*Session title: TA10 – Non Ionizing Radiation*

# *EFFECTS OF AN ACUTE AND A SUB-CHRONIC 900 MHz GSM EXPOSURE ON BRAIN ACTIVITY AND BEHAVIORS OF RATS*

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*Abstract*

*Radio frequencies are suspected to produce health effects. Concerning the mobile phone technology, according to position during use (close to the head), possible effects of radio frequencies on the central nervous system have to be evaluated. Previous works showed contradictory results, possibly due to experimental design diversity.*

*In the framework of RAMP 2001 project, we evaluated possible effect of a 900 MHz GSM exposure on the central nervous system of rat at a structural, a functional and a behavioral level after acute or sub-chronic exposures. Rats were exposed using a loop antenna system to different SAR levels and durations, according to results of the French COMOBIO 2001 project. A functional effect was found (modification of the cerebral activity and increase of the glia surface) after an acute exposure, even at a low level of brain averaged SAR (1.5 W/kg). No cumulative effect was observed after a sub-chronic exposure (same amplitude of the effect). No structural or behavioral consequence was noted.*

*We do not conclude on the neurotoxicity of the 900 MHz GSM exposure on the rat brain. Our results do not indicate any health risk.*

*Introduction*

*Low power electromagnetic fields (EMF) are suspected to produce health effects. Among them, radiofrequencies (0.1 MHz to 300 GHz) were used in many technologies and specifically in the mobile phone communication.*

*According to position of mobile phones during use (close to the head), the possible interaction of radiofrequencies within the brain is a particular interest of research. Many studies have been done to measure potential changes in structure, function (modification of neurotransmitter release, blood-brain barrier permeability or glia evolution) or behavior after an exposure to a non-thermal specific absorption rate (SAR, under 4 W/kg). But several results were contradictory (D'ANDREA et al. 2003a; D'ANDREA et al. 2003b; HOSSMANN and HERMANN 2003). The diversity in the exposure set-up, strain of animals or protocols used could explain this variation in effects.*

*In the framework of the RAMP 2001 project, we evaluated possible effects of a 900 MHz Global System of Mobile communication (GSM) exposure on the central nervous system of rat. Aim of our work was to perform a global study*

*on the rat brain after exposure using a acute exposure set-up (loop antenna system). To determine a potential cumulative neurotoxicity, effects of acute and sub-chronic exposures were measured on brain structure, functions (neuronal activity and glia evolution) and behaviors (motor co-ordination, anxiety and explorative capacities, learning and memory).*

*This study was in the continuity of a national French project (COMOBIO 2001), using the same exposure set-up, and wanting to reproduce and complete its previous results.*
*In particular, Bontempi et al. (BONTEMPI et al. 2002) found a different modification of the rat brain activity according to SAR after an acute 2 hours 900 MHz GSM exposure. They found an increase in different brain areas at a level of brain averaged SAR of 1.5 W/kg*
*and a decrease at a higher SAR level (6 W/kg). No consequence was observed on learning and memory. Moreover, Mausset-Bonnefont et al. (MAUSSET-BONNEFONT et al. 2004) described a strong glial reaction in cortex and striatum after an acute 15 minutes 900 MHz GSM exposure at a 6 W/kg SAR brain average, without modification of locomotor activity.*

*Results obtained in our study are resumed here, showing principal effects observed*
*on structure, brain activity, glia evolution and behavior after an acute or a sub-chronic*
*900 MHz GSM exposure at different SAR levels in comparison to control animals. Results*
*are discussed and human extrapolation is commented to evaluate potential health effect*
*and validity of current local exposure limit, 2 W/kg (1999/519/CE 1999; DÉCRET_2002-775 2002).*

## *Methods*

### *Exposure set up and dosimetry*

*Rats (Sprague Dawley male adults) were placed in Plexiglas rockets (5 mm thick, 6 cm diameter, 15 cm length) with a truncated cone (3 cm length) in which the animal inserted*
*its head and above which an individual loop antenna was placed. The loop antenna was connected to a radio frequency generator (RFS900-64 type, RFPA, Bordeaux, France), emitting a 900 MHz GSM electromagnetic field (1/8 duty factor) pulsed at 217 Hz. 4 rats could be exposed in the same time in an anechoic chamber. The SAR calculations were made with homogenous phantoms and non-homogenous numerical models (LEVEQUE et al. 2004).*

### *Structure*

*After exposure, rats were sacrificed and their brains were removed. The general aspect was noted and some brain slices were colored using a histological staining (Thionin) to detect potential macroscopic morphology modifications of the nervous central system. The nuclear coloration obtained with Thionin allows verifying the integrity of cells and tissue.*

*Function*

*c-Fos protein. The cartography of the rat cerebral activity was obtained looking at the c-Fos protein expression (DRAGUNOW and FAULL 1989). An immunostaining protocol was performed on brain slices and the number of positive nuclei/ mm2 was measured in 24 brain areas according to the Bontempi et al. previous study (BONTEMPI et al. 2002).*

*First, a parametric study was performed after an acute exposure. To determine the effect of the SAR level on the brain activity, 5 groups of rats were exposed for 2 hours at one of 5 different SAR levels (0; 0.75; 1.5; 3 and 6 W/kg). In a second experiment, to determine the effect of the exposure duration on the brain activity, 6 other groups were exposed to 3 different durations (15, 30 or 45 minutes) at a 0 or 1.5 W/kg SAR. These results were compared to those obtained after a 120 minutes exposure. Then, the cartography of c-Fos positive nuclei was evaluated after a sub-chronic exposure. Animals were exposed 45 min at a 1.5 W/kg SAR or 15 min at a 6 W/kg SAR during 1 month (7 days/ week). The protocols and studies are detailed in the following publications (BRILLAUD et al. 2005b, BRILLAUD et al. 2005c).*

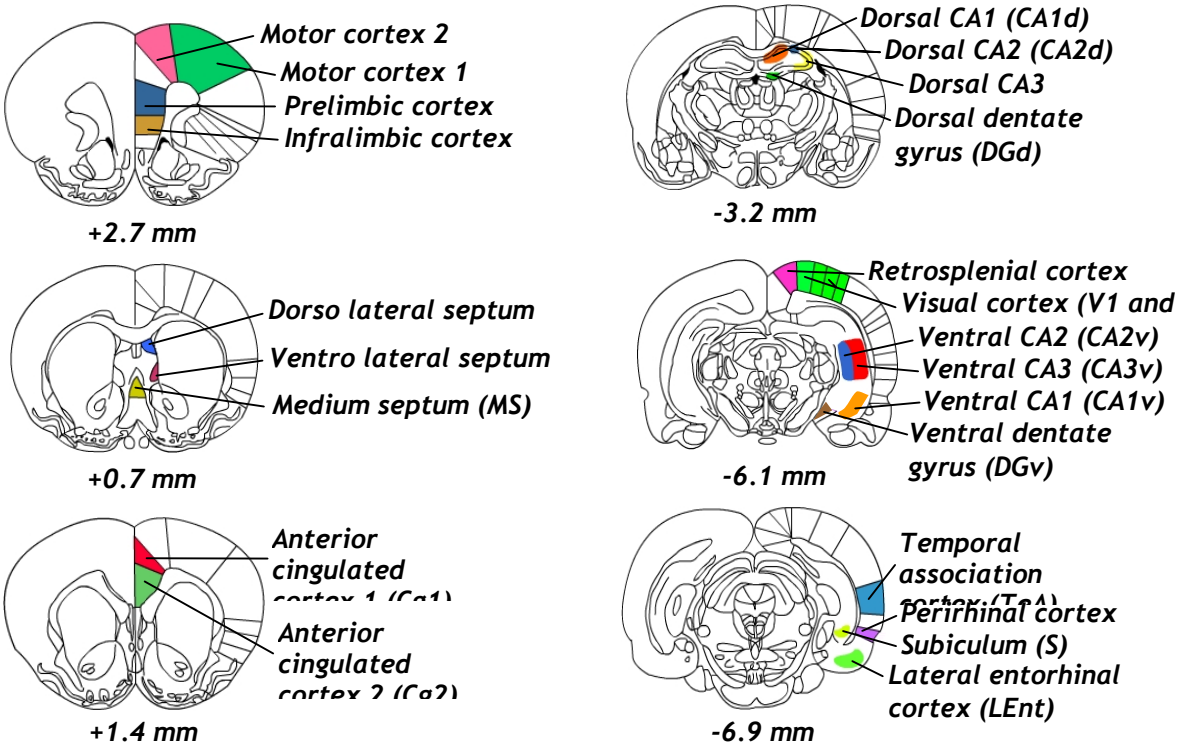


*Figure 1: Representation of rat brain coronal sections (adapted from the stereotaxic atlas of Paxinos G. and Watson C., 1998, fourth edition) indicating the 24 regions of interest selected to count the number of c-Fos positive nuclei. Numbers*

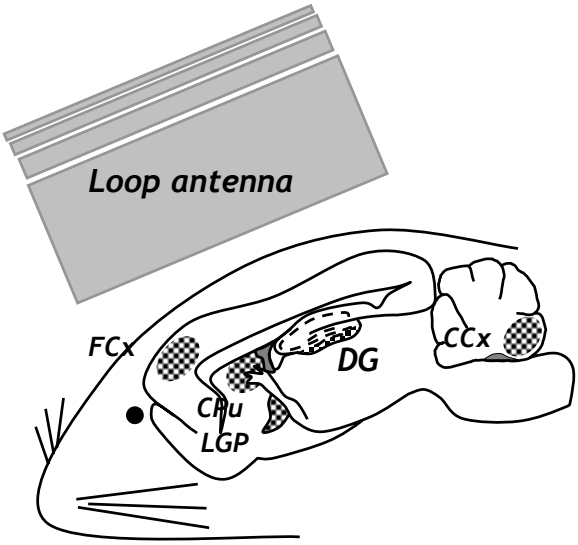


*Figure 2: Representation of brain structures analysed in the rat brain to measure the surface of GFAP stained area: FCx = Frontal Cortex; CPu = Caudate Putamen; LGP = Lateral Globus Pallidus; DG = Dentate Gyrus of hippocampus; CCx = Cortex of Cerebellum (+2.5 mm sagittal section)*

*GFAP protein The glia properties were analyzed looking at the evolution of the astrocytes, staining a structural protein, the Glial Fibrillary Acidic Protein (GFAP) (ENG et al. 2000). Indeed, astrocytes can react to an aggression of the central nervous system by a hypertrophy (cell size increase) and/or a hyperplasy (cell proliferation). An immunostaining protocol was performed on brain slices and the stained GFAP area/ mm2 was measured in 5 brain structures according to the Mausset-Bonnefont et al. previous study (MAUSSET-BONNEFONT et al. 2004).*

*First, the evolution of the GFAP stained area was measured 2, 3, 6 and 10 days after an acute 6 W/kg exposure (15 min). To evaluate the SAR impact, another acute exposure was performed (45 min at 1.5 W/kg) and the GFAP marker was analyzed the third day after the exposure. Then, evolution of the GFAP stained area was evaluated after a sub-chronic exposure. Animals were exposed 45 min at a 1.5 W/kg SAR or 15 min at a 6 W/kg SAR, during 2 months (5 days/ week). The protocols and studies are detailed in the following publications (BRILLAUD et al. 2005a; BRILLAUD et al. 2005b; BRILLAUD et al. 2005c).*

*Behavior*

*The motor co-ordination of rats was tested using a rota-rod test (Dunham and Miya 1957; Watzman and Barry 1968). Animals were placed on a rotating axis at a definite speed. The staying time on axis is measured in 3 consecutive trials (maximal duration = 60 seconds).*

*The anxiety level and explorative capacities of rats were evaluated by an elevated plus maze test (PELLOW et al. 1985; RODGERS and DALVI 1997). This test is formed of two open arms (without lateral protection) and two closed arms (with lateral protections) disposed in a "+" configuration. Rat behavior was observed during 8 min. The time spent in each types of arm (open or closed) is measured and allows to evaluate the anxiety level of animal (more animal stays in open arms, less it is considered as anxious). The total number of entries is an index of the explorative capacities of rat.*

*The radial-maze test evaluates learning and memory performances of rats (OLTON 1987; SLANGEN et al. 1990). The apparatus is compound of 8 arms situated in starlike shape around a central platform. In the selected protocol, testing the working memory, the 8 arms were baited and all arms were open. During the learning task, animals were tested 1 trial/ day until they learned to visit each arm only one time. When this criterion was reached, the animal memory was evaluated: the rats were tested in the maze after successive delays (1, 2, 3 and 6 days). Parameters measured were the total trial time, the number of errors (visit of a previously visited arm) and the total number of visits.*

*First, animals were tested just after an acute exposure (45 min 1.5 W/kg or 15 min 6 W/kg) using the rota-rod and the elevated plus maze tests. Then, rats were tested after*

*a sub-chronic exposure (45 min 1.5 W/kg or 15 min 6 W/kg/ day). Two experiments were done. In the first experiment, animals were daily exposed during 1 month and they were tested every day in a radial-maze task to evaluate learning and memory capacities. In a second experiment, animals were exposed 2 months (5-days/ week) and their motor capacities, the anxiety level and their explorative capacities were evaluated at the end of exposure time. The protocols and studies are detailed in the following (BRILLAUD et al. 2005b; BRILLAUD et al. 2005c).*

*Results*

*Structure*

*No morphologic change of brains and no macroscopic modification were noted after sacrifice whatever the exposure duration or the SAR.*

*Function*

*c-Fos protein Results of the parametric study showed a modification of the cerebral activity after a 2 hours exposure according to the SAR level. After a 120 min exposure at a 1.5 W/kg SAR, the number of positive c-Fos nuclei increased in cerebral areas closed and distant from antenna. After a 120 min exposure at a 6 W/kg SAR, the number of c-Fos positive nuclei decreased in some cerebral areas. No effect was observed at SAR levels of 0.75 and 3 W/kg. The involved structures are known to intervene in mnesic processes. Comparing the different durations, the cerebral activity modification seemed to appear beyond 45 minutes of exposure.*

*After the sub-chronic 45 min exposure at a 1.5 W/kg SAR, an increase of the c-Fos staining was noted with an equivalent amplitude from that obtained after an acute exposure for 120 min. The brain areas involved were analogous. After a sub-chronic 15 min exposure at a 6 W/kg SAR, the cerebral activity modification was like the one observed after the 1.5 W/kg acute exposure (same involved areas, same amplitude of the effect).*

*The results are resumed in Figure 3.*

*GFAP protein Results of the 15 min acute exposure at a 6W/kg SAR showed a strong increase in the GFAP stained area 2 days after exposure in the frontal cortex and the caudate putamen. This increase was still present 3 days after exposure but the amplitude was decreased. No significant effect was notified 6 and 10 days after exposure. An increase of the GFAP stained area was visible in the cerebellum cortex, only 3 days after exposure. No modification of GFAP stained area was observed in the dentate gyrus and the lateral globus pallidus, whatever the day after exposure. An increase of the GFAP stained area was found in the frontal cortex and the lateral globus pallidus 3 days after the 45 min acute exposure at a 1.5 W/kg SAR. The amplitude of the effect is much lower than the one obtained after an acute 15 min exposure at a 6 W/kg SAR.*

*After a sub-chronic 45 min 1.5 W/kg exposure, no effect was highlighted on the GFAP stained area evolution. After a sub-chronic 15 min 6 W/kg*

*exposure, a weak increase of the GFAP stained area was observed in the frontal cortex, the lateral globus pallidus and the cerebellum cortex. The amplitude of the effect was smaller than the one obtained after the acute similar exposure.*

*The results are resumed in Figure 4.*

*Behavior*

*No behavioral effect was found after a acute 900 MHz GSM exposure.*

*No motor, anxiety and learning effect was found after a sub-chronic 900 MHz GSM exposure. At the higher SAR (6 W/kg), no memory consequence was noted. After the 45 min 1.5 W/kg exposure, a weak effect was measured during the memory task after 2 days of delay. Exposed animals spent more time to finish the trial, with more errors. However, because of the low significance level of this result, this effect could be due to hazard.*

*Results were resumed in Figure 5.*

*Discussion*

*Structure*

*An acute or a sub-chronic 900 MHz GSM exposure does not modify the structure of the rat brain, even at a high SAR exposure level (6 W/kg).*

| | SAR (W/kg) | Duration (min) | Global effect | Brain areas involved |
|---|---|---|---|---|
| ***Acute exposure*** | *0.75* | *120* | = | / |
| | *1.5* | *15, 30, 45* | = | / |
| | | *120* | ↗ | IL, PrL, Cg1, M2, CA1v, S, RSA, V2, PRh, TeA |
| | *3* | *120* | = | / |
| | *6* | *120* | ↘ | IL, CA1v, S, TeA |
| ***Sub-chronic exposure*** | *1.5* | *45* | ↗ | M2, M1, DGd, CA2d, DGv, CA2v, RSA, V2, V1, Lent, PRh, TeA |
| | *6* | *15* | ↗ | PrL, Cg1, M2, DGd, CA1d, CA1v, CA2v, S, RSA, V1, Lent, PRh, TeA |

***Figure 3: Summary of the results found on the cerebral activity (c-Fos positive nuclei count) after acute and sub-chronic exposures at different durations and SAR levels (significance of the brain area initials are***

| | SAR (W/kg) | Duration (min) | Global effect | Brain structures involved |
|---|---|---|---|---|
| ***Acute exposure*** | *1.5* | *45* | + | FCx, LGP |
| | *6* | *15* | +++ | FCx, CPu, CCx |
| ***Sub-chronic exposure*** | *1.5* | *45* | = | / |
| | *6* | *15* | ++ | FCx, CCx |

***Figure 4: Summary of the results found on glia evolution (GFAP stained area measurement) after acute and sub-chronic exposures at different durations and SAR levels (significance of the brain areas initials are indicated in***

| | SAR (W/kg) | Duration (min) | Global effect | Behaviors tested |
|---|---|---|---|---|
| ***Acute exposure*** | *1.5* | *45* | = | motor co-ordination, anxiety/ exploration |
| | *6* | *15* | = | |
| ***Sub-chronic exposure*** | *1.5* | *45* | = | motor co-ordination, anxiety/ exploration, learning/ memory |
| | *6* | *15* | = | |

***Figure 5: Summary of the results found on behavior (rota-rod, plus maze and radial maze tests) after acute and sub-chronic exposures at different durations and SAR levels.***

*Function*

*c-Fos Results obtained after the acute exposure confirmed those obtained by Bontempi et al. (BONTEMPI et al. 2002): an acute exposure can modify the cerebral activity. This effect is dependent on the SAR level, and appears only after minimal exposure duration. It could be due to an interaction of radio frequencies with a biological system such as a neurotransmitter system. Data obtained after the sub-chronic exposure showed equivalent amplitude of the effect than after an acute exposure (max + 150%), whatever the exposure parameters (SAR and duration). These results do not suggest a cumulative effect.*

*GFAP Results obtained showed that an acute exposure could induce an activation of the astrocytes in the rat brain. This effect seemed to be temporary (astrocytes hypertrophy) and had a spatial and a temporal propagation. It could be explained by an interaction of radio frequencies with a biological process (such as a neurotransmitter pathway), involving a cascade of reactions. The effect is dependent on the SAR level, perhaps with a thermal threshold. Our results confirm those obtained by Mausset-Bonnefont et al. (MAUSSET-BONNEFONT et al. 2004). Data obtained after the sub-chronic exposure suggest an adaptive effect and not a cumulative one (amplitude decrease of the effect).*

*Behavior*

*In spite of modifications of the GFAP and c-Fos markers in cerebral structures involved in motor, sensitive and cognitive functions, no behavioral effect was found after an acute or a sub-chronic 900 MHz GSM exposure. Results are in agreement with the Mausset-Bonnefont et al. ones concerning the motor behavior, and the Bontempi et al. ones concerning the learning and memory (BONTEMPI et al. 2002; MAUSSET-BONNEFONT et al. 2004). The weak effect observed on memory (2 days of delay, 45 min exposure at a 1.5 W/kg SAR) could show a momentary deterioration of consolidation processes, correlated to the cerebral activity results (increase of c-Fos positive nuclei in the areas involved in these processes). But the low significance level of this result does not allow concluding.*

*Conclusion*

*A functional effect was found (modification of the cerebral activity and increase of the glia) after an exposure to a 900 MHz GSM signal, even at a low SAR level (1.5 W/kg). However, this modification seems to be a temporary one, and no cumulative effect was observed after a repeated exposure. Furthermore, it is no correlated to structural or behavioral consequences. This effect does not seem to be a neurotoxic one.*

*Extrapolation of these results to human requires precautions. First, the brain conformation (more developed cortex in human brain) and the exposure localization*

*(medio-sagittal exposure for rat vs. lateral exposure with phone) are not equivalent, involving different brain areas nearest to the antenna during exposure. Moreover, the SAR calculation is not comparable for the rat brain and for human. Indeed, for rat, the local SAR calculation was performed using 1g of tissue, equivalent to the total brain weight. For human, the local SAR calculation was performed using 10g of tissue, including superficial tissue (skin, bone) and a small part of the brain (weight average of human brain = 1.8 kg).*

*To reach a brain averaged SAR of 1.5 W/kg in the total human brain, the local SAR to apply would be higher than the current limit of local exposure (2 W/kg) (1999/519/CE 1999; DÉCRET_2002-775 2002).*

*But considering a local averaged SAR in the human brain, with a 2 W/kg local SAR exposure (10g), the averaged SAR calculated on 1 g of tissue is about 2.8 to 3.6 W/kg in superficial tissue and 1 W/kg in brain tissue (superficial cortex). In our studies, a functional modification was found starting from a 1.5 W/kg SAR level in the rat brain. A biological effect can be suspected at an analogous regional SAR in human brain, with a potential propagation of the effect. A 1.5 W/kg SAR value averaged on 1 g in the human brain would need a 3 W/kg local SAR exposure calculated on 10 g of tissue, which is more than the current exposure limit.*

*Our results do not indicate any health risk of mobile phone use. But the question of the validity of the safety margin can be asked before the apparition of a biological effect, even if a potential health consequence of such effect is not attested.*

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