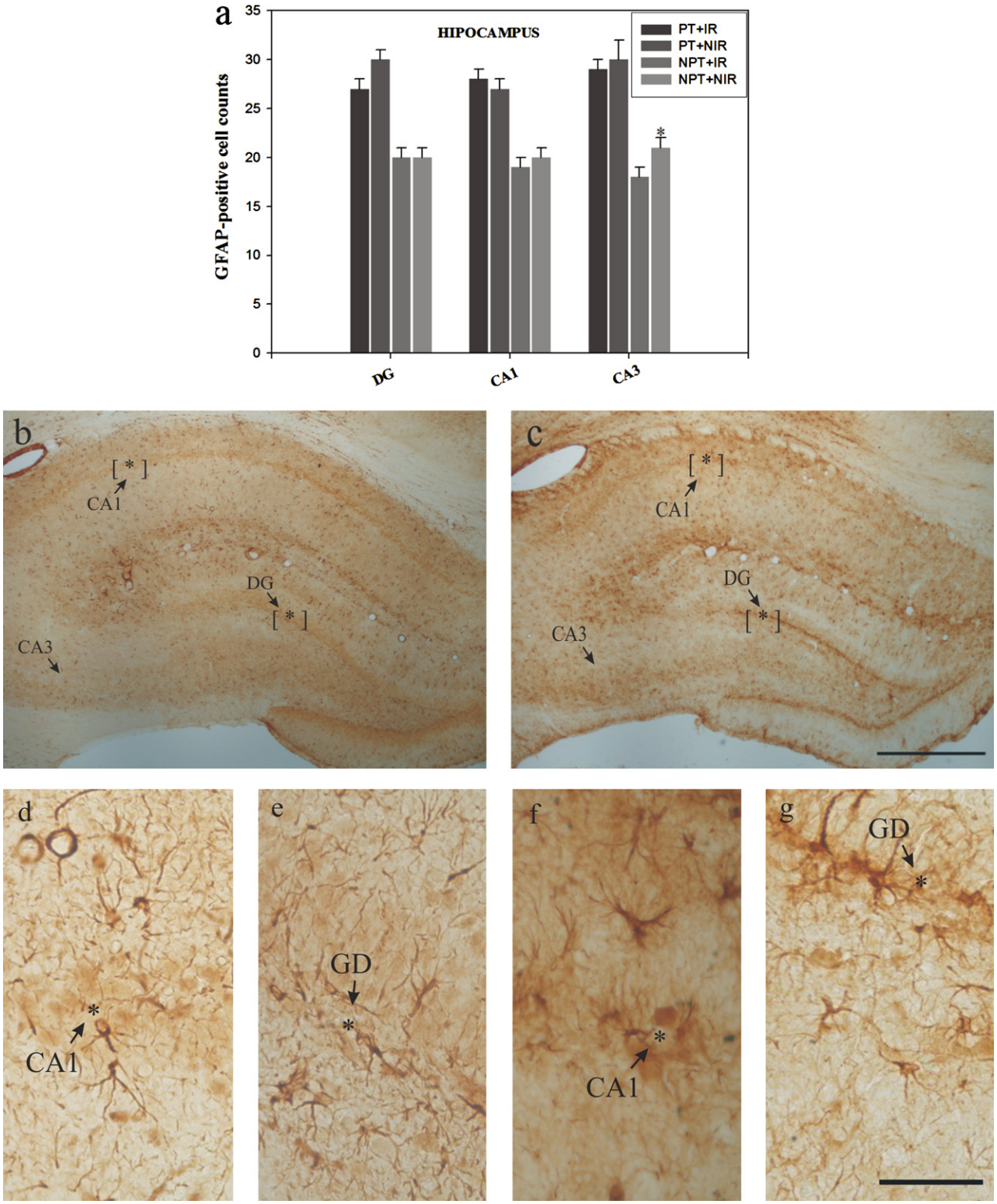

Fig. 7. . Histograms and microphotographs showing the GFAP-positive cell counts in hippocampal areas (average of seven or eight rat groups). Histograms in (a) hippocampus. *Average significant differences ($p < 0.05$) between PT + IR and NPT + IR, PT + NIR and NPT + NIR. The photographs show the GFAP immunomarkers in the hippocampus (b and c), CA1 (d and f) and dentate gyrus (e and g) for PT + IR and PT + NIR rats. Scale bar (b and c) = 500 μm; scale bar (d–g) = 30 μm.

hippocampus (Willoughby et al., 1995) than in other anatomical areas 90 min and 24 h after radiation, as prior publications indicate (Dragunow and Robertson, 1987; Morgan and Curran, 1991b; Kiessling and Gass, 1993; Willoughby et al., 1995; López-Martín et al., 2006). c-fos expression in all regions of the cerebral cortex is inversely related to c-fos activation in the hippocampus. There is evidence of greater c-fos expression in the neocortex and paleocortex when picrotoxin doses induce partial crises; in contrast with lower c-fos expression in these regions when higher doses of picrotoxin induce generalized crises (Willoughby et al., 1995). After evaluating the degree of neuronal activation in the various cortical regions for the timeframes studied, only the

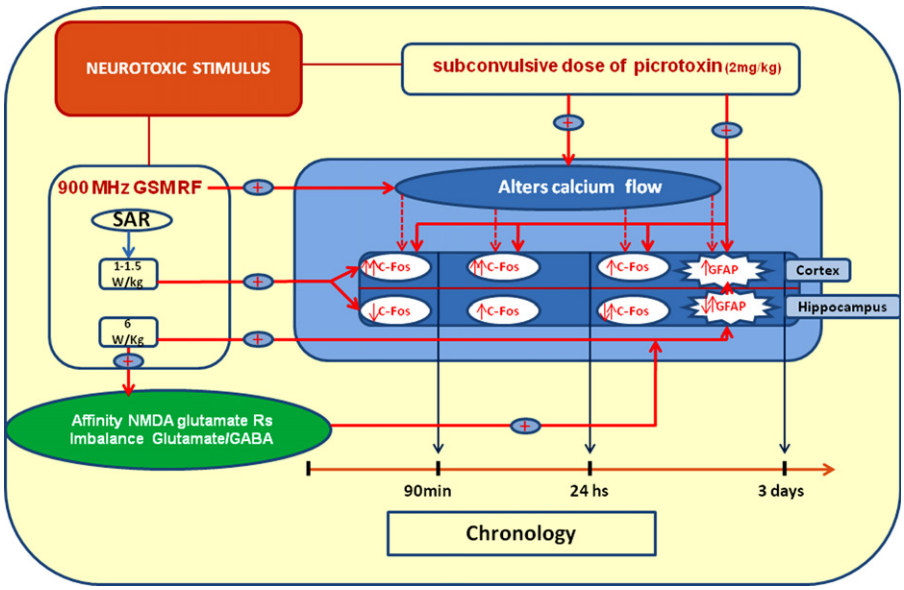

Fig. 8. Model illustrating the hypothetical link between the results on paper and pathological findings. The effects of radiation (SAR 1–1.5 W/kg) are summarized in the cortex and hippocampus, while those of picrotoxin (2 mg/kg) can be seen in the neuronal marker c-fosand GFAP glia marker, 90 min, 24 h and 3 days after exposure. The solid red arrows indicate continued activation of different mechanisms. Dashed red arrows indicate possible triggers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

entorhinal cortex showed low reactiveness to changes. Greater susceptibility to picrotoxin treatment is probably related to the cellular typology involved in the increased excitability phenomena described in other works (Hartmann et al., 2004; Semyanov and Kullmann, 2002). This determines a greater irradiation effect as we will see in the following section.

After 24 h the effects of picrotoxin are more intense in the seven anatomical areas studied, but diminish after three days. The temporal evolution is related to the decrease in the neuronal activation induced by picrotoxin (Nutt et al., 1982), as well as to the intensity and duration of the stimulus (Bullitt et al., 1992).

4.2. The influence and temporal evolution of treatment and GSM radiation on c-fos expression

As far as we know, this is the first immunohistochemical study of the effects of mobile phone radiation for epilepsy or seizure-proneness on the brains of animal models, although DC magnetic fields and extremely low frequency fields (ELF-MFs) have been observed to result in an increasing epileptiform activity (Persinger and Belanger-Chellew, 1999) or may alter the convulsion susceptibility of seizure response to bicuculline and picrotoxin in NMDA mice (Sung et al., 2003).

A comparison of the c-fos counts in rat brain indicates that irradiation produces a statistically significant increase over time both generally and in all regions and areas studied. Particularly significant are the responses in the neocortical region ($p = 0.173$) and the hippocampus ($p = 0.110$), where simultaneous statistical evaluation of the three factors shows no significant, effect-causing interaction. Thus, the entorhinal cortex stands out as the least sensitive anatomical area in compensating for changes after neuronal activation, and is where the most significant results of irradiation, treatment and time appear ($p < 0.001$). This again indicates a marked response to irradiation by certain regions of the limbic system such as the paleocortex (especially the entorhinal cortex) and the subcortical areas (CA1 and CA3), which has already been described in the subconvulsive model (López-Martín et al., 2006, 2009).

In this experiment, the increase of c-fos that also appears in the groups of irradiated animals not treated with picrotoxin is noteworthy in the somatosensory cortex, the limbic cortex and the hippocampus, which also occurs in other models (Jorge-Mora et al., 2010). This suggests that the application of power above 1 W, or a SAR of 1.45 or 1.38 W/kg, may itself determine a greater stimulus in animal brain but does not reach thermal levels, especially not in short periods of exposure (90 min).

In many cases the data favouring an increase in c-fos activation were related to an increase in temperature induced by radiation at a high SAR (Morrissey et al., 1999). c-fos expression increased in various regions of the cortex and in periventricular areas when the rats were exposed to 9.3 W/kg over their entire body (Mickley et al., 1994). Studies using sub-thermal SAR levels show negative results in many cases (Fritze et al., 1997). However, radio frequency (RF) was not modulated with GSM for all the powers tested due to limitations in the amplifier, as some authors indicate. In contrast with continuous or non-pulsated radiation, RF with GSM shows a modulation in the wave amplitude at low frequencies of 217 Hz and 8.24 Hz. Neuronal ion channels (sodium, potassium and calcium) may be affected by interference with the membrane voltage, which can alter both channel gating and channel properties. Such alterations cause microvolt changes in the neuronal membrane potential (Mathie et al., 2003). Several studies describe alterations in the flow of calcium in nerve tissue under the influence of low-amplitude modulated radiofrequency waves. The maximum effects were seen at a critical modulation frequency of 16 Hz (Bawin et al., 1975; Blackman et al., 1980; Adey et al., 1982; Kittel et al., 1996). Intracellular calcium is one of the most important intracellular messengers in many biological processes. $[Ca^{2+}]_i$ is known to be involved in the transcription of early gene expression by activating calcium response elements in gene promoting regions (Morgan and Curran, 1991a). It is involved in neuronal excitacion and seizures induced by diverse convulsant agents (Solà et al., 1999). The low frequency GSM radiation used in this experiment could thus alter calcium flow (Paulraj et al., 1999), which would explain the activation of c-fos in nerve tissue (Thompson et al., 1995), even in non-treated groups, in contrast with the negative data provided by other authors (Fritze et al., 1997) or in picrotoxin-treated animals (López-Martín et al., 2009) see Fig 8.

After 90 min c-fos increases in the irradiated groups and the effect of irradiation is in picrotoxin-treated animals much greater

in the neocortex and particularly in the paleocortex, piriform, entorhinal cortex ($p < 0.044$ and $p < 0.001$), CA1 ($p < 0.039$) and CA3 ($p < 0.001$), where there is a positive statistical interaction between radiation and treatment. This might suggest that the electrochemical instability of animal brains has a facilitating effect linked to irradiation (Stewart, 2000; Hossmann and Hermann, 2003). Studies of epileptic patients found that pulse-modulated RF fields can alter electrophysiological activity in the awake human brain (Relova et al., 2005). Maby et al. (2004, 2005) described how the influence of GSM RF on AEPs can induce some common spectral components, but both found it difficult to draw conclusions about these changes. Under conditions of GSM radiation exposure at 900 MHz, a decrease in the GABA-A receptors to the radioactive [^{3}H] Mucinol ligand, as well as a decrease in the affinity of the NMDA glutamate receptors to their radioactive [^{3}H] TPC ligand in various zones (but not the hippocampus) was described by Mausset-Bonnefont et al. (2004). Given that the GABAergic system is an inhibitory neurotransmission system and that the glutamatergic system is an excitatory system, it is possible that an imbalance occurs between the two systems in rats exposed to GSM radiation. This imbalance may potentiate the blockage of GABA-A channels due to the picrotoxin and induce a greater c-fos expression in the treated irradiated group than in the treated non-irradiated group. This contrast is significantly greater in the non-treated irradiated groups. These findings are in consonance with other studies done at 0.225 W (López-Martín et al., 2006). However, co-exposure to magnetic fields (MMF) and bicuculline decreased the GABA levels in the cortex, hippocampus and hypothalamus (Jeong et al., 2005). After MF exposure, the glutamate level was increased and GABA was decreased in NMDA and picrotoxin-induced seizures (Sung et al., 2003). ELF-MFs may also alter convulsion susceptibility through the GABAergic mechanism, which involves glutamate and GABA levels.

The increased neuronal activation continued in almost all areas 24 h after radiation. In all areas except the piriform cortex, increased neuronal activation was observed 24 h after radiation.In this sense, the radiation factor was not significant ($p < 0.341$) but treatment with picrotoxin did prove to be a significant factor ($p < 0.001$).

However, the slight difference in counts between irradiated and non-irradiated animals, whether picrotoxin-treated or not, indicates a state of hyper-excitability in which the action of the GABA-A antagonist seems to persist longer than the irradiation. Other authors also describe neurotoxic effects in the frontal cortex 24 h after exposing rat brain to 900 MHz with a SAR of up to 6 W/kg (Mausset-Bonnefont et al., 2004).

The decline after 3 days of the effect of radiation in the hippocampus and piriform is also described by other authors who used GSM radiation at 900 MHz (Brillaud et al., 2007). However, disappearance of the neuronal activation measured by c-fos is slower in the limbic regions, and especially in the entorhinal and somatosensory cortices, than the process caused by other drugs in rat cerebral tissue (Popovici et al., 1990; Motte et al., 1997).

The combined effect of the non-competitive antagonist picrotoxin and radiation depends on the temporal modification of the excitability of the GABA-A receptors and may be related to the sub-unit interaction of the receptor with picrotoxin (Bell-Horner et al., 2000) or the spontaneous activity of the drug with the receptor (Mortensen et al., 2003). The entire three-day study presented here has shown that GSM irradiation with non-thermal SAR appears to have acute effects on c-fos neuronal activation see Table 8. The effects were compensated in all regions studied except the paleocortex areas, where there appeared to be later recovery. This effect was enhanced by the combined action of picrotoxin and radiation, which leads us to suspect greater limbic cortex vulnerability caused by the joint and potentially toxic action of the two agents.

4.3. Influence of picrotoxin treatment on GFAP

GFAP is an extensively used marker for evaluating astrocytic responses to injury processes in the nerve tissue (de Armond et al., 1980; O'Callaghan and Millar, 1983).

Treatment with picrotoxin had significant effects on the entire brain and on its various regions and areas ($p < 0.001$). In our study glial activation, as a function of immunomarked cells for the GFAP protein, appears in the treated groups of all areas studied, presenting statistically significant differences with respect to the non-treated groups, independently of radiation, in all anatomical areas examined ($p < 0.001$ in all areas). The results showing glial activation to be induced by picrotoxin are consistent with earlier studies that used different epileptic models (Tashiro et al., 2002). The changes in GFAP expression become less persistent as the inducing stimulus diminishes. The increase in GFAP expression after seizure activity is less persistent over time when the seizure is less intense, and is always less than that produced by a lesion in the nerve tissue (Torre et al., 1993). A single dose of convulsive drugs in rats causes GFAP to increase on the second day of treatment, and it returns to normal levels after the fourth day (Torre et al., 1993). For this reason we chose a survival time of three days to evaluate the effects of GFAP expression, as has been done in other studies involving microwaves (Mausset-Bonnefont et al., 2004).

There is evidence of possible glial activity in epilepsy in the absence of degeneration or neuronal death (Khurgel et al., 1995). Our study involved a sufficiently low dose of picrotoxin to avoid causing appreciable cytotoxic effects (Torre et al., 1993) or neuronal death associated with repeated doses (Franke and Kitter, 2001). Our seizure model produced sub-clinical seizure activity without inducing convulsions (in most rats), in contrast with previous neuro-physiological studies using analogous administration guidelines (López-Martín et al., 2006). By applying a seizure model that does not cross the cytotoxicity threshold in cerebral tissue, we managed to avoid an excessively intense effect on glial activity, which might mask the effects of GSM irradiation as studied here.

In our study, increased GFAP expression in regions of the cortex reflects glial activation with low doses of picrotoxin. This affects not only the neurons but also the astrocytes themselves due to the rapid increase in their intra-cellular K^+ levels (Amzica et al., 2002). This is due to the fact that the astrocytes in homeostatic responses recapture the K^+ liberated by extra-cellular neurons during the seizure crisis (Heinemann et al., 1977). The discovery of functionally active NMDA glutamate receptors in cortical astrocytes (Lalo et al., 2006) is noteworthy; their activation is evident in trans-membrane currents during astrocytic response to neuronal activation. The seizure model used in our study produces a blockage of the GABA-A channels so that activation of the glutamatergic system prevails over the GABAergic inhibitor system, with the related increase in neuronal activation. Thus it appears that activation of the NMDA receptors in the astrocytes may explain glial activation when picrotoxin is administered see Fig 8.

4.4. The influence and temporal evolution of irradiation and picrotoxin treatment on GFAP

A comparison of GFAP expression after three days in the groups treated with picrotoxin shows statistically significant differences between radiated and non-radiated animals in the neocortex and paleocortex, and subtle differences in the dentate gyrus ($p = 0.05$).

This finding is coherent with the results published by other authors that indicate an increase in GFAP expression in the cortex, hippocampus and striatum (Mausset-Bonnefont et al., 2004) three days after GSM irradiation of the animals. Although the SAR levels used in this experiment were far below those used by others (1.5 W/kg as opposed to 6 W/kg), important glial activation was observed in both the neocortex and paleocortex regions and even in some regions of the hippocampus such as the dentate gyrus. This would indicate an irradiation effect on the cerebral tissue that is related to the SAR and to potentiation by other toxic agents linked to irradiation, such as picrotoxin see Fig 8 (López-Martín et al., 2009). In addition, the effect of the descending gradient of radiation is clearly an influencing factor, and its influence is greater in the cortex than in other parts of the brain. The joint action of irradiation and the drug has less effect in the deepest regions of the brain such as the hippocampus (Huber et al., 2003, 2005; Christ et al., 2005).

In the paleocortex region, piriform and entorhinal cortices ($p = 0.002$ and $p = 0.01$), the statistical interaction of picrotoxin with irradiation indicates potentiation of the irradiation in the presence of the electrochemical lability caused by the picrotoxin treatment (Nutt et al., 1982).

In addition to the decrease in affinity of the NMDA–GABA-A receptors after GSM radiation at 900 MHz (Mausset-Bonnefont et al., 2004), there could be an astrocytic activation mechanism in the presence of irradiation, derived from an excitatory–inhibitory balance in the cerebral neurotransmission systems that act on the NMDA receptors of the cortical astroglia (Lalo et al., 2006). Another possible mechanism causing radiation effects on the brain is the action on calcium metabolism that is manifest in several studies at low frequencies of amplitude with GSM modulation of 217 Hz and 8.24 Hz, with maximum effects around the critical modulation frequency of 16 Hz (Bawin et al., 1975; Blackman et al., 1980; Kittel et al., 1996; Adey et al., 1982). GSM modulation may cause the fluctuations of this element that occur within the astrocytes during seizure crises (Solà et al., 1999), which appear to be transmitted as waves through the astrocytic syncytium (Cornell-Bell and Finkbeiner, 1991; Innocenti et al., 2000).

The results of our study of glial activation in the presence of irradiation show an adaptive response of cerebral tissue to GSM radiation with a SAR below the basic established limit (Royal Decree 1066/28-9-2001). The intensity of this response could vary in relation to the SAR reached in various zones of the brain, and its effects might be potentiated by the existence of cerebral electrochemical lability.

Although anatomical differences in cerebral morphology and size between rats and humans imply important differences in dosimetry, our results indicate that the c-fos protein and glia markers are activated by the combined effect of irradiation stress at non-thermal SARs and the toxic action of picrotoxin on cerebral tissues. The tendency towards electrical instability in the neural pathways of persons with epilepsy suggests that they may be especially sensitive to electromagnetic radiation. The nature of this sensitivity and the implications of these findings (in the brain tissues of picrotoxin-treated rats) for epileptic human subjects using mobile phones are not necessarily the same. However, recent studies have found a significant increase in EEG activity within the alpha, beta and gamma bands when epileptic patients were exposed to electromagnetic radiation in a controlled manner (Relova et al., 2010). In our study, neurotoxic markers found in subconvulsive models of brain tissue in rats subjected to irradiation are an expression of how exposure to mobile phone RF fields can induce (potentially reversible) changes in brains with a physiology susceptible to electrical instability. The findings of this study suggest the need for further and rigorous examination of the effects of mobile telephone RF on epileptic patients.

Conflict of interest statement

None declared.

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References

Adey WR, Bawin SM, Lawrence AF. Effects of weak amplitude modulated microwave fields on calcium efflux from awake cat cerebral cortex. Bioelectromagnetics 1982;3:295–307.
Ammari M, Gamez C, Lecomte A, Sakly M, Abdelmelek H, De Seze R. GFAP expression in the rat brain following sub-chronic exposure to 900 MHz electromagnetic field signal. Int J Radiat Biol 2010;86(5):367–75.
Amzica F, Massimini M, Manfridi A, Amzica F, Massimini M, Manfridi A. Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. J Neurosci 2002;22(3):1042–53.
Bawin SM, Kaczmarek LK, Adey WR. Effects of modulated VHF fields on the central nervous system. Ann NY Acad Sci 1975;247:74–81.
Bell-Horner CL, Dibas M, Huang RQ, Drewe JA, Dillon GH. Influence of subunit configuration on the interaction of picrotoxin-site ligands with recombinant GABA(A) receptors. Brain Res Mol Brain Res 2000;76(1):47–55.
Belyaev YI, Baeuréus CK, Terenious O, Roxstron-Lindquist K, Malmgren OGL, Sommer HW, et al. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. Bioelectromagnetics 2006;27:295–306.
Bernardi P, Cavagnaro M, Pisa S, Piuzzi E. Specific absorption rate and temperature elevation in a subject exposed in the far-field of radio-frequency sources operating in the 10–900-MHz range. IEEE Trans Biomed Eng 2003;50(3):295–304.
Blackman CF, Benane SG, Elder JA, House DE, Lampe JA, Faulk JM. Induction of calcium-ion efflux from brain tissue by radiofrequency radiation: effect of sample number and modulation frequency on the power-density window. Bioelectromagnetics 1980;1(1):35–43.
Brillaud E, Piotrowski A, de Seze R. Effect of an acute 900 MHz GSM exposure on glia in the rat brain: a time-dependent study. Toxicology 2007;238(1):23–33.
Bullitt E, Lee CL, Light AR, Willcockson H. The effect of stimulus duration on noxious-stimulus induced c-fos expression in the rodent spinal cord. Brain Res 1992;580(1–2):172–9.
Burkhardt M, Spinelli Y, Kuster N. Exposure setup to test effects of wireless communications systems on the CNS. Health Phys 1997;73(5):770–8.
Christ A, Chavannes N, Nikolski N, Gerber H-U, Pokovik K, Kuster N. A numerical and experimental comparison on human head phantoms for compliance testing of mobile telephone equipment. Bioelectromagnetics 2005;26:125–37.
Cornell-Bell AH, Finkbeiner SM. Ca^{2+} waves in astrocytes. Cell Calcium 1991;12(2–3):185–204.
de Armond SJ, Eng LF, Rubinstein LJ. The application of glial fibrillary acidic (GFA) protein immunohistochemistry in neurooncology. A progress report. Pathol Res Pract 1980;168(4):374–94.
Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, et al. Continuous exposure to 900 MHz GSM-modulated EMF alters morphological maturation of neural cells. Neurosci Lett 2009;455(3):173–7.
Dimbylow PJ, Mann SM. SAR calculations in an anatomically realistic model of the head for mobile communication transceivers at 900 MHz and 1.8 GHz. Phys Med Biol 1994;39(10):1537–53.
Dragunow M, Robertson HA. Generalized seizures induce c-fos protein(s) in mammalian neurons. Neurosci Lett 1987;82(2):157–61.
Eddleston M, Mucke L. Molecular profile of reactive astrocytes—implications for their role in neurologic disease. Neuroscience 1993;54(1):15–36.
Finne JW, Cai Z, Manavis J, Helps S, Blumbergs PC. Microglial activation as a measure of stress in mouse brains exposed acutely (60 minutes) and long-term (2 yrs) to mobile telephone radiofrequency fields. Pathology 2010;42829:151–4.
Finnie JW, Cai Z, Blumbergs PC, Manavis J, Kuchel TR. Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to global system for mobile communication microwaves. Pathology 2006;38(4):333–5.
Franke H, Kitter H. Morphological alterations of neurons and astrocytes and changes in emotional behavior in pentylenetetrazol kindled rats. Pharmacol Biochem Behav 2001;70:291–303.
Fritze K, Wiessner C, Kuster N, Sommer C, Gass P, Hermann DM, et al. Effects of GSM microwave exposure on the genomic response of the rat brain. Neuroscience 1997;81:627–39.
Hartmann K, Stief F, Draguhn A, Frahm C. Ionotropic GABA receptors with mixed pharmacological properties of $GABA_A$ and $GABA_C$ receptors. Eur J Pharmacol 2004;497:139–46.
Hayes RL, Yang K, Raghupathi R, McIntosh TK. Changes in gene expression following traumatic brain injury in the rat. J Neurotrauma 1995;12(5):779–90.

Heinemann U, Lux HD, Gutnick MJ. Extracellular free calcium and potassium during paroxismal activity in the cerebral cortex of the cat. Exp Brain Res 1977;27(3–4):237–43.
Hossmann KA, Hermann DM. Effects of electromagnetic radiation of mobile phones on the central nervous system. Bioelectromagnetics 2003;24:49–62.
Huber R, Schuderer J, Graf T, Jutz K, Borbély AA, Kuster N, et al. Radio frequency electromagnetic field exposure in humans: estimation of SAR distribution, effects on sleep and heart rate. Bioelectromagnetics 2003;24:262–76.
Huber R, Treyer V, Schuderer J, Berthold T, Buck A, Kuster N, et al. Exposure to pulse-modulated radio frequency electromagnetic fields affects regional cerebral blood flow. Eur J Neurosci 2005;21:1000–6.
Innocenti B, Parpura V, Haydon PG. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. J Neurosci 2000;20(5):1800–8.
Jeong JH, Choi KB, Choi HJ, Song HJ, Min YS, Ko SK, et al. Extremely low frequency magnetic fields modulate bicuculline-induced-convulsion in rats. Arch Pharm Res 2005;28(5):587–91.
Jorge-Mora T, Alvarez-Folgüeiras M, Leiro J, Jorge Barreiro FJ, Ares-Pena FJ, López-Martín E. Exposure to 2.45 GHz microwave radiation provokes cerebral changes in induction of HSP-90α/β heat shock protein in rat. Prog Electromagn Res PIER 2010;100:351–79.
Khurgel M, Switzer RC 3rd, Teskey GC, Spiller AE, Racine RJ, Ivy GO. Activation of astrocytes during epileptogenesis in the absence of neuronal degeneration. Neurobiol Dis 1995;2(1):23–35.
Kiessling M, Gass P. Immediate early gene expression in experimental epilepsy. Brain Pathol 1993;3(4):381–93.
Kittel A, Siklow L, Thuroczy G, Somosy Z. Qualitative enzymehistochemistry and microanalysis reveal changes in ultrastructural distribution of calcium and calcium activated ATP-ases after microwave irradiation of the medial habenula. Acta Neuropathol 1996;92:362–8.
Kovács KJ. Measurement of immediate-early gene activation—c-fos and beyond. J Neuroendocrinol 2008;20:665–72.
Lalo U, Pankratov Y, Kirchhoff F, North RA, Verkhratsky A. NMDA receptors mediate neuron-to-glia signaling in mouse cortical astrocytes. J Neurosci 2006;26(10):2673–83.
López-Martín E, Bregains J, Jorge-Barreiro FJ, Sebastian-Franco JL, Moreno-piquero E, Ares-Pena FJ. An experimental set-up for measurement of the power absorbed from 900 MHz GSM standing wave by small animals, illustrated by application to picrotoxin-treated rats. Prog Electromagn Res PIER 2008;87:149–65.
López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. J Neurosci Res 2009;87(6):1484–99.
López-Martín E, Relova-Quinteiro JL, Gallego-Gómez R, Peleteiro-Fernandez M, Jorge-Barreiro FJ, Ares-Pena FJ. GSM radiation triggers seizures and increases cerebral c-fos positivity in rats pretreated with subconvulsive doses of picrotoxin. Neurosci Lett 2006;298:139–44.
Maby E, Le Bouquin Jeannès R, Faucon G, Liégeois-Chauvel C, De Sèze R. Effects of GSM signals on auditory evoked responses. Bioelectromagnetics 2005;26(5):341–50.
Maby E, Le Bouquin Jeannès R, Liégeois-Chauvel C, Gourevitch B, Faucon G. Analysis of auditory evoked potential parameters in the presence of radiofrequency fields using a support vector machines method. Med Biol Eng Comput 2004;42(4):562–8.
Martinez M, Calvo-Torrent A, Herbert J. Mapping brain response to social stress in rodents with c-fos expression: a review 2002. Stress 2002;5(1):3–13.
Mathie A, Kennard LE, Veale EL. Neuronal ion channels and their sensitivity to extremely low frequency weak electric field effects. Radiat Prot Dosimetry 2003;106(4):311–6 (review).
Mausset-Bonnefont AL, Hirbec H, Bonnefont X, Privat A, Vignon J, de Seze R. Acute exposure to GSM 900-MHz electromagnetic fields induces glial reactivity and biochemical modifications in the rat brain. Neurobiol Dis 2004;17:445–54.
Mickley GA, Cobb BL, Mason PA, Farrell S. Disruption of a putative working memory task and selective expression of brain c-fos following microwave-induced hyperthermia. Physiol Behav 1994;55(6):1029–38.
Morgan JI, Curran T. Proto-oncogene transcription factors and epilepsy. Trends Pharmacol Sci 1991a;12(9):343–9.
Morgan JI, Curran T. Stimulus-transcription coupling in the nervous system:involvement of the inducible proto-oncogenes fos and jun. Annu Rev Neurosci 1991b;14:421–51.
Morrissey RW, Raney S, Heasley E, Rathinavelu P, Dauphinee M, Fallon JH. Iridium exposure increases c-fos expression in the mousebrain only at levels which likely result in tissue heating. Neuroscience 1999;92:1539–46.
Mortensen M, Wafford KA, Wingrove P, Ebert B. Pharmacology of GABA-A receptors exhibiting different levels of spontaneous activity. Eur J Pharmacol 2003;476:17–24.
Motte JE, da Silva Fernandes MJ, MarescauXC Nehlig A. Effects of pentylenetetrazol-induced status epilepticus on c-fos and HSP-72 immunoreactivity in the immature rat brain. Brain Res Mol Brain Res 1997;50(1–2):79–84.
Neumann-Haefelin T, Wiessner C, Vogel P, Back T, Hossmann KA. Differential expression of the immediate early genes c-fos, c-jun, junB, and NGFI-B in the rat brain following transient forebrain ischemia. J Cereb Blood Flow Metab 1994;14(2):206–16.
Nutt DJ, Cowen PJ, Batts CC, Grahame-Smith DG, Green AR. Repeated administration of subconvulsant doses of GABA antagonist drugs. I. Effect on seizure threshold (kindling). Psychopharmacology 1982;76:84–7.
O'Callaghan JP, Millar DBI. Nervous system-specific proteins as biochemical indicators of neurotoxicity. Trends Pharmacol Sci 1983;4:388–90.
Paulraj R, Behari J, Rao AR. Effect of amplitude modulated RF radiation on calcium ion efflux and ODC activity in chronically exposed rat brain. Indian J Biochem Biophys 1999;36:337–40.
Paulraj R, Behari J. Radio frequency radiation effects on protein-kinase C activit in rats' brain. Mutat Res/Fund Mol Mech Mutagen 2004;545:127–33 2004.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1986.
Persinger MA, Belanger-Chellew G. Facilitation of seizures in limbic epileptic rats by complex 1 microTesla magnetic fields. Percept Mot Skills 1999;89(2):486–92.
Popovici T, Represa A, Crepel V, Barbin G, Beaudoin M, Ben-Ari Y. Effects of kainic acid-induced seizures and ischemia on c-fos-like proteins in rat brain. Brain Res 1990;536(1–2):183–94.
Relova JL, Peleteiro M, Pertega S, Vila JA, López Martín E, Ares F. Changes in the electroencephalographic spectrum of epileptic patients induced by mobile phone. In: Proceedings of Bioelectromagnetics 2005: a joint meeting of the Bioelectromagnetics Society and European Bioelectromagnetics Association. Dublin, Ireland; 2005;391–4.
Relova JL, Peleteiro M, Pertega S, Vila JA, López Martín E, Ares F. Effects of cell-phone radiation on electroencephalographic spectra of epileptic patients. IEEE Antennas Propag Mag 2010;52(6):173–9.
Royal Decree 1066/28-9-2001. 1999:36217–27.
Schmid & Partner Engineering AG. Reference manual for the SEMCAD simulation platform for electromagnetic compatibility, antenna design and dosimetry. 2009 Available from: http://www.semcad.com.
Semyanov A, Kullmann DM. Relative picrotoxin insensitivity distinguishes ionotropic GABA receptor-mediated IPSCs in hippocampal interneurons. Neuropharmacology 2002;43:726–36.
Solà C, Barrón S, Tusell JM, Serratosa J. The Ca^{2+}/calmodulin signaling system in the neural response to excitability. Involvement of neuronal and glial cells. Prog Neurobiol 1999;58(3):207–32 (review).
Stewart SW. Mobile phones and health, radiofrequency fields from mobile phone technology. Chapter 4, Independent Expert Group on Mobile Phone. 2000.
Sung JH, Jeong JH, Kim JS, Choi TS, Park JH, Kang HY, et al. The influences of extremely low frequency magnetic fields on drug-induced convulsion in mouse. Arch Pharm Res 2003;26(6):487–92.
Tashiro A, Goldberg J, Yuste R. Calcium oscillations in neocortical astrocytes under epileptiform conditions. J Neurobiol 2002;50(1):45–55.
Thompson MA, Ginty DD, Bonni A, Greenberg ME. L-type voltage-sensitive Ca^{2+} channel activation regulates c-fos transcription at multiple levels. J Biol Chem 1995;270(9):4224–35.
Thorlin T, Rouquette JM, Hamnerius Y, Hansson E, Persson M, Björklund U, et al. Exposure of cultured astroglial and microglial brain cells to 900 MHz microwave radiation. Radiat Res 2006;166(2):409–21.
Torre ER, Lothman E, Steward O. Glial response to neuronal activity: GFAP-mRNA and protein levels are transiently increased in the hippocampus after seizures. Brain Res 1993;631(2):256–64.
Trastoy-Rios A, Lopez-Martin E, Jorge-Barreiro FJ, Sebastián-Franco JL, Ribas Ozonas B, Moreno-Piquero E, et al. Experimental setup for exposures to a 900 MHz-GSM radiation: application to small animals. In: Proceedings of international conference on Electromagnetic Fields, Health and Environment – EHE06 in Madeira Island (Portugal). 2006;119–23.
Willoughby JO, Mackenzi L, Medvedev A, Hiscock J. Distribution of fos-positive neurons in cortical and subcortical structures after picrotoxin induced convulsions varies with seizure type. Brain Res 1995;683:73–87.
Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett 2007;412:34–8.