Behavioral and biochemical effects of GSM 900MHz in neuroinflammation and gestational inflammation models

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***Abstract* - The widespread use of mobile phones raises the question of the effects of electromagnetic fields (EMF, GSM 900 MHz) on the brain. Effects of these EMFs during development stages and in vulnerability states as inflammation have been only poorly studied. Our aim was to assess EMF effects in adolescent rats subjected to neuroinflammation (Lipopolysaccharide (LPS) or artificial cerebrospinal fluid (aCSF) diffusion in lateral ventricle or in hippocampus (1.25 µg/h for 4 weeks from day 25) or to gestational inflammation (LPS or saline injection in peritoneum (0.1 mg/kg) on gestational day (GD) 15, GD17, and GD19). Sprague Dawley rats (32 days) were repeatedly exposed to various specific absorption rates (0, 1.5 or 6 W/kg; 45 min/d, 5 d/week, for 4 weeks). Endpoints were emotional memory, sensori-motor reactivity, exploration and anxiety (using fear conditioning, startle inhibition, open field and elevated plus maze) as well as cerebral Interleukine-1 beta (IL-1β) and glial fibrillary acidic protein (GFAP). The neuro-inflammation model was characterized by increased levels of IL-1beta and GFAP, reduced startle level and by the increase of startle inhibition and the decrease of short term emotional memory. Gestational inflammation was linked to teratological effects, but no behavioral modifications. Effect of GSM exposures were shown by a decrease of GFAP in 1.5 W/kg-LPS compared to 0W/kg-LPS exposed rats. There was no GSM or LPS effect on anxiety. GSM EMFs at 900MHz did not induce neurobiological effects by itself and impacted only one biochemical parameter in vulnerable. These data suggest no specific vulnerability of adolescents with or without pre-existing inflammation in response to mobile phone EMF exposure.**

I. Introduction

The widespread use of mobile phones raises the question of the effects of electromagnetic fields (EMFs) from the Global System for Mobile Communication (GSM) 900 MHz to the brain. Up to date, most experimental researches assessing this question focused on healthy adult rodent models. These studies showed that glial fibrilary acidic protein (GFAP) [1], [2] or cytokines [3], [4] were increased. Moreover, studies showed a possible impact of EMF ted on emotional memory [3] and anxiety [7]. However, these biochemical and behavioural results were not always reproduced [3], [5], [6] and [10].

Meanwhile, because teenagers represent an increasing part of the cell phone users, concerns are currently raised in the case of cerebral vulnerability, as development and maturation. Moreover, we may hypothesize that adolescent brain vulnerability to environmental insult would be worsened by a previous developmental toxicological exposure, which is a suspected cause for the etiology of psychopathologies diagnosed during adolescence [12].

In the same way, adolescent subjected to neuro-inflammation, which is the common feature of most neuro-pathologies may be more vulnerable in response to co-exposed GSM. In this way, we assessed behavioural (fear conditioning, startle reflex inhibition, elevated plus maze and open field) and biochemical (IL-1β and GFAP) effects of repeated EMFs GSM 900 MHz exposures in adolescent rat models of gestational inflammation or neuro-inflammation.

II. Material and Method

A. Neuroinflammation model

At post-natal day 25 (P25): Sprague Dawley rats were implanted with Alzet osmotic pump (0.25 µl/h for 28 days; 5 µg/µl LPS or artificial cerebrospinal fluid (vehicle)). Infusions were performed in one lateral cerebral ventricle (Antero-posterior: -1.3 mm to Bregma, medo-lateral: ±1.5 mm, and dorso-ventral: -3.5 mm) or in the hippocampus (Antero-posterior: -2.5 mm to Bregma, medo-lateral: -3 mm, and dorso-ventral: 7.0 mm).

B. Gestational inflammation model

Pregnant Sprague Dawley rats were injected in intra-peritoneal daily with 0.1 mg/kg of LPS or saline on gestational day (GD) 15, GD17, and GD19

C. GSM 900MHz exposure

Exposure set-up was performed as previously described [13]. A radio frequency power source (900-64 type, Radio Frequency Power Amplifier, France) emitting a 900 MHz EMF (1/8duty factor) pulse modulated at 217 Hz was connected to a four-output divider. Each output was connected to a loop antenna allowing local exposure of four animals simultaneously in an anechoic chamber.

Exposure dosimetry was previously performed Leveque et al. [14] and confirmed experimentally in our laboratory by Ammari et al. [15]. Presented Specific Absorption Rate (SAR) values correspond to adult rat's exposure.

From P32 to P62, healthy and vulnerable models of rats were placed in a Plexiglas rocket on which an individual loop antenna was fixed for head exposure. Rats were repeatedly exposed (45 min/day; 5 day/week; during 1 month) to various specific absorption rates (SAR) of EMFs GSM 900MHz (0, 1.5 or 6W/kg).

D. Behavioral tests

Fear conditioning paradigm (P56-P57), startle inhibition paradigm (PPI) (P60 to P62), elevated plus maze (P63) and open field (P63 or P64) were tested at the exposition month end for intracerebroventricular inflammation model and gestational inflammation model.

Fear conditioning occurred in four standard fear conditioning apparatus purchased from Bioseb (France). Four freezing boxes (25×25×25cm). Freezing response was defined as complete immobilization of the rat, except for respiration [16]. It was measured in response to stimuli presentation (context and tone) by amplitude of movement of the animal and was scored as percentage of time spent freezing.

Training phase began with a 2 min habituation period (no auditory or aversive stimulus), followed by five training cycles. For one cycle, the three consecutive steps were: 28 s of sound (Frequency: 2 kHz; 100 dB), 2 s of sound + electrical shock (0.25 mA) and a 30 s exploration period (no auditory or aversive stimulus).

To test contextual memory, animals were introduced for 3 min in the boxes used for the training. To avoid extinction, the 3h contextual memory test ended with two training cycles.

To perform the cue memory test, animals were introduced in boxes with modified environment (different box size, walls color and floor texture) for 2 min and then were exposed to the sound for 3 min and 2 min of exploration period.

Prepulse inhibition occurred in the same four standard fear conditioning apparatus. For this test, rats were placed in a restraining tube. Throughout the session, a background noise level of 60 decibel (dB) was maintained.

Rats were run in squads of four. Each rat was put into the PPI chamber for a 5-min acclimatization period with 60 dB background noise. Following this period, 10 startle pulses (120 dB, 40 msec duration) were presented with an average intertribal interval of 15 sec. Then, no stimulus (background noise, 60 dB), prepulses alone (75, 80 or 85 dB, 20 msec duration), startle pulses alone, and prepulses followed 80 msec later by startle pulses were presented six times randomly distributed over the next 15 min. The percentage of PPI induced by each prepulse intensity was calculated as 100[(SP-SPP)/SP], with SP being the average startle amplitude after the startle pulses and SPP being the average startle response after the combination of a certain prepulse and the startle pulse.

Elevated plus maze test was performed according to previously described methods [17]. The apparatus was composed of a plus shaped acryl maze with two opposite open arms and two opposite closed arms (50 cm in length, 10 cm width, and 31 cm in height), extending out from a central platform (10 cm × 10 cm) and elevated 50 cm above the floor. Rats were placed in the centre of the maze, the head facing an open arm and were allowed to explore for 5 min. Data were presented as percentage of time spent in the open arm compared to the time spent in the four arms during the first 30 seconds of the test.

Open field test was performed according to previously described methods [18]. Movements were monitored for 30 min using an automated apparatus consisting of four Plexiglas boxes (60 × 60 × 40 cm) equipped with lower 16 sets and top 8 sets (for vertical activity evaluation) of evenly spaced infrared beams located along two adjacent sides of one chamber (Imetronic, France). The time spent in the centre area of the apparatus, considered as a model of stress-induced inhibition of exploration behavior was monitored simultaneously.

A. Sacrifice

At P64, rats were sacrificed. Brains were quickly removed. The right medio-lateral parts of each brain (containing the injection site) were used for ELISA analyses (IL-1β and GFAP).

B. Biochemistry

Brain structures were homogenized by sonication in a Trizma base/NaCl/Triton buffer containing a 4% protease inhibitor (Roche). Samples were centrifuged (13000 rpm/20 min/4 °C) and the supernatant was collected. Bradford test was used to measure total protein content in each sample.

IL-1β were quantified using commercialized sandwich ELISA kits (Rat IL-1β/IL-1 F2 DuoSet ELISA Development system, R&D systems) according to the manufacturer's instructions.

GFAP were quantified a sandwich ELISA assay. Plates were coated in-house with the capture antibody (20334-Dako), detection antibody (ab 10062-abcam) and as tertiary antibody, we used a poly monoclonal anti-IgG-HRP (ab 6728-abcam). Glial fibrillary acidic protein purified from human brain (EMD Biosciences) was used as standard.

C. Statistics

Statistical analyses were performed using the Prism 5 (GraphPad Software, inc). Values were given as mean±standard deviation of mean (SEM) per group. D'Agostino and Pearson test was used for variance homogeneity. Analyses were performed using two-way analysis of variance (ANOVA). The main effects were analyzed using Bonferroni's post-hoc corrected t-test. Effects were considered significant when $p<0.05$.

III. Results

A. Gestational LPS

LPS gestational injections reduced the viable pups number (t-test: p=0.0291) (Fig: 1).

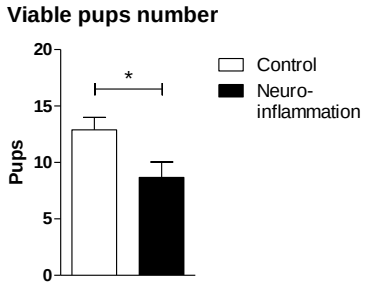

Fig. 1. LPS gestational injections effects on the viable pups number.
* p=0,0291 (t-test analyse).

B. Behavior

The percentage of time spent freezing during the short-term contextual memory test was decreased (two-way ANOVA: p=0.0252) in the neuroinflammation model compared to the control group, but there was no difference in the gestational inflammation model (Fig 2).

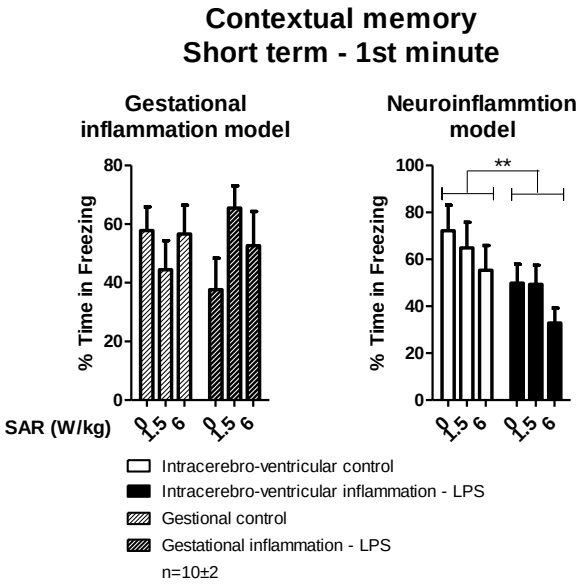

Fig. 2. GSM 900MHz and LPS effects on freezing behaviour during the first minute of short term contextual memory test. ** p<0.01: LPS treatment versus vehicle treatment. (Two-way ANOVA analyses).

There is no difference of time spent freezing during the long term contextual memory (fig. 3) and cue memory (fig. 4) tests. No effect of EMF was detected on freezing behaviour (fig. 2,3 and 4).

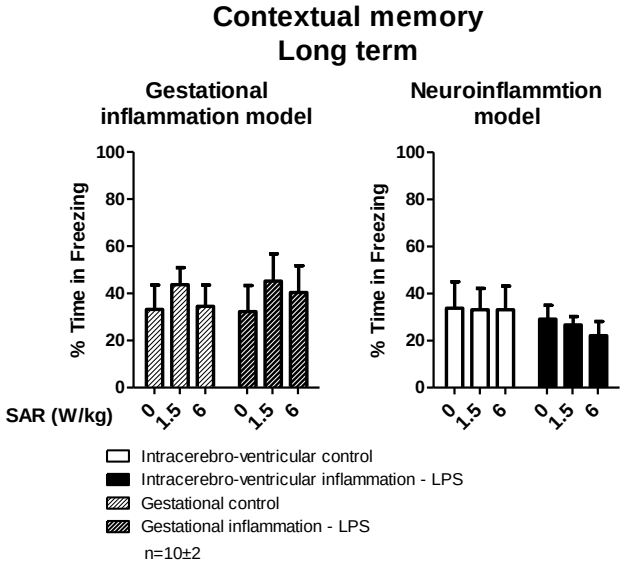

Fig. 3. GSM 900MHz and LPS effects on freezing behaviour during the long term contextual memory test.

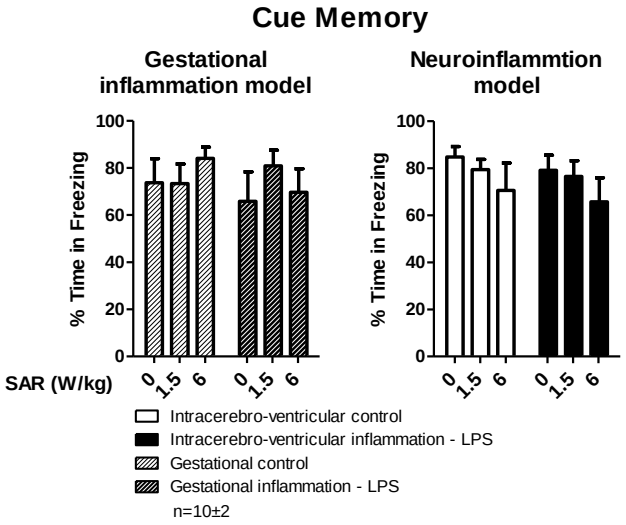

Fig. 4. GSM 900MHz and LPS effects on freezing behaviour during the cue memory test.

Startle amplitude in rats subjected to neuro-inflammation was decreased compared to control rats (two-way ANOVA: p=0.0055), but there was no effect of gestational inflammation (fig 5).

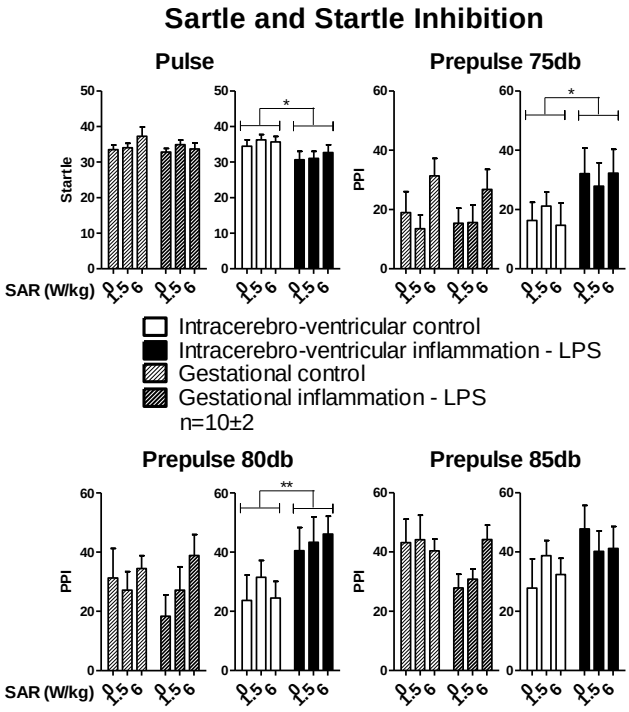

Fig. 5. GSM 900MHz and LPS effects on startle and startle inhibition. * p<0.05, ** p<0.01: LPS treatment *versus* vehicle treatment (Two-way ANOVA analyses).

Global analysis of the three days of prepulse inhibition test didn't show any effect of inflammation or EMF for both, gestational inflammation and neuroinflammation model.

When the third days was analysed separately, for the neuroinflammation model, the percentage of PPI was increased

in LPS treated rats when compared to control rats following the prepulse at 80db (two-way ANOVA: p=0.0029) and at 75db (two-way ANOVA: p=0.0253) (fig 5). No EMF effect was detected for this model (fig 5).

In the gestational model, no LPS effect was detected (Fig 5).

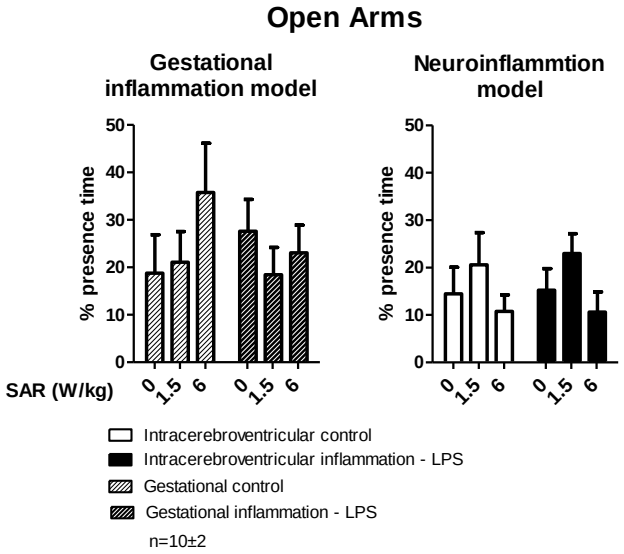

Fig. 6. GSM 900MHz and LPS effects presence time in open arm of elevated plus maze.

No LPS or GSM effect was obtained for both vulnerability models regarding the percentage of time spent in the open arm of the elevated plus maze (fig. 6) and the open field test (fig. 7, 8 and 9).

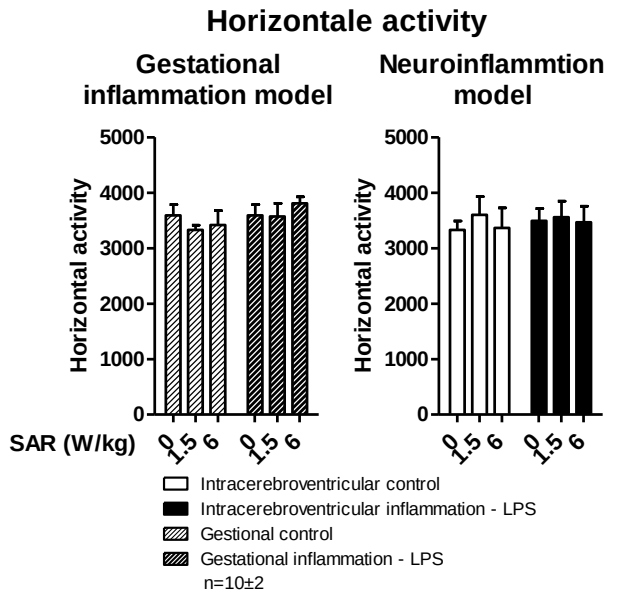

Fig. 7. GSM 900MHz and LPS effects on horizontal activity in open field test.

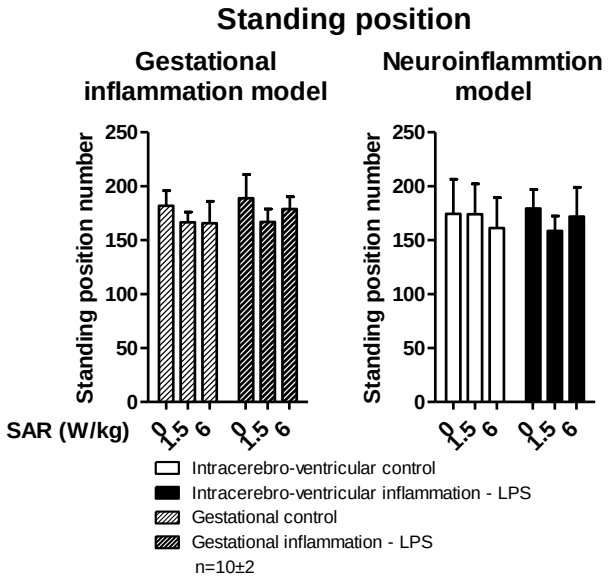

Fig. 8. GSM 900MHz and LPS effects on standing position in open field test.

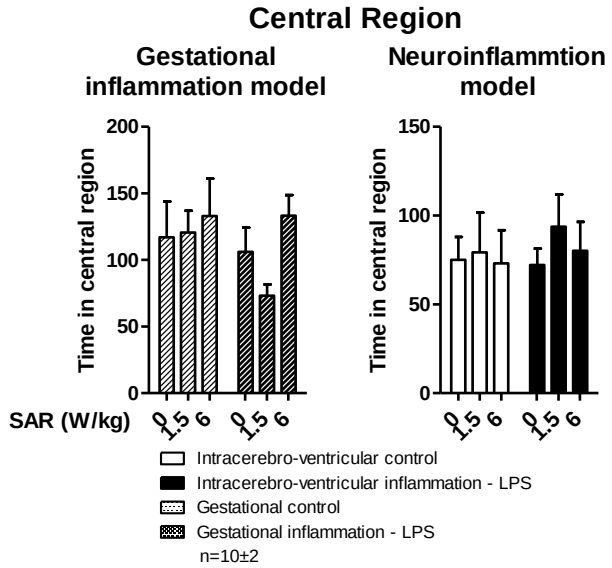

Fig. 9. GSM 900MHz and LPS effects on presence time in central region of open field test.

C. Biochemistry

In the model of neuroinflammation using intra-hippocampal diffusion, GFAP and IL-1β were significantly increased (two-way ANOVA: $p<0.0001$) in LPS treated rats when compared to vehicle treated rats (Fig 10).

Significant GSM effect was shown by the decrease of GFAP in the 1.5W/kg-LPS-exposed rats when compared to the 0W/kg-LPS-exposed group (Fig 10).

Further biochemical data for neuro-inflammation and gestational inflammation are in progress.

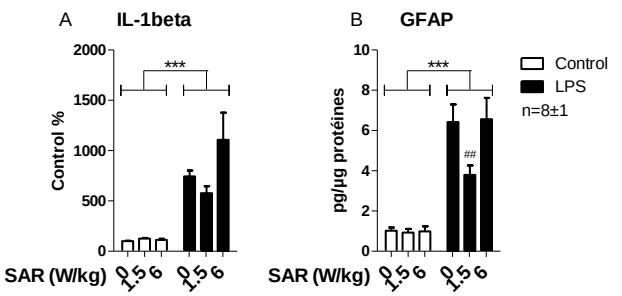

Fig. 10. GSM 900MHz and LPS effects on cerebral (A) IL-1β and (B) GFAP. * p<0.05, ** p<0.01, ***p<0.001: LPS treatment *versus* vehicle treatment; ## p<0.01: LPS 1,5W/kg exposed versus LPS 0W/kg and versus LPS 6 W/kg (post-oc Bonferroni analyses).

IV. Conclusion

Our vulnerability models were characterized and validated by gestational LPS-induced foeto-toxicity, by intra-hippocampal LPS-induced elevated cytokins and GFAP and by intracerebroventricular LPS-induced startle response to novelty and reduced short term emotional memory.

We did not show any behavioural effect of GSM in healthy adolescent rats after one month head-only exposure.

Our vulnerability models, gestational inflammation model and intracerebroventricular neuroinflammation model, did not show higher behavioural sensibility to GSM.

These data suggest no specific vulnerability of adolescents with or without pre-existing inflammation in response to mobile phone EMF exposure.

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