Final Report:
Effect of GSM-1800 and UMTS exposure on radical stress in the rat brain

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Introduction

Radical stress (oxygen and nitrogen free radicals) is known to contribute to the alteration of cells and their membranes. This phenomenon is known to be involved in ageing and a number of neuro-degenerative diseases, such as Alzheimer disease or amyotrophic lateral sclerosis.

In the last few years, some authors have suggested that radiofrequency radiation could play a role in the generation of radical stress in cells or animals, but possible bias in the exposure scheme have been identified. However, there is no clear evidence to date that such a phenomenon occurs in the brain.

The objective of the present project was to investigate whether radiofrequency fields linked to mobile telephony, i.e. GSM-1800 and UMTS signals, can induce radical stress in the rat brain.

In the present project, biological samples had already been exposed and were ready to use for staining. However, a description of the whole experiment, i.e. animal exposure and samples preparation is described bellow.

Exposure Systems

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Setup

Exposure was done using the RFR head-only exposure system available in our group, i.e. the loop antenna (Figure 1). The characterisation of the SAR absorbed in the brain (BASAR[1]) had been performed at 900 MHz[2]. As part of the project, the same approach was used at 1800 (GSM-1800) and 1960 MHz (UMTS) in collaboration with Dr Philippe Lévêque (XLIM, University of Limoges, France) using experimental measurements and numerical simulations (FDTD). The Finite Difference Time Domain (FDTD) method was used to calculate the SAR distribution. This method is particularly well adapted to this objective and has become a very powerful and popular tool in bioelectromagnetic studies. FDTD has been often used to analyze and design exposure setups for *in vitro* and *in vivo* biological experiments[3,4,5].

Since the computational volume is limited, Absorbing Boundary Conditions (ABC) are needed to simulate free space and avoid spurious reflections from the edges. Large efforts have been carried out to improve the ABC used in FDTD. In this study, the Perfectly Matched Layer (PML) ABC were used[6,7]; these ABC limit to less than 40 dB the spurious reflections induced by the restriction of the computational domain.

[1] Brain-averaged SAR
[2] Lévêque, et al. (2004).
[3] Burkhardt, et al. (1996).
[4] Laval et al. (2000).
[5] Watanabe, et al. (1996).
[6] Gedney (1996).
[7] Bérenger (1994).

In this project, the local BASAR level of 2.0 W/kg was used. This level was chosen to be relevant to human exposure to mobile telephones, but since the head of the rat (50 g) is much smaller than that of humans, for which the usual definition of the local SAR is that averaged over 10 g (ICNIRP), the local SAR in the heads of rats had been defined in our laboratory as the BASAR.

BASAR determination

Temperature measurements were done using a gel phantom with a Vitek probe and gave 6.00±0.55 W/kg/W at 1.3 cm from the inner side of the rocket directly below the loop.
At the location of these measurements the FDTD calculations gave 8.2±0.3 W/kg/W and 10.9±0.3 W/kg/W for a homogeneous numerical phantom at 1800 and 1960 MHz, respectively. Calculation of the BASAR using the 7-tissue phantom gave 11.5 and 14.3 W/kg/W at 1800 and 1960 MHz, respectively (Figure 2). If the BASAR is considered as the "local" SAR level, then the level set in the exposures was 2.6 W/kg.

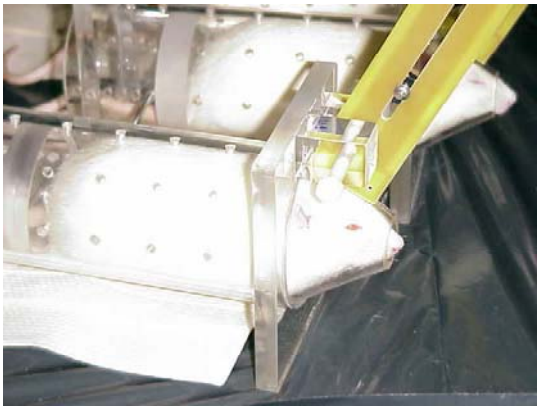

Figure 1: Rat exposure to GSM-1800 or UMTS. Rats are restrained within the rocket and the loop antennas operate at 1800 or 1960 MHz

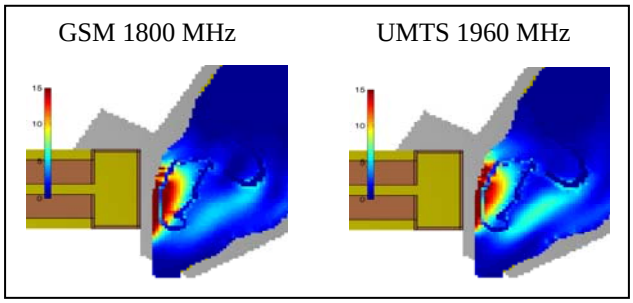

Figure 2: Schematic view of the exposure system with the loop antenna at 1800 and 1960 MHz, set for FDTD calculations with the 7-tissue numerical phantom in place in the rocket.

Biological parameters

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Study design

Animals

Six- to ten-week-old rats (young adults) and seventeen-month-old (elderly) male Wistar-Han rats were purchased from Janvier (Le Genest Saint Isle, 53940 France).

They were housed under controlled temperature (22°C) and lighting conditions (monitored light-dark cycles 08:00-20:00), and supplied with water and food (UAR 04, Safe, France) *ad libitum.*
Animals were kept for one week in the animal facility before starting any experimental procedure.
Cages were cleaned twice a week. Bedding during all the experimental process was softwood. All national regulations were implemented in housing and handling of animals.

Training of animals

Care was taken to avoid stressing the animals. Therefore, upon acclimation period, rats were randomly distributed in each experimental group and progressively trained over one week (0.5, 1, 1.5, and 2 hours/day) to the rocket-type exposure setup. Then rats were enrolled in single and repeated exposures to GSM-1800 and UMTS.

Exposure protocol

Rats were randomly divided into 5 groups: sham-exposed group, and 4 groups exposed to the GSM-1800 or UMTS mobile telephony-related signal using either a single 2-hour exposure or a repetitive scheme of 2 hours per day, 5 days per week, during 4 weeks. Two SAR levels were used: 0.0 W/kg (sham) and 2.6 W/kg. Each experimental group consisted in a total of 8 rats.

Controls

As mentioned above, the sham-exposed group was used as a control for handling and restraining in a rocket (0 W/kg). Other control groups were cage controls and positive controls.
Cage control rats stayed in the animal facilities during the whole experiment (8 rats / group).
Different treatments have been used as positive controls (cerebral intraventricular LPS, cerebral intraventricular Quinolinic acid, subcutaneous Kainic acid, intravenous LPS, subcutaneous Pilocarpine, two venous occlusion ischemia). The search for positive controls needed more time than expected as treatments gave very disperse results and we wanted to find the more reproducible treatments for each parameter. The treatment selected as positive control for the identification of the presence of 4-HNE was an intravenous injection of LPS (E. Coli O55:B5, 10 mg/kg, sacrifice 8 hours after injection, n=8).
For the two other markers, a great effort was made to find a valid positive control; it appeared that the treatment protocol is highly critical for a significant different staining to be observed and reproducibility is hard to achieve given the high toxicity of these treatments. Almost 80% of the animals did not survive long enough (the time needed is usually 24 hours after the treatment). However, given the delay in the programme already, we decided to give here preliminary data, although in each case, we had only one animal per group. We will finalise this part of the work in the coming submitted manuscripts. The indications we have now from this large screening is that (i) pilocarpine injection (400 mg /kg, sacrifice 3-6 hours after *status epilepticus*) was found effective for the induction of 3-Nitrotyrosine (n=1, to be completed) and (ii) ischemia by two vessels occlusion (30 minutes) and reperfusion (24 hours) was found an effective inducer of 8-oxo-dG (n=1, to be completed).
The brains of positive control rats were handled as described bellow.

Samples

Rats were ethically sacrificed: At the time of sacrifice, rats were euthanized using isoflurane inhalation. Rat brains were fixed using intracardiac perfusion with phosphate buffer (0.1 M) followed by 4% paraformaldehyde. Brains were kept in the fixative solution overnight at 4°C, then cryo-preserved in 20 % sucrose in phosphate buffer for 48 hours at 4°C and frozen to –80°C using isopentane. To ensure blinding of the experiments, brains were coded before slicing and analysis.

Serial 10-µm brain sections were prepared from 3 brain zones named Z1 (bregma –0.30 to –0.80 mm), Z2 (bregma –3.30 to –4.50 mm), and Z3 (bregma –7.30 to –8.00 mm) for frontal, median and posterior zones, respectively (Figure 3).

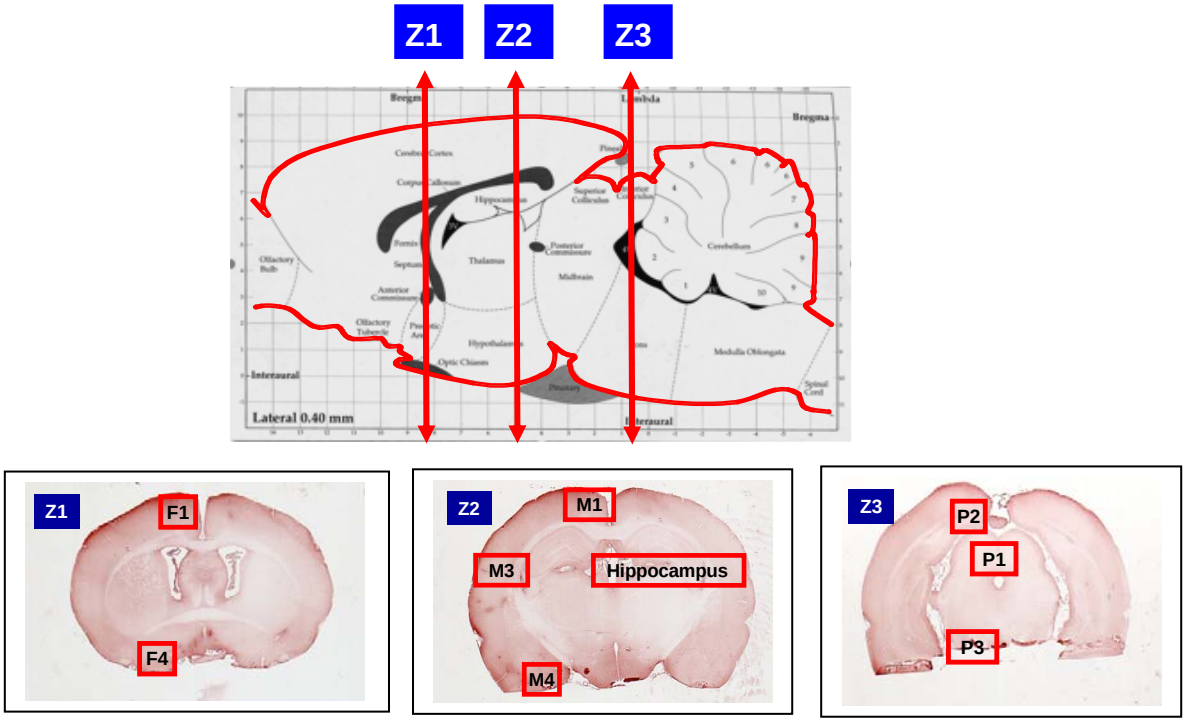

Figure 3: Rat brain zones and regions considered in the study. For details see text.

Different regions in the brain, for a total of 12 regions, were then considered in each slice. The regions selected were:

- In Z1: the motor cortex (F1) and the medial forebrain bundle (F4),
- In Z2: the retrosplenial cortex (M1); the auditory cortex (M3), the cortical amygdaloid nucleus (M4), and the hippocampus with the Cornu Ammonis field 1 (CA1), the Cornu Ammonis field 2 (CA2), the Cornu Ammonis field 3 (CA3), the Cornu Ammonis field 4 (CA4), and the Dentate Gyrus (DG)
- in Z3: the dorsomedial periaqueducal gray (P1), the retrosplenial cortex (P2), and the Pontine nuclei (P3)

Protocols

Three markers were used to investigate radical stress in rat brain by immuno-histochemistry: anti 4-HNE antibody for revealing lipid peroxydation, anti 3-

Nitrotyrosine antibody for protein nitration, and anti 8-oxo-dG antibody for DNA oxidation.

Lipid peroxidation: 4-hydroxynonenal (4-HNE) staining

Lipid peroxidation is detected using mouse anti-4-HNE antibodies (HNEJ-2, 1/100°, overnight, 4°C, GENTAUR®) and FITC-labelled second antibodies (FITC-antimouse 1/250°, 1 hour, room temperature, SIGMA®).
Coverslips were mounted on slides before microscopy observation. In each brain region of interest, one X400 representative microscopic photograph was taken using a camera on a microscope (Zeiss). From these photographs, analysis was performed on the brain regions selected using visual quantification. 4-HNE staining intensity was scored as following, based on fluorescence intensity and area (Figure 4): score 1: no staining; score 2: between 1 and 10% of maximal staining; score 3: between 10 and 30%; score 4: between 30 and 50%; score 5: between 50 and 80%; score 6: between 80 and 100% of maximal staining.
Scores were averaged of two slices per sample. Then, the analysis was performed, in each group, in each sub-region and on the total score for the cortex, the hippocampus and the brain. Thus, the maximal score to be expected was “48” for the cortex, “30” for the hippocampus and “78” for the brain.
In some cases, some slices were lost during staining or some hippocampal sub-regions were non-analysable, so that some groups have less than 8 samples (n<8).

Figure 4 : Representative images and their corresponding 4-HNE scores based on fluorescence intensity and area (score 1, 4, and 6) in the rat cortex.

Protein Nitration: 3-Nitrotyrosine

Protein oxidation was detected using rat anti-3NT antibodies (1/500°, 1 hour, room temperature, GémacBio®) and avidin-biotin-peroxidase antirabbit second antibodies (VECTASTAIN ABC kit, 1 hour, room temperature, VECTOR®). The presence of 3-NT was revealed using DAB-Nickel (peroxidase substrate kit, VECTOR®). Coverslips were mounted on slides before microscopy observation. In each brain region of

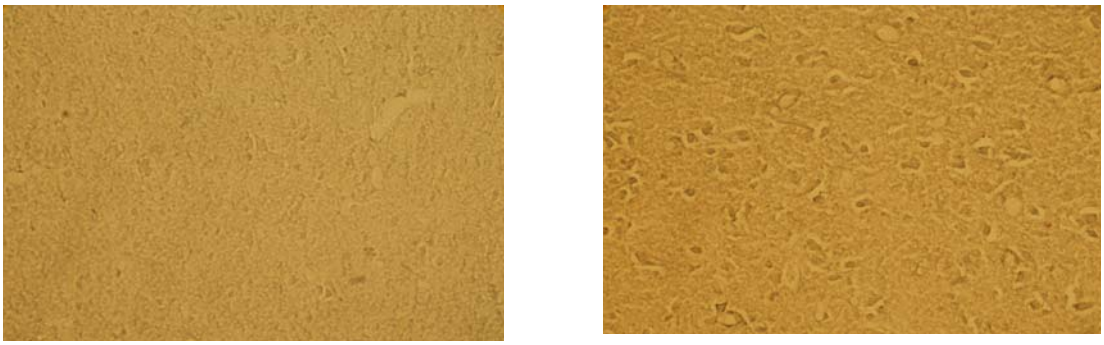

Figure 5: Representative images of 3-NT negative (left) and positive (right) rat brains. Motor cortex is shown here. 3-NT positive cells appear brown.

interest, one X400 representative microscopic photograph was taken using a camera on a microscope (Zeiss). From these photographs, analysis was performed on the brain regions selected using the Aphelion image analysis software. 3-NT staining intensity was scored automatically based on mean intensity and area (Figure 5). Analysis was then performed on the whole brain, on the superior part of the brain, i.e. cortex (F1+M1+P2), on the hippocampus (HC=CA1+CA2+CA3+CA4+DG), on the midbrain (M3+HC+P1) and the inferior part of the brain (F4+M4+P3).

DNA oxidation: 8-Oxo deoxyGuanine (8-oxodG)

DNA oxidation is detected using mouse anti-8-oxo-dG antibodies (1/25, overnight, 4°C, GENTAUR®) and biotinylated antimouse second antibodies (LABS2 system HRP kit, 10 minutes, room temperature, Dakocytomation®). The presence of 8-oxodG is revealed using VECTASTAIN ABC kit, VECTOR®) and DAB-Nickel (peroxidase substrate kit, VECTOR®). Coverslips were mounted on slides before microscopy observation. In each brain region of interest, one X400 representative microscopic photograph was taken using a camera on a microscope (Zeiss). From these photographs, analysis was performed on the brain regions selected using the Aphelion image analysis software. 8-oxo-dG staining intensity was scored automatically based on mean intensity and area (Figure 6). Analysis was then performed on the whole brain, on the superior part of the brain, i.e. cortex (F1+M1+P2), on the hippocampus (HC=CA1+CA2+CA3+CA4+DG), on the midbrain (M3+HC+P1) and the inferior part of the brain (F4+M4+P3).

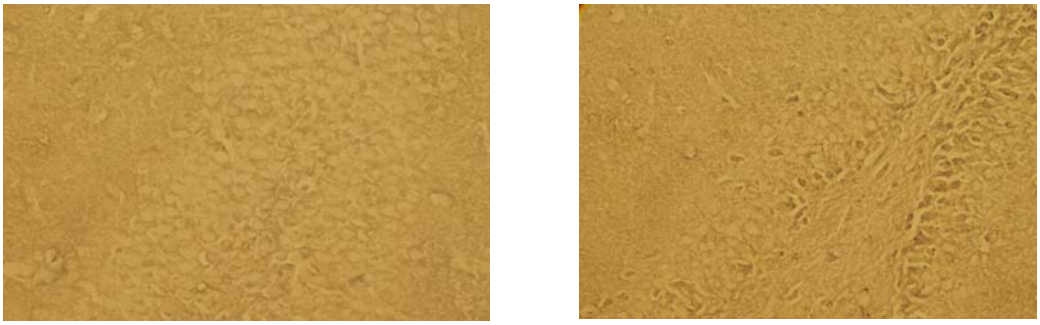

Figure 6 : Representative images of 8-oxodG negative (left) and positive (right) rat brains. The Dentate Gyrus of the hippocampus is shown. 8-oxodG positive nuclei appear brown.

Statistics

Statistical comparison between sham- and exposed groups used the Kruskall-Wallis test. Statistical comparison between Cage- and positive controls used the Mann and Whitney test. $P < 0.05$ was considered as significant. Outliers were excluded using the outlier calculator from Graphpad software (http://www.graphpad.com).

Results

Lipid peroxidation

Significant lipid-peroxidation was observed in the whole-brain of positive-control rats (score 42.4) as compared to cage-control animals (score 31.8) ($p < 0.05$). The maximum score was obtained in some regions only.
Figure 7 shows the 4-HNE scores obtained in sham-exposed and RF-exposed animals. The 4-HNE score was found similar in sham-exposed (score 36.7) versus cage control rats ($p=0.43$).
As compared to sham-exposure, a single exposure to either signal did not influence background lipid peroxidation (medium level) in the brains of the young-adult rats. After repeated exposure to GSM-1800, no effect was observed on lipid peroxidation, while repeated exposures to UMTS were found to significantly decrease lipid peroxidation in the rat brains as compared to sham-exposed rats (score 23.9, $p < 0.001$). The decrease was seen in every single brain region of UMTS-exposed rats (data not shown).
In elderly rats, no significant difference could be seen in the brains after either a single or a repeated exposure to GSM-1800 and UMTS as compared to sham-exposed rats. For details, see Annex 1.

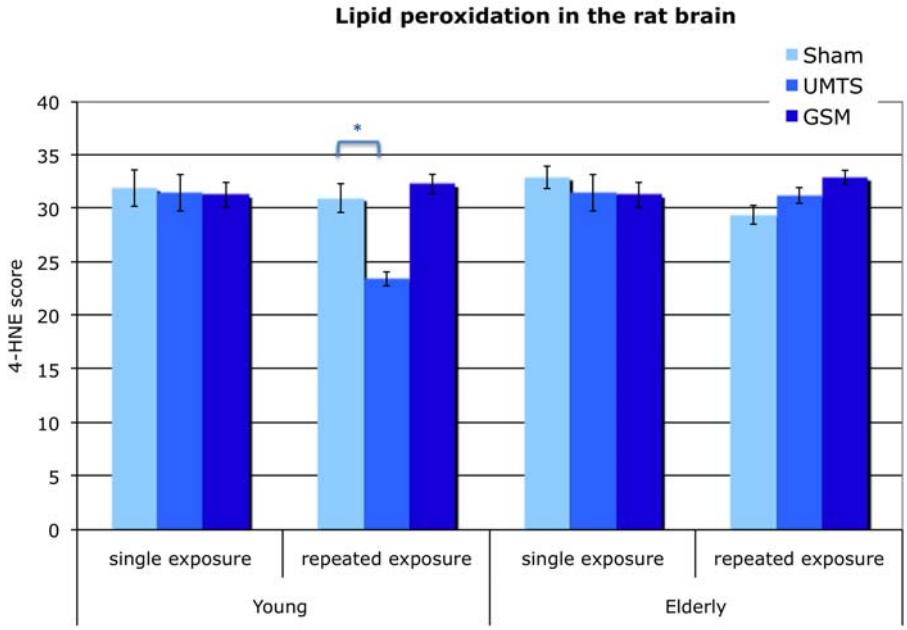

Figure 7 : 4-HNE score in the brains of rats sham-exposed and exposed to GSM-1800 or UMTS. Young : 12 week-old rats ; elderly : 17 month-old rats, single = single 2-hour exposure, repeated =2 h/d, 5 d/w, 4 w exposure. n =7-8 rats/group. p<0.001, UMTS vs Sham, Kruskall-Wallis test.

Protein nitration: 3-Nitrotyrosine

Protein nitration was found similar in cage control and sham-exposed rats in any condition. For instance, the mean staining intensity (arbitrary units, AU) was 1071 ± 167 AU and 1135 ± 205 AU, respectively in young animals and 880 ± 282 AU and 696 ± 97 AU, respectively in elderly rats (single exposure-equivalent). No difference was seen either in the protein oxidation in young and elderly rat brains.

Figure 8 shows the averaged 3-NT staining intensity obtained in the brain of sham-exposed and RF-exposed animals. As compared to sham-exposure, neither GSM-1800 exposure nor UMTS were found able to alter protein oxidation in young and elderly rats, and we found no differences in either zone of the brain. By contrast, we found that pilocarpine was able to increase protein nitration locally in the brain of a 12-week-old rat (Z2, 1136 AU) as compared to 12-week-old cage-control animals (Z2, 789.4±155.5 AU). More data are needed with pilocarpine injections in order to perform statistical analysis.

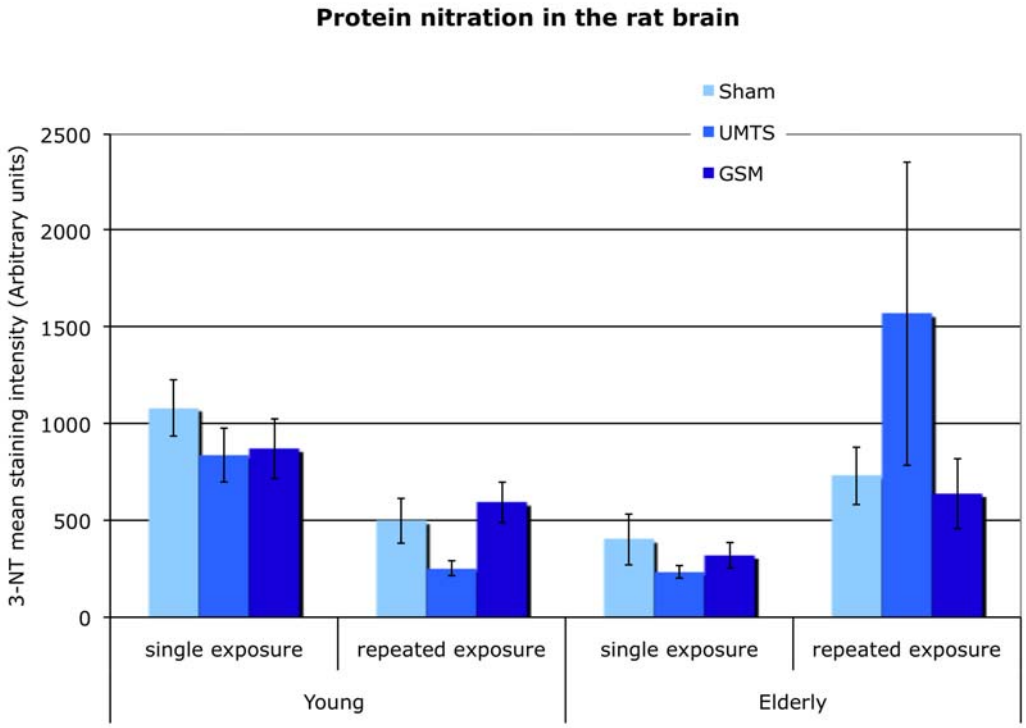

Figure 8 : 3-NT mean staining intensity (Arbitrary units) in the brains of rats sham-exposed and exposed to GSM-1800 or UMTS. Young : 12 week-old rats ; elderly : 17 month-old rats, single exposure = single 2-hour exposure, repeated exposure =2 h/d, 5 d/w, 4 w exposure.

DNA oxidation: 8-Oxo deoxyGuanine (8-oxodG)

No difference was seen in DNA oxidation between cage control and sham-exposed rats in any condition. For instance, the 8-oxodG mean-staining intensity (arbitrary units) was 7489 ± 1679 and 8461 ± 1242, respectively in young animals and 12053 ± 2744 and 13170 ± 2119, respectively in elderly rats (single exposure-equivalent). No difference could be seen either in the DNA oxidation in young and elderly rat brains.
By contrast, we found that ischemia was able to increase DNA-oxidation in the whole-brain of a 12 week-old rat (17506 AU) as compared to 12 week-old cage-control animals (7489 ± 1679 AU). More data are needed with ischemia in order to perform a statistical analysis.

Figure 9 shows the 8-oxodG staining intensity obtained in the hippocampus of sham-exposed and RF-exposed animals. As compared to sham-exposure, a single

exposure to either signal did not influence background DNA oxidation in the brains of the young-adult rats. After repeated exposure to RF, a significant difference between groups was found in the rat hippocampus ($p<0.001$, Kruskall Wallis) and the post-test revealed that repeated exposures to UMTS significantly decreased DNA oxidation as compared to sham-exposed rats (2678 ± 532 AU versus 24378 ± 6049 AU, $p<0.05$). The increase in DNA oxidation observed after GSM-1800 exposure was not found significant as compared to sham-exposure (95920 ± 24212 AU).

In elderly rats, no significant difference in DNA oxidation was seen in any brain zones after either a single or a repeated exposure to GSM-1800 and UMTS as compared to sham-exposed rats.

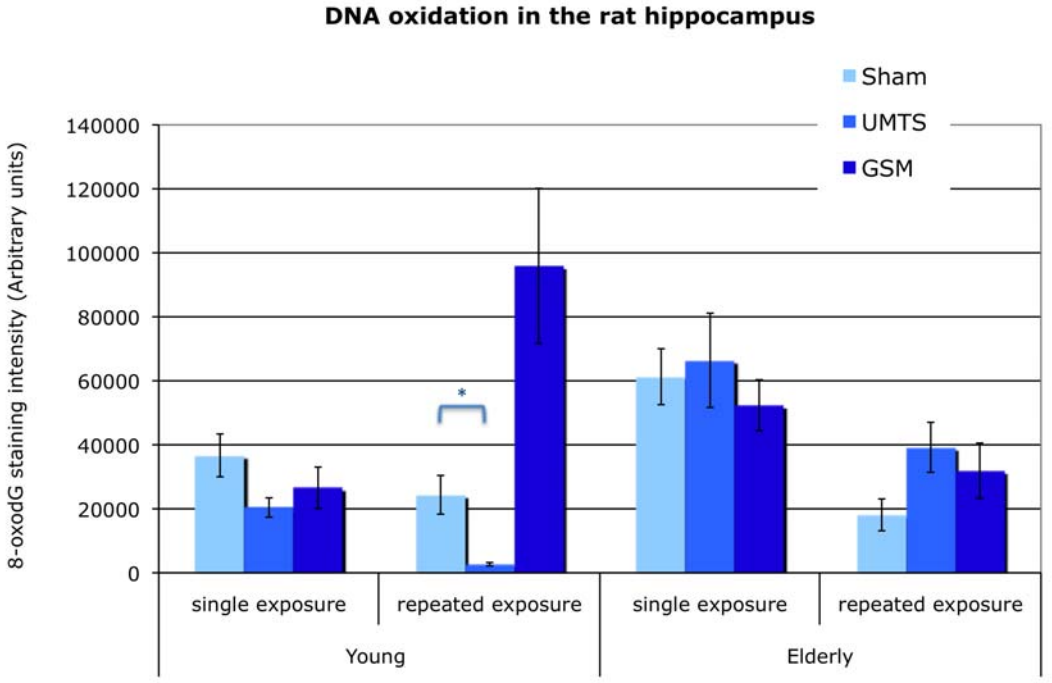

Figure 9 : 8-oxodG staining intensity (Arbitrary units) in the hippocampus of rats sham-exposed and exposed to GSM-1800 or UMTS. Young : 12 week-old rats ; elderly : 17 month-old rats, single exposure = single 2-hour exposure, repeated exposure =2 h/d, 5 d/w, 4 w exposure.

Discussion

Radical stress (oxidative and nitrogen free radicals) is known to contribute to the alteration of cells and their membranes. This phenomenon is for instance involved in ageing and a number of neurodegenerative diseases, such as the Alzheimer disease or amyotrophic lateral sclerosis (Reynolds et al., 2007).

In the last few years, some papers have suggested that radiofrequency radiation could play a role in the generation of radical stress in cells or animals, but there is no robust evidence of this phenomenon. In vitro, the Polish group of Stopczyk et al. (2002, 2005) found decreased SOD1 activity and correlated increase in MDA in human blood platelets exposed to GSM-900 (SAR unknown, Abstract only available in English). More recently, the group of Simko examined the effect of 1800 MHz RFR on Reactive Oxygen Species. No significant differences in free radical production were detected after RFR exposure and no additional effects on superoxide radical anion production were detected after co-exposure to RFR + phorbol ester or RFR + LipoPolySaccharides in human Mono Mac 6 and K562 cells (Lantow et al., 2006a) or in human umbilical cord blood-derived monocytes and lymphocytes (Lantow et al., 2006b) when exposed to continuous wave or different GSM signals (GSM-DTX and GSM-Talk) up to 2 W/kg for 30 or 45 minutes.

In vivo, contradictory data were reported on radical stress. Also to mention is the fact that most studies investigating radical stress *in vivo* used GSM-900 RFR and genuine mobile phones as exposure set-up. MDA (malondialdehyde) level was found to increase significantly with a reduction in GSH (reduced glutathione) concentration and an increase in SOD (superoxide dismutase) activity in the blood of rats exposed to 945 MHz at 3.67 W/m^2 or SAR of 11.3 mW/kg (Yurekli et al., 2006). The Turkish group of Ozguner repeatedly found radical stress in different organs (retina, kidney, heart, liver, nasal mucosa) of rats exposed to 900 MHz RFR (30 minutes/day, 10 days to 3 months) at 0.016 W/kg whole-body SAR. In these studies, the rats were restrained in a plastic tube while habituation to theses tubes is never mentioned in the papers (Ozguner et al., 2004, 2005a, 2005b, 2005c, 2006; Oktem et al., 2005).

Regarding radical stress in the animal brains, Köylü et al. (2006), from the Ozguner group, found that exposure at a brain-averaged SAR of 4 W/kg increased lipid peroxidation in the rat cortex and hippocampus. Exposure lasted 30 minutes / day, 5 days / week for 2 weeks. Lipid peroxidation was also detected in the rat brain after 1 hour of exposure to 2450 MHz RFR at 6 mW/cm^2 (Aweda, 2003), or after exposure to RFR 1 hour/day for 7 days at peak brain SAR of 2 W/kg (Ilhan et al., 2004). In this last case, exposure was “head-mainly” using genuine mobile phones and it cannot be ruled out that the effects seen on radical stress in the rat brains were due to stress as there is no mention of habituation to the rocket. Meral et al. (2007) detected an increase in lipoperoxidation and a decreased activity of GSH and catalase in the brains of guinea pig exposed to a genuine GSM-900 mobile phone at 0.95 W/kg 12 hours/day for 30 days.

By contrast, Irmak et al. (2002) showed that local exposure to genuine GSM-900 mobile phone (30 minutes/day for 7 days) at 0.02 mW/cm² (SAR unknown) had no significant effect on the antioxidant enzyme level in the brains of rabbits. Ferreira et al. (2006) found no alteration in non-enzymatic antioxidant defense and lipid and protein oxidative damage in the rat frontal cortex and hippocampus after whole-body exposure to 834 MHz RFR from genuine mobile phone, 7.5 hours/day for 7 days. Rats were aged 1 or 2.5 months and whole-body SAR varied from 0.36 to 0.98 W/kg.

In the present study, we observed no effects of a GSM-1800 signal (2.6 W/Kg

BASAR) on radical stress in the brains of young and elderly rats. Although an increase in DNA oxidation was found after repeated exposure in the brain of young animals, it was not statistically significant.
By contrast, repeated exposures to UMTS (2.6 W/kg BASAR) were able to significantly decrease radical stress by decreasing lipid peroxidation in every single brain zone tested and DNA oxidation in the hippocampus of young rats.

Discrepancy with part of the available literature may be due to different exposure pattern as the loop antenna partially exposed the rat head by contrast to mobile phones that exposed the whole animal. The frequencies used here are also higher, leading to more superficial RFR absorption.
4-hydroxynonenal (4-HNE) is a neurotoxic by-product of lipid peroxidation of arachidonic acid, and has become of interest in a variety of neurological diseases involving oxidative stress (Williams et al., 2006). For instance, it exists in increased concentrations in Alzheimer's disease (AD) patients and is found in amyloid β peptide (Aβ) plaques associated with AD. It was recently shown that 4-HNE covalently modifies Aβ, triggering its aggregation, which may be important in the pathogenesis of AD (Siegel et al., 2007).
8-oxo-deoxyguanosine (8-oxo-dG) is a known mutagen and a possible correlation exists between its accumulation and pathological processes like cancer, degenerative diseases and aging (Dorszewska et al., 2007 ; Souza-Pinto et al., 1999).
Therefore, the decrease in lipid peroxidation and DNA oxidation observed after repeated exposure to UMTS in the brains of young rats is suggestive of a beneficial effect. Of interest is the finding, in the same animals, that repeated exposures to UMTS were able to increase Hsp70 expression in the hippocampus of young rats, as well as microglial activation, while GSM-1800 did not (Laclau et al., BEMS2006). Hsp70 expression is known as a neuroprotector in the brain (Calabrese et al., 2006). Both effects are thus likely to be correlated.
However, the underlying mechanisms are unknown at this stage, given the SAR level (non thermal level) and the absence of effect of GSM-1800 at similar frequency and SAR level.

In the present study, we found no statistical difference in the radical stress in elderly rats as compared to their young counterparts. Young rats were 3-months old and old animals were 16-17 months old. Although it is generally reported that radical stress increases with age, Hamilton et al. (2001) showed that this increase was significant between rats or mice aged of 24 months and their 18- and 6-months old counterparts. The increase was not significant for the 18- as compared to the 6-months old animals. Thus, our data are in agreement with this study and even suggest that exposure to mobile phone- related RFR signals does not accelerate ageing in rats.

In summary, we tested single and repeated exposures to GSM-1800 and UMTS on radical stress in the brains of young adult and elderly rats. We showed no effects of a single exposure in both rat populations. Repeated exposures to GSM-1800 did not alter radical stress in the rat brains. We found that repeated exposure to UMTS decreased oxidative stress (lipoperoxidation and DNA oxidation) in the brains of young adult rats, while no effects could be seen in those of elderly rats.

We found no evidence that exposure to GSM-1800 and UMTS could lead to deleterious effects through radical stress induction.

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Dissemination

The Swiss Research Foundation on Mobile Communication was acknowledged in the following scientific contributions (posters)

Lagroye I, Haro E, Hurtier A, Billaudel B, Taxile M, Ladevèze E, Athané A, Veyret B. Effects of mobile telephony signals exposure on radical stress in the rat brain. *Poster submitted at the thirsty Annual Technical Meeting of the Bioelectromagnetics Society, 2008.*

Lagroye I., Haro E., Ladevèze E., Madelon C., Billaudel B., Taxile M., Veyret B. Effects of mobile telephony signals exposure on radical stress in the rat brain. Twenty-nine Annual Technical Meeting of the Bioelectromagnetics Society, Kanazawa, Japan (June 2007);

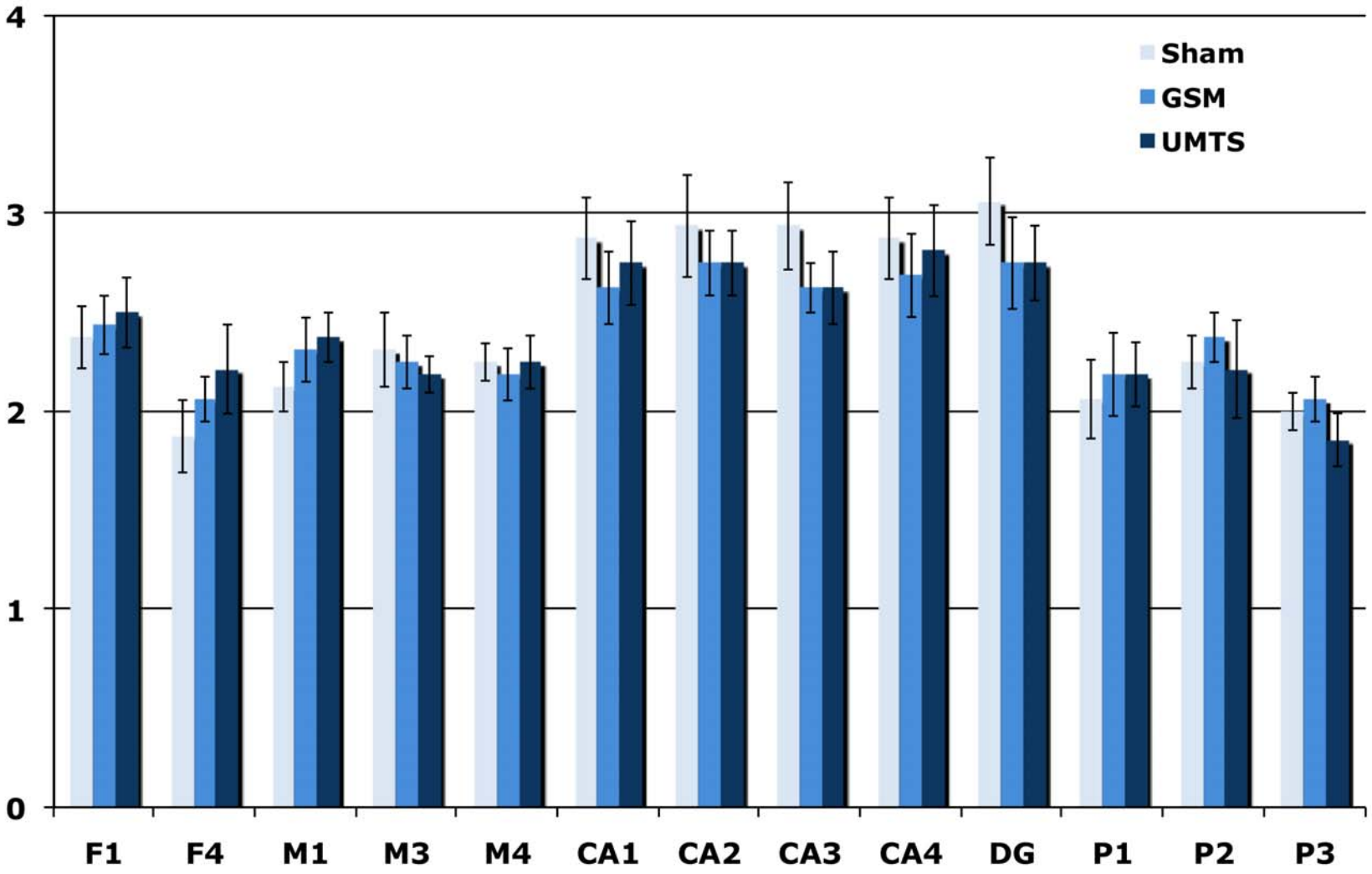
Lagroye I., Haro E., Ladevèze E., Billaudel B., Taxile M., Veyret B. Effects of GSM-1800 exposure on radical stress in rat brain. 8th International Congress of the European BioElectromagnetics Association, Bordeaux, France (April 2007).

ANNEXES

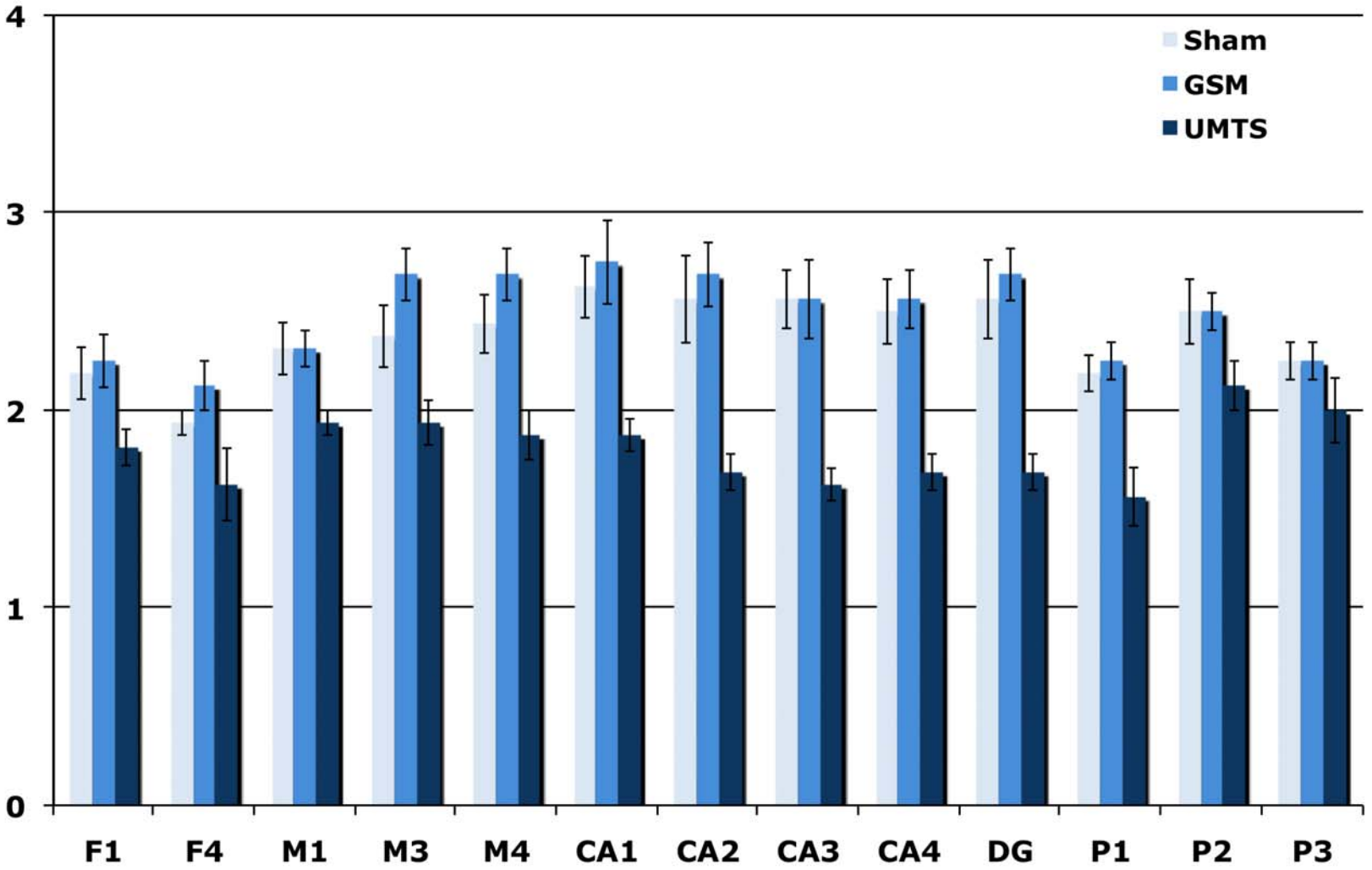
Annex 1 : Effects of GSM-1800 and UMTS on lipid peroxidation

Annex 2 : Effects of GSM-1800 and UMTS on protein nitration

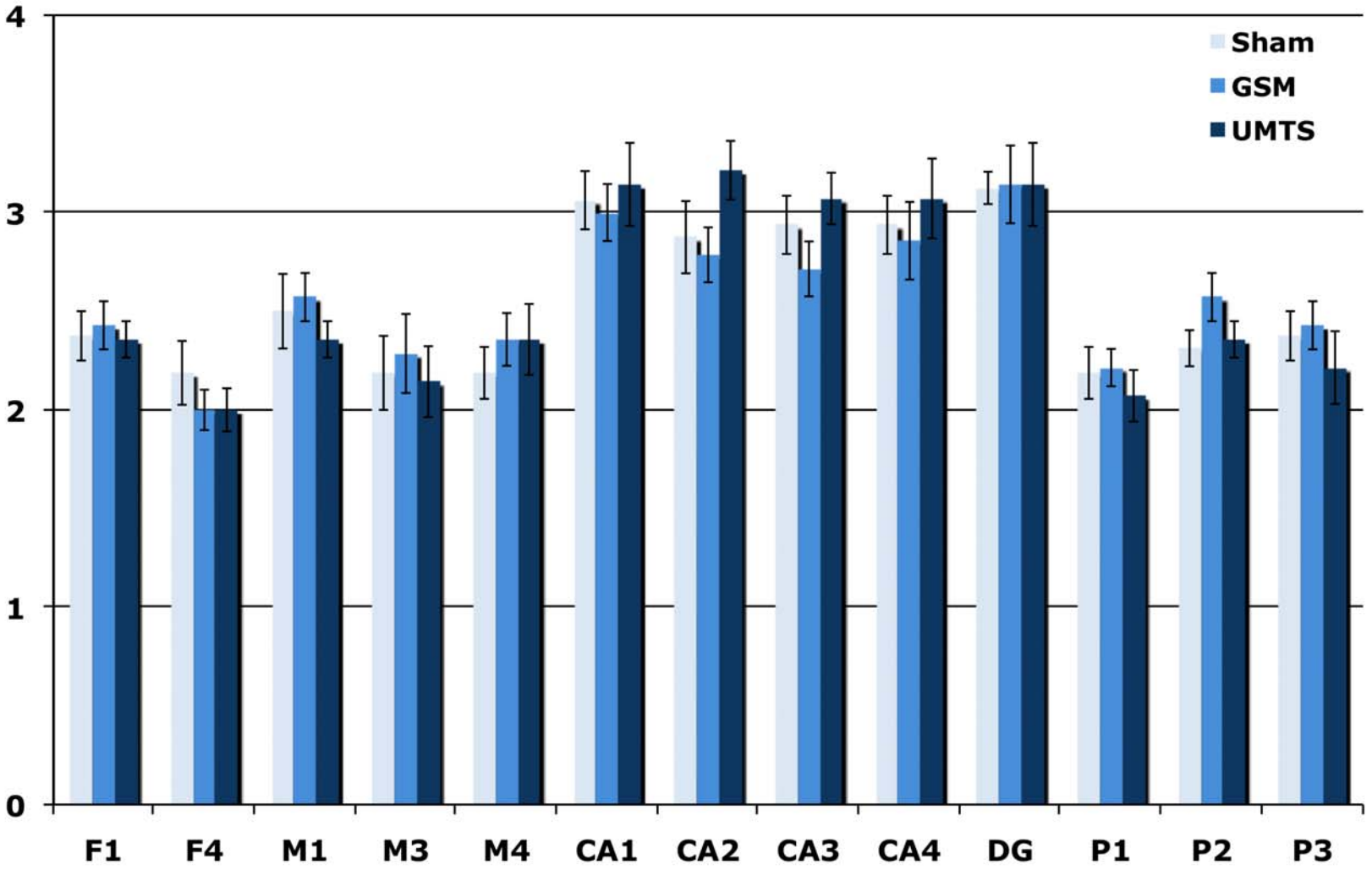
Annex 3 : Effects of GSM-1800 and UMTS on DNA oxidation



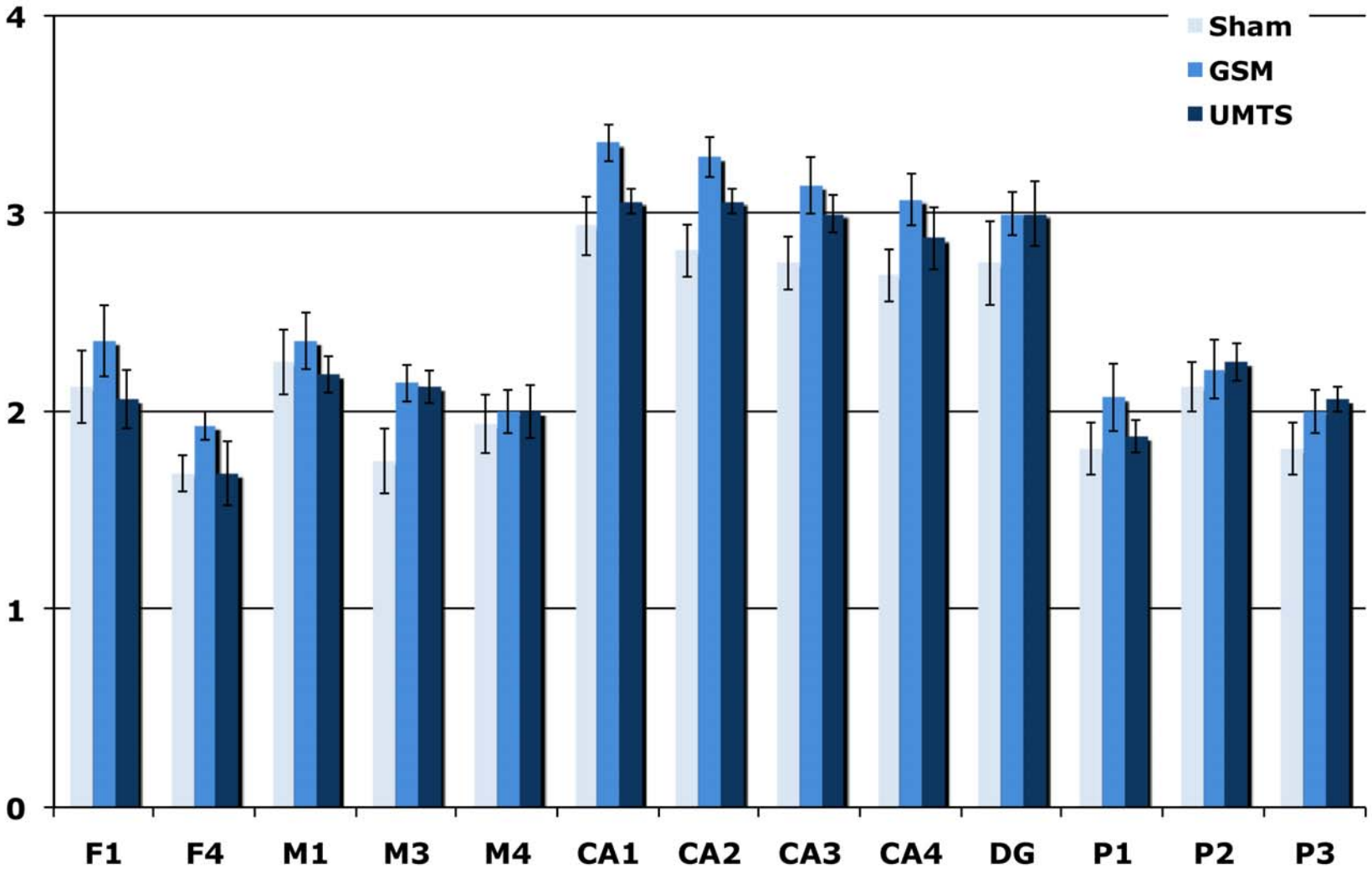
Annex 1a : Effects of a single 2-hour UMTS and GSM-1800 exposure on lipid peroxidation in the different brain zones of young rats (12 weeks-old). Lipid peroxidation is detected by mean of 4-HNE intensity staining.



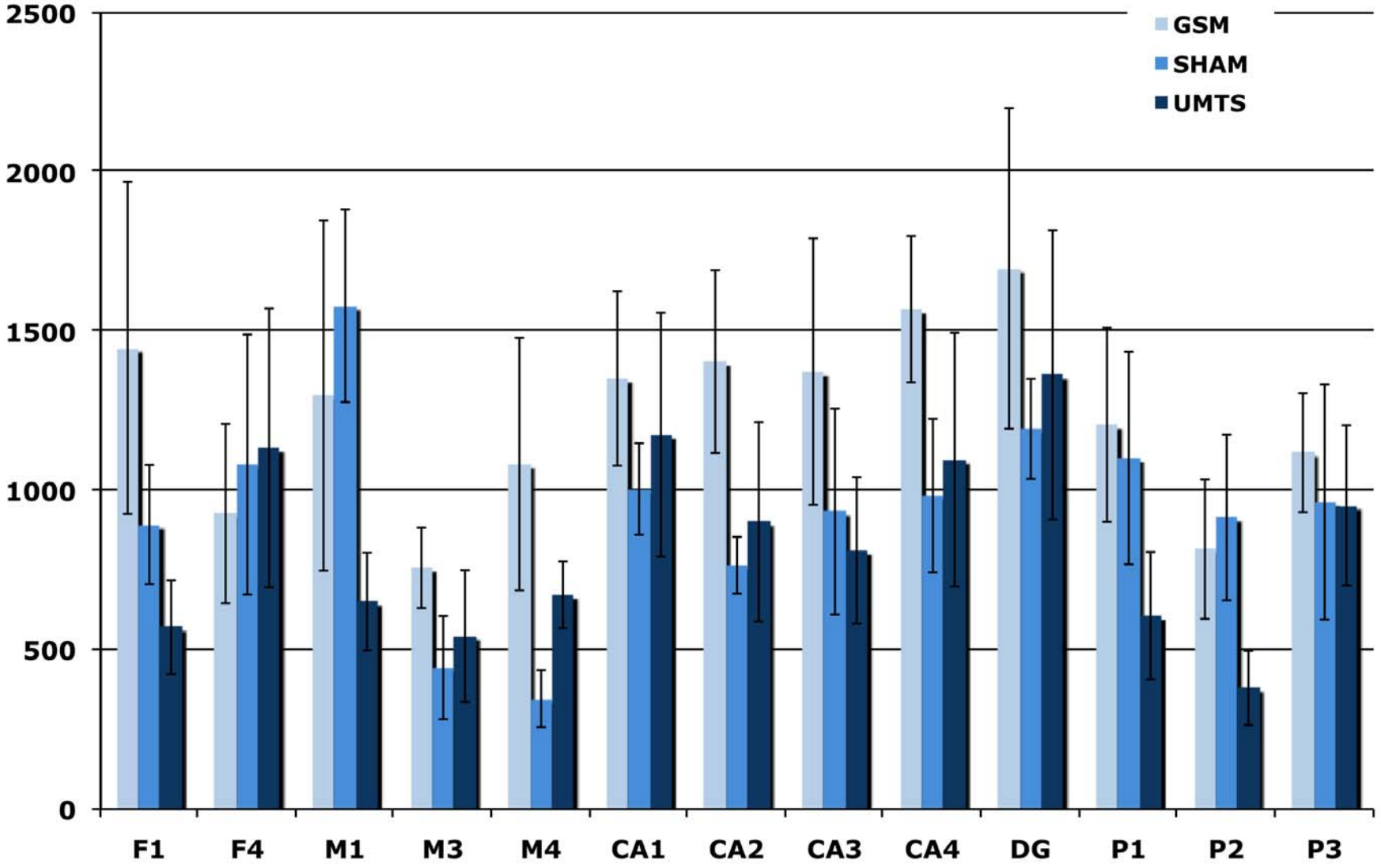
Annex 1b : Effects of repeated UMTS and GSM-1800 exposures (2 hours/day, 5 days/weeks, 4 weeks) on lipid lipoperoxidation in the different brain zones of young rats (12 weeks-old). Protein nitration is detected by mean of 4-HNE intensity staining.



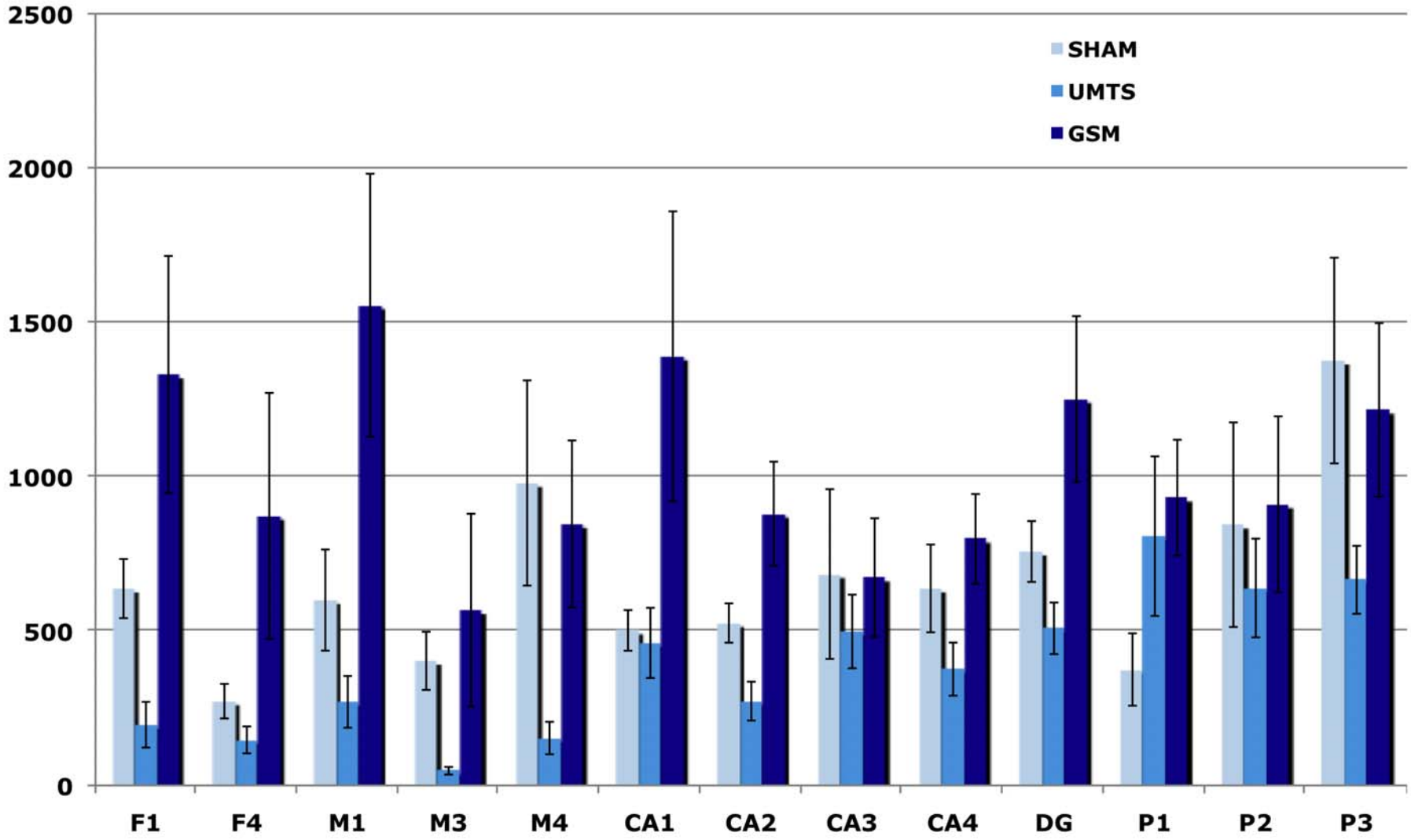
Annex 1c : Effects of of a single 2-hour UMTS and GSM-1800 exposure on lipid peroxidation in the different brain zones of elderly rats (16 months-old). Lipid peroxidation is detected by mean of 4-HNE intensity staining.



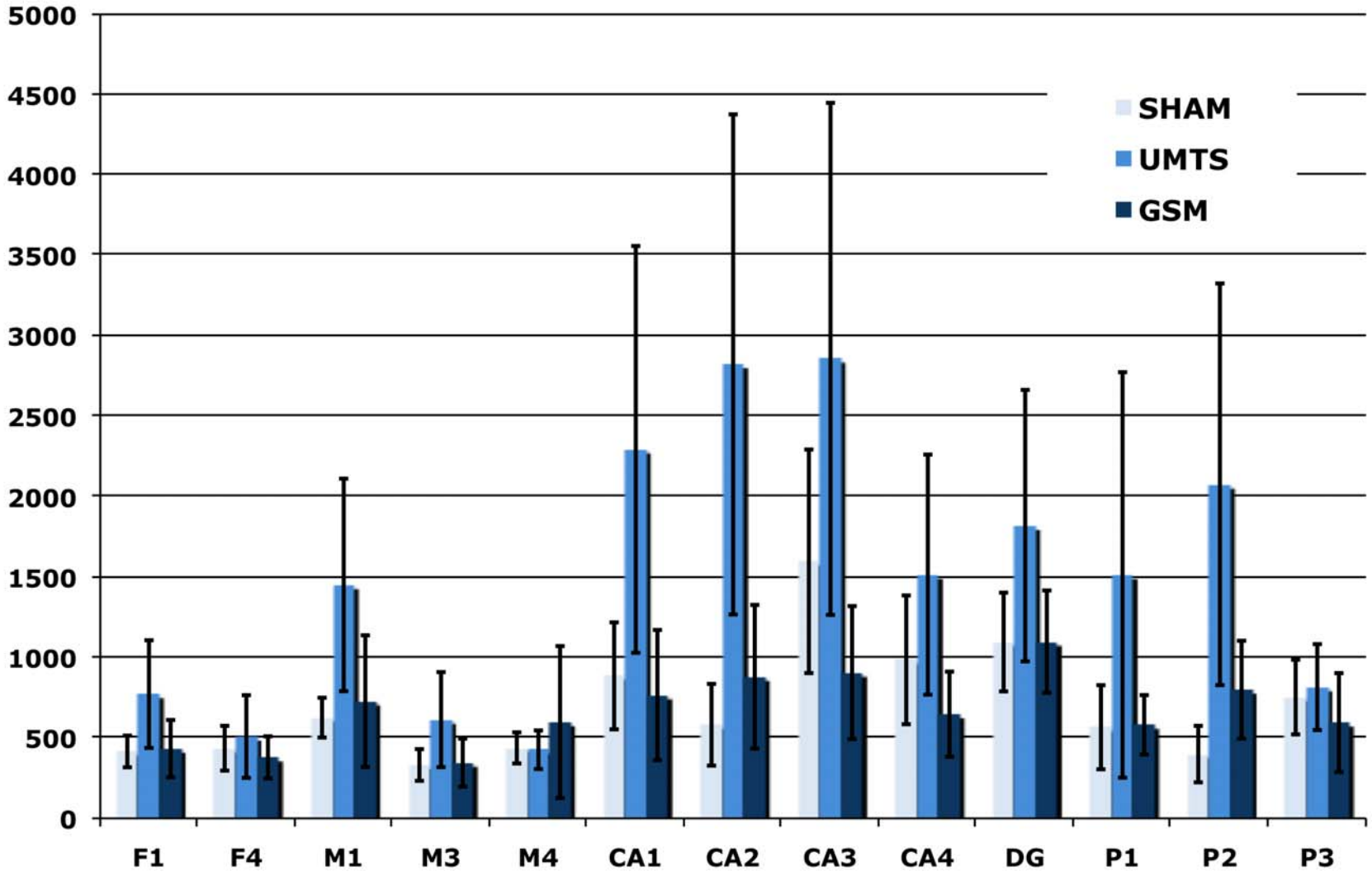
Annex 1d : Effects of repeated UMTS and GSM-1800 exposures on lipid peroxidation in the different brain zones of elderly rats (16 months-old). Lipid peroxidation is detected by mean of 4-HNE intensity staining.



Annex 2a : Effects of a single 2-hour UMTS and GSM-1800 exposure on protein nitration in the different brain zones of young rats (12 weeks-old). Protein nitration is detected by mean of 3-NT intensity staining.



Annex 2b : Effects of repeated UMTS and GSM-1800 exposures (2 hours/day, 5 days/weeks, 4 weeks) on protein nitration in the different brain zones of young rats (12 weeks-old). Protein nitration is detected by mean of 3-NT intensity staining.



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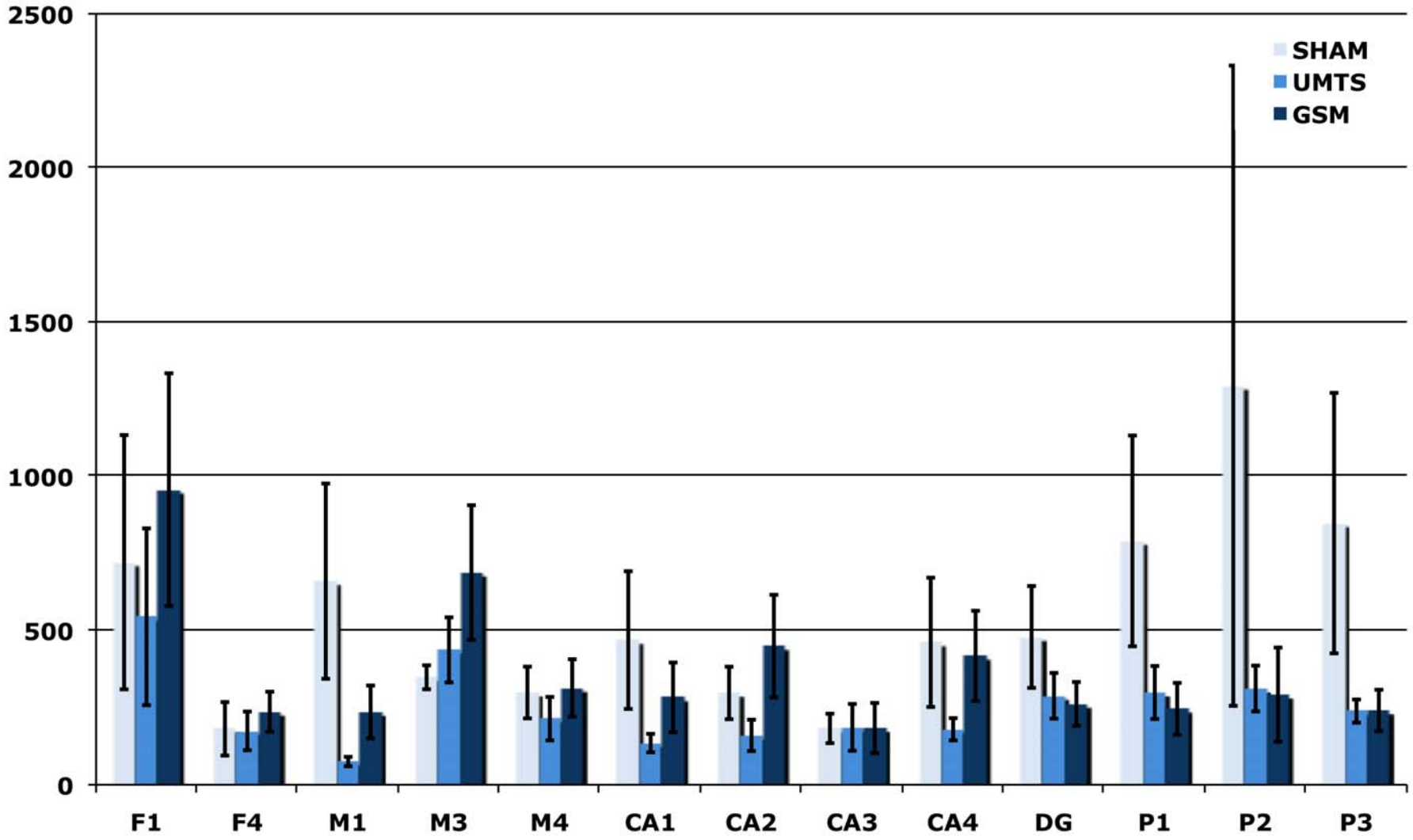

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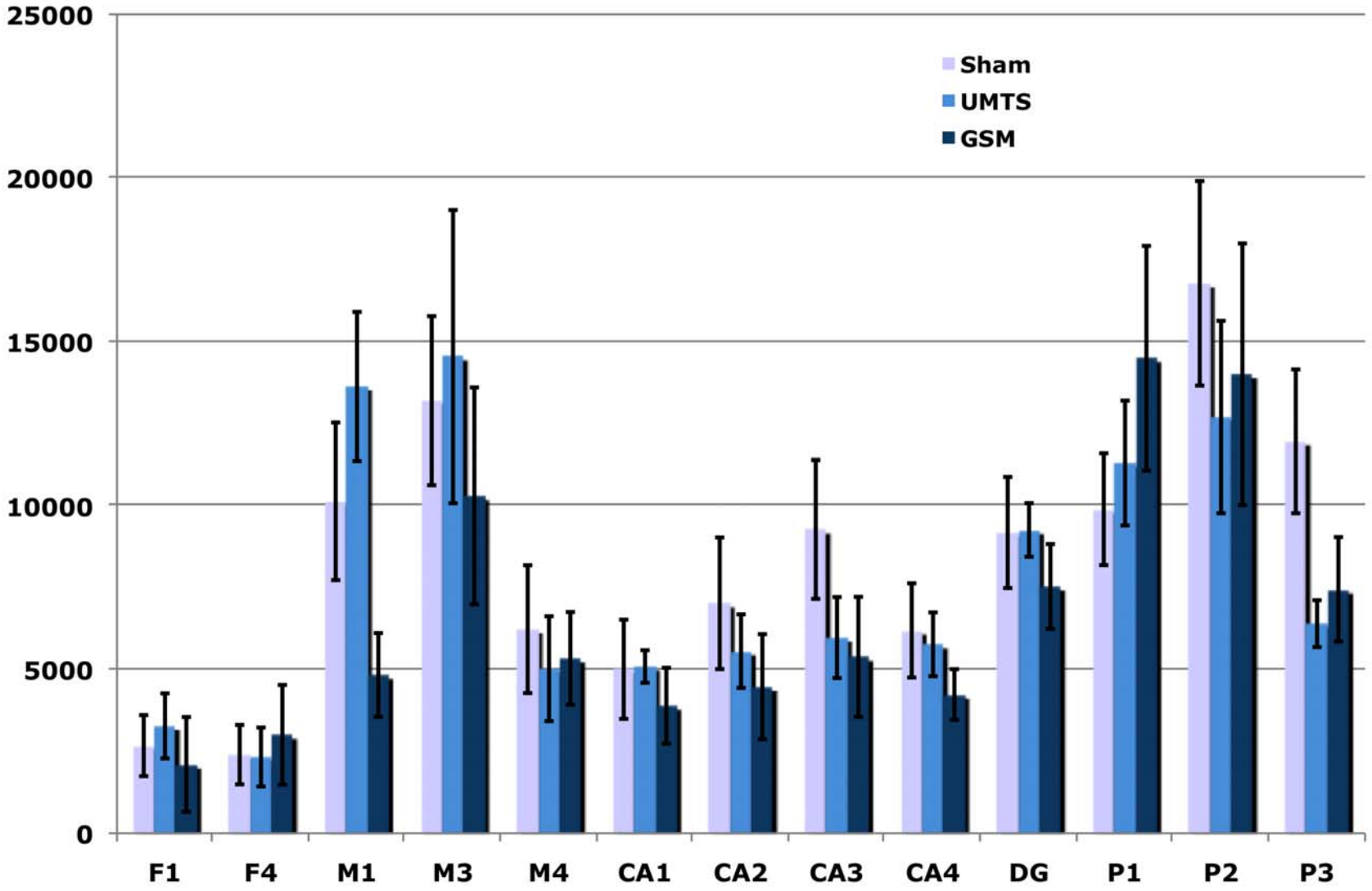
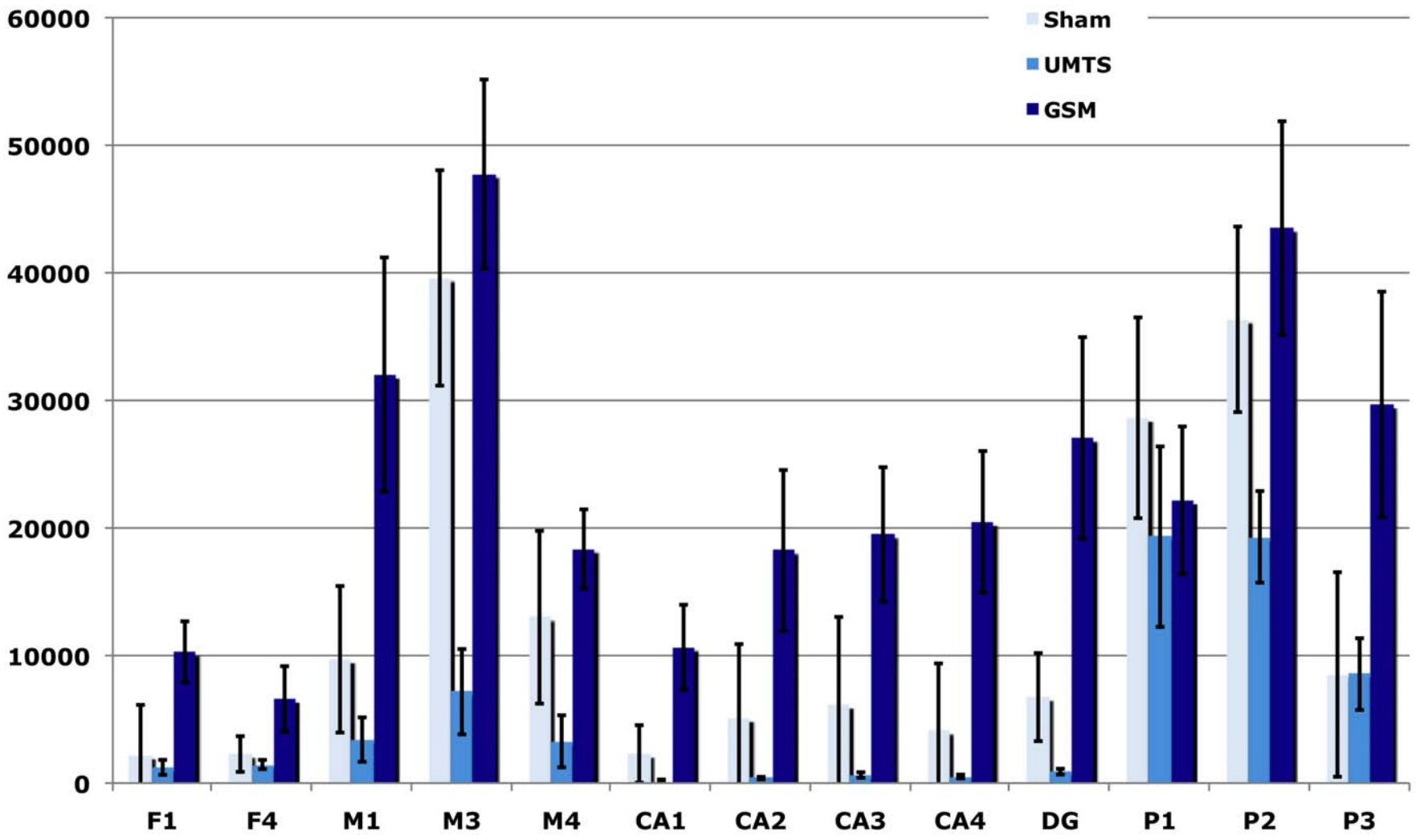
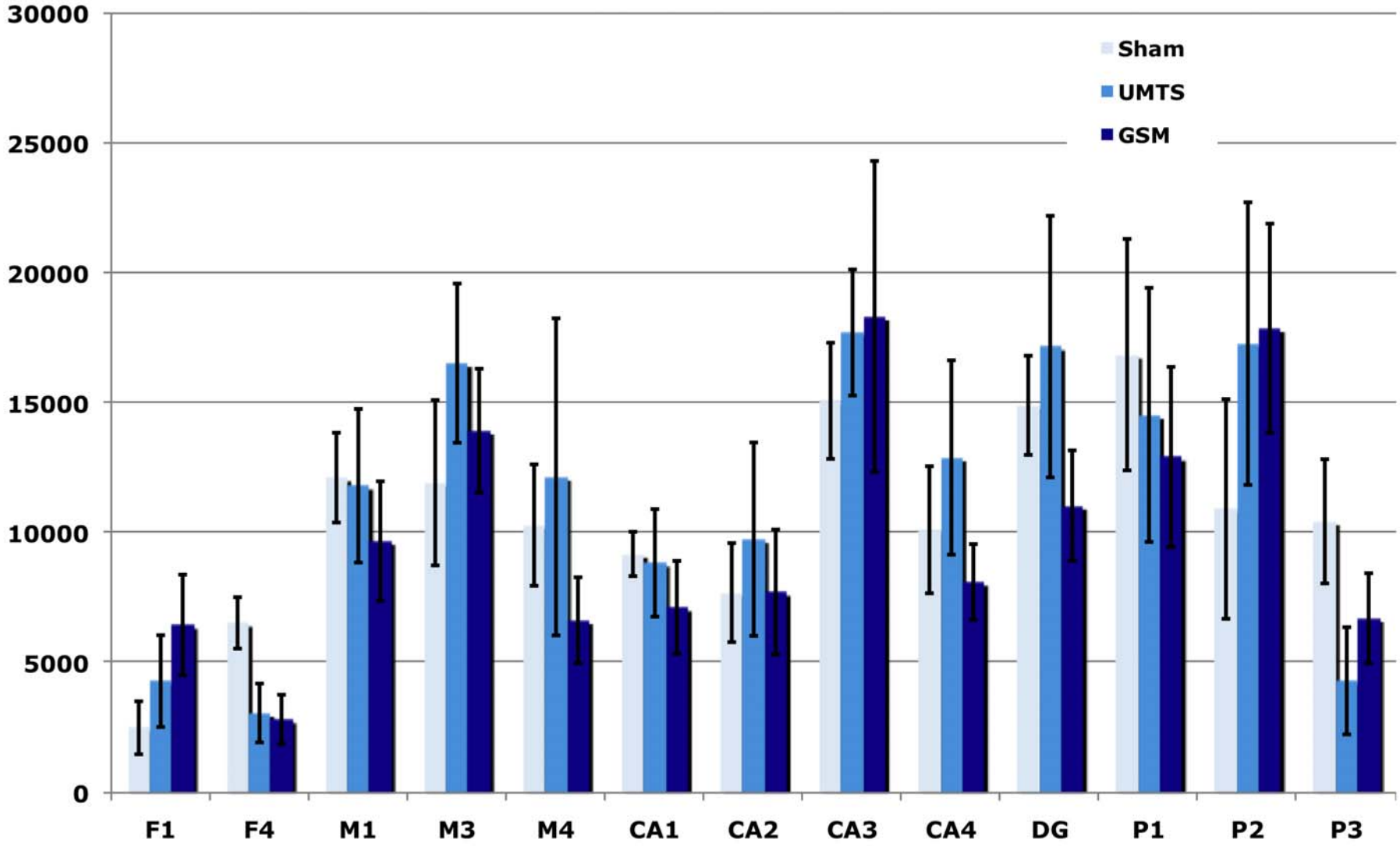


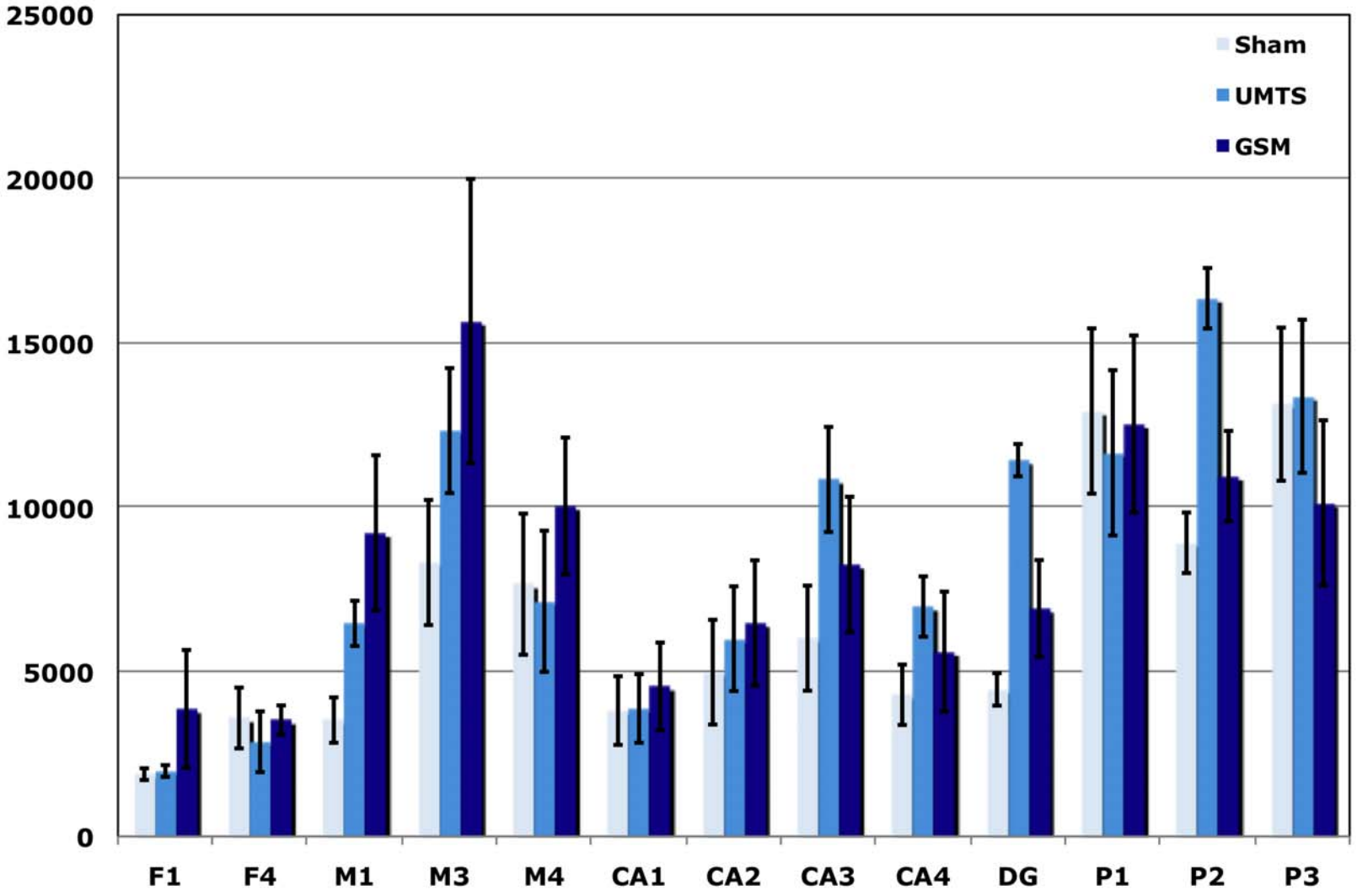
Annex 3a : Effects of a single 2-hour UMTS and GSM-1800 exposure on DNA oxidation in the different brain zones of young rats (12 weeks-old). DNA oxidation is detected by mean of 8-oxodG intensity staining.



Annex 3b : Effects of repeated UMTS and GSM-1800 exposures (2 hours/day, 5 d/weeks, 4 weeks) on DNA oxidation in the different brain zones of young rats (12 weeks-old). DNA oxidation is detected by mean of 8-oxodG intensity staining.



Annex 3c : Effects of of a single 2-hour UMTS and GSM-1800 exposure on DNA oxidation in the different brain zones of elderly rats (16 months-old). DNA oxidation is detected by mean of 8-oxodG intensity staining.



Annex 3d : Effects of Effects of repeated UMTS and GSM-1800 exposures (2 hours/day, 5 d/weeks, 4 weeks) on DNA oxidation in the different brain zones of elderly rats (16 months-old). DNA oxidation is detected by mean of 8-oxodG intensity staining.