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# Sex dependant impact of perinatal 5G electromagnetic field exposure in the adolescent rat behaviour.

Raphaël Bodin<sup>a</sup>, Chandreshwar Seewooruttun<sup>b</sup>, Aurélie Corona<sup>b</sup>, Stéphane Delanaud<sup>b</sup>, Amandine Pelletier<sup>b§</sup> Anne-Sophie Villégier<sup>a§\*</sup>

PERITOX Laboratory (UMR\_I 01), UPJV/INERIS

<sup>a</sup> INERIS, MIV/TEAM, Verneuil-en-Halatte, France

<sup>b</sup> University of Picardie Jules Verne, CURS, Amiens, France

<sup>§</sup>Both authors have equally contributed

\*Corresponding author :

Anne-Sophie Villégier

anne-sophie.villegier@ineris.fr

(+33) 03 44 55 62 61

## Abstract:

The fifth generation (5G) network is currently being worldwide spread out, raising questions about the potential impact of this new technology, particularly on immature organisms. The current study aimed to investigate the effects of daily 5G electromagnetic field (EMF) perinatal exposure on the neurodevelopment of rats. The exposure level was set to the limit of whole-body public exposure defined by the International Commission on Non-Ionizing Radiation Protection. The mother rat specific absorption rate (SAR) was 0.07 W/kg for 22h/day at 3500 MHz continuous wave from gestational day (GD) 8 to post-natal day (PND) 21. Clinical observations were performed on weight, length, sex ratio, number of pups per litter and number of stillborn in sham and EMF-exposed groups (n=7). The age of pinna ear detachment, incisor eruption and eye opening were recorded. Behavior was assessed on righting, gripping and negative geotaxis reflexes at PND 3 or 7 and on stereotyped and horizontal movements in the open field at PND 43. Our results indicated that both male and female pups showed delayed incisor eruption in the EMF-exposed group compared to the sham group (+1 day). Regarding activity in the open field, adolescent females showed less stereotyped movements (-70%) while adolescent males showed more stereotyped movements (+50%) compared to the sham-exposed adolescent rats. Thus, the present study suggested that perinatal exposure to 5G at SAR level below reglementary threshold led to perturbations in the descendants seen in juveniles and adolescents.

Keywords: 5G communications; Radiofrequency fields; Behavioural tests; Incisors eruption; Electromagnetic radiation ; Neurodevelopment.

## INTRODUCTION

The fifth generation of mobile networks (5G) is being rolled out around the world. In 2020, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) has published new guidelines to limit exposure to electromagnetic fields (EMF). In this guideline, the exposure limit for the general public remains at 0.08 W/kg for the frequency range from 0.1 MHz to 300 000 MHz (radiofrequency (RF) range). The aim of this threshold is to avoid the thermal effects induced by RF exposure, occurring from 4 W/kg (ICNIRP, 2020).

The perinatal period is a critical window in which the organism is particularly sensitive to environmental factors. During this period, it is well known that the ability of an immature organism to excrete and metabolize chemicals is greatly reduced compared to a fully developed organism (National Research Council, 1993 ; Spielberg S.P. et al. 1992 ; Rodier P.M. et al. 1995). Consequently, neurodevelopmental impairments are observed in male and/or female offspring rodents after perinatal chemical or stress exposure (Bock et al. 2011 ; Enayati et al. 2020 ; Goel et al. 2014 ; Hiroi et al. 2016 ; Luft et al. 2020). Concerning physical factors, some data have shown that the biological barriers of an immature organism such as the skull are less efficient to prevent their penetration. In fact, local specific absorption rate (SAR) from cell phone used against the head may be 10-fold higher in the skull of children (3-14 years) compared to adults (Fernandez et al. 2018). This deeper penetration of RF may be a reason for specific or more severe effects in children.

A multicentric study on 55 507 pregnant women and their children showed no association between fetal growth or birth weight and the number of phone calls per day (Tsarna et al. 2019). However, the authors did show a link between the increasing number of phone calls per day and both shorter pregnancy duration and increased risk of preterm birth. Taken separately from this multicentric study, the Danish cohort suggested an association between RF exposure and headaches in children (Sudan et al. 2012). Vrijheid et al. (2010) showed a link between cell phone use by mothers during pregnancy (assessed by a form) and higher mental but lower psychomotor development scores. Higher environmental exposure to RF was also associated with lower scores for verbal expression/comprehension or reduced visuomotor coordination in children (Calvente et al. 2015). Controversial experimental results indicate that the neurodevelopmental effects of RF at a SAR below 4 W/kg are still a matter of debate. After birth, the weight of pups was either not modified in protocols with 0.16 to 4 W/kg SARs and RFs (1600, 1950 MHz or Wi-Fi signal at 2450 MHz) (Anderson et al. 2004 ; Sambucci et al. 2011 ; Ogawa et al. 2009) or impacted in protocols with lower SARs (1 to 5 mW/kg) and RFs from 900 MHz, an antenna park (80-900 MHz) or 2450 MHz (Alimohammadi et al. 2018 ; Magras et al. 1997 ; Sangun et al. 2014). The study of Poullietier de Gannes et al. (2012) reported no evidence of teratology during pre- or post-natal development following the *in-utero* exposure to a 2450 MHz Wi-Fi signal at SAR of 0.08 W/kg or 0.4 W/kg. Similarly according to Shirai et al. (2017) gestational exposure to multiple frequencies (two of 800 MHz band, two of 2000 MHz band, one of 2400 MHz band, two of 2500 MHz band and one of 5200 MHz band) at SAR of 0.4 W/kg did not induce embryotoxicity or effects on physical development in the offspring's.

Pups' behavior data suggested hyperactivity and lower memory performance after *in utero* exposure from a 800-1900 MHz cellular phone at SAR of 1.6 W/kg, 24h/day (Aldad et al. 2012). To the opposite, Petidant et al. showed reduced activity in the open field (OF) test after a perinatal exposition at 900 MHz (Petidant et al. 2018). A transitory deleterious effect on neonatal reflexes was shown in rats exposed during the perinatal period at 2450 MHz by a higher delay for righting from post-natal day (PND) 3 to PND 6 and negative geotaxis only at PND 5 (Othman et al. 2017). Another study with multiple RF exposures (800 MHz band, 2000, 2400, 2500 and 5200 MHz band) also showed no effect for OF and the water-maze test (Shirai et al. 2017).

Recently, two studies reported increased corticosterone and modifications in appetite and brain oxidative status in adult rodents exposed for 1 to 4 months to 3500 MHz 5G signal (Perov et al. 2022 ; Bektas et al. 2022). Because this signal is becoming ubiquitous during the neurodevelopment, new insights are required to evaluate the risk for immature organisms of long-term exposures at the ICNIRP limits. The present study affords new insights on clinical and behavioral endpoints on the developing rat under chronic 5G exposure: weight, body size, neonatal reflex performance and activity in the OF test, age of eye opening, pinna detachment and incisor eruption. Here, the 3500 MHz 5G signal exposure in rat was chronic (22h/day) from the beginning of pregnancy (gestational day (GD) 8) until pups weaning (PND 21). The exposure level in the range of 0.08 W/kg mimicked the ICNIRP limit for whole-body exposure of the public.

Here, our hypothesis was that clinical endpoints will detect differences in the rat neurodevelopment according to the presence or the absence of 0.07 W/kg 3.5 GHz RF exposure in the perinatal environment. The aim of the present study was to investigate the effects of daily twenty-two hour exposure from gestational day (GD) 8 to postnatal day (PND) 21 to a 3.5 GHz signal on neurodevelopment in rats. The exposure level was chosen to mimic the ICNIRP limit for whole body exposure of the general public.

## METHODS

### 1. Animals

Fourteen pregnant female rats (Sprague Dawley, 10-12 weeks old) were purchased from Janvier (France). Protocols complied with the decree on vertebrate animal experiments (French State Council, 1987) and were approved by the Regional Ethical Committee N. 96 (CREMEAP) and the French Ministry of Research. Rats were received at GD 3. The experiments began after a 5-day acclimatization period in their home cages.

### 2. Procedures and experimental groups

The pregnant rats were randomly assigned to the sham- (control) or RF-exposed groups ( $n = 7$ ). Two independent exposure chambers containing 7 rat cages were used, one for the sham group, the other for the RF-exposed group. They were identical in size (2.4 m in length  $\times$  1.6 m in width  $\times$  2.43 m in height), controlled air-conditioned, soundproofing, wall temperatures ( $24.1 \pm 0.2$  °C) and lighting (12 h/12 h light/dark cycle, lights on at 7 a.m. and off at 7 p.m., 200 lux).

Pregnant rats were exposed to continuous wave (CW) from GD 8 until PND 21, 22h a day (Fig. 1). Exposure was off for 2 h per day for daily care and measures. At birth, litters were adjusted to 6 pups (3 males and 3 females) by reduction or adoption. Before birth, the experimental design planned for each litter and each sex, that rat number 1 will be sacrificed at PND 9, and that rats number 2 and 3 will be tested in the OF at PND 21 and PND 43, respectively. After birth, at PND 1, these numbers were semi-randomly allocated for each sex by labelling 1, 2 or 3 ink marks on the pup back during the sexing step. Technically, the 1<sup>st</sup> male randomly picked by a blinded experimenter was labelled 1. The 2<sup>nd</sup> male semi-randomly picked was labelled 2, and so on for male and female pups. All rats underwent the neonatal tests on the righting and gripping at PND 3 and on the righting, gripping and negative geotaxis at PND 7. Two rats per litter (one male and one female) were sacrificed at PND 9. Two rats per litter (one male and one female) were tested in the OF at PND 21 and then sacrificed. After weaning, rats from the same litter were housed 4 or 3 per cage per gender and experimental group. Two rats per litter (one male and one female) were tested in the OF at PND43 and then sacrificed.

### 3. Exposure and field measurements

A generator (ANAPICO ASPIN4010 – 9kHz - 4000 MHz) was set at 3500 MHz band (CW) RF at the SAR of  $70 \pm 8$  mW/kg. It was coupled with an amplifier RFPA (RF10003800-10, band pass 1000-3800 MHz) connected to a four-output divider (NARDA band pass 2000-8000 MHz) with 4 antennas (Antenna Laird multi band CFS60383 600 MHz à 3800 MHz) equally placed above cages. Each antenna measured 23 cm $\times$ 14 cm $\times$ 5 cm and was a broadband, directional, vertically polarized gain antenna designed for indoor radio installations. The power was set so that the mean electric field at the point of measurement was 62.2 V/m. The SAR was calculated as described by Mai *et al.* (2020).

### 4. Physical development

Pup body weight and length were measured every 4 days from birth. The mothers' weight was measured every day from GD 8 to delivery and then every 4 days. Averaged daily gain (ADG) and fractional growth rate (FGR) were obtained with the following calculations:  $ADG = ((\text{body weight day} + 1) - \text{body weight}) / 4$  days and  $FGR = ((\text{body weight day} + 1) - \text{body weight}) / \text{birth weight}$  (Laporte *et al.* 2018). Each pup was observed every day to determine the age of eye opening, incisor eruption, and pinna detachment.

### 5. Neonatal reflex tests

Surface righting was assessed by the latency to right from a supine position. Grip strength was assessed using the latency to fall from a wire (1 mm in diameter) grasped by both forepaws. Negative geotaxis was tested using the aptitude (success or failure) to turn 180° when placed head downward on a 30° inclined plane. In these 3 tests, the observation stopped after 30 seconds (s) even if the behaviour did not occur (censored data).

## 6. Open field

The rats were placed for 5 minutes in the arena of a Plexiglas box (60 × 60 × 40 cm). Horizontal activity and stereotyped movements were automatically recorded with the interruption of the 4 lower and/or 4 upper infrared beams located at the adjacent sides of the arena. Stereotyped movements were defined as repetitive motor responses that are invariant and have no purpose or goal (for example repeating back and forth displacements on a short distance).

## 7. Statistical analysis

Means ± standard error were analysed using GraphPad Prism (Version 8.0.2, GraphPad Software, Boston, Massachusetts USA). In complete data sets, three and two-ways ANOVAs were performed. Age, RF exposure and sex were considered as between subject factors. When significant effects, Sidak's or Tukey's t-test post-hoc were performed. When data sets were not complete (for missing water intake data), we performed mixed effects analysis and multiple comparison tests. Independent t-test was used for the number of pups at birth and the number of pups born alive and Chi-square test was used for the sex ratio of litters at birth. Log Rank, Contingency and Chi-square with Yates' correction analyses, were performed for the neonatal reflexes. Survival test with Gehan-Breslow-Wilcoxon test was performed for the occurrence of clinical signs (incisor eruption, pinna detachment or eye opening). Significant effects were reported when p-value was inferior to 0.05.

# RESULTS

## 1. Physical development

The weight of mothers was presented in Figure 2a. The two-way ANOVA indicated no effect of 3500 MHz exposure on mothers' weight ( $p > 0.05$ , Table 1). As expected, there was a significant increase of weight with age during the gestation period ( $F_{24,288} = 265.1$ ,  $p < 0.0001$ , Table 1). There was no effect of the 5G exposure on the mothers ADG or FGR during the pre- or post-natal period when analyzed with two-way ANOVAs test (data not shown). Only an expected age effect was significant for ADG ( $F_{4,288} = 170.7$ ,  $p < 0.0001$ ) and FGR ( $F_{4,291} = 139.6$ ,  $p < 0.0001$ ).

The mothers water intake per cages was presented in Figure 2b. The mixed-effects analysis and multiple comparison test indicated the mothers water intake was not impacted by 3500 MHz exposure ( $p > 0.05$ , Table 1). However, mothers water intake was impacted by the interaction between age and 3500 MHz exposure ( $F_{30,345} = 1.878$ ,  $p = 0.0043$ , Table 1) and an effect of age ( $F_{30,345} = 17.84$ ,  $p < 0.0001$ , Table 1).

The size of litters on PND 0 is presented in Figure 2c. The independent t-test indicated that the 3500 MHz exposure had no impact on the size of the litters. The sex balance of litters is presented in Figure 2d. The Chi-square indicated no difference on the sex ratio between the sham (ratio males/females = 1.117) and RF-exposed groups (ratio males/females = 0.8654). The percent of pups born alive is presented in Figure 2e. The independent t-test test indicated that the 3500 MHz exposure had no impact on the percent of pups born alive.

Data reported in Figure 3a and 3b present body weight of male and female pups, respectively. The three-way ANOVA indicated a significant weight increased with age ( $F_{5,368} = 1529$ ,  $p < 0.0001$ , Table 1), and sex effect ( $F_{1,368} = 13.60$ ,  $p = 0.0003$ , Table 1) but no effect of the 3500 MHz exposure ( $p > 0.05$ , Table 1).

Data reported in Figure 3c and 3d present body length of male and female pups, respectively. The three-way ANOVA test showed an increase in length with age ( $F_{4,315} = 2741$ ,  $p < 0.0001$ , Table 1) and in males compared to females (sex effect:  $F_{1,315} = 5.315$ ,  $p = 0.0218$ , Table 1) but no RF exposure or interaction.

Figure 4a and 4b represent the age of ear detachment for males and females, respectively. Median for ear detachment was 3 days in both males and females (RF and sham-exposed groups). The Log-rank test indicated no effect of the 3500 MHz exposure ( $p > 0.05$ ).

Figure 4c and 4d represent the age of incisor eruption for males and females, respectively. Median for incisor eruption was 8 days in both males and females sham-exposed groups and 9 days in both males and females RF-exposed groups. The Log-rank test indicated that the incisors eruption was delayed in the 5G exposed group in both male ( $\text{Chi}^2 = 5.373$  ;  $p = 0.015$ ) and female pups ( $\text{Chi}^2 = 5.913$  ;  $p = 0.020$ ).

Figure 4e and 4f represent the age of eyes opening occurrence for males and females, respectively. Median for eyes opening was 14 days in both males and females (RF and sham-exposed groups). The Log-rank test indicated no effect of the 3500 MHz exposure ( $p > 0.05$ ).

## 2. Neonatal reflexes

Figures 5a and 5b presented righting for females at PND 3 and PND 7, respectively. Figures 5c and 5d presented righting for males at PND 3 and PND 7, respectively. Pups righted significantly faster at PND 7 compared to PND 3 ( $p < 0.0001$ ). The log-rank test indicated that the 3500 MHz exposed pups righting reflexes were not modified compared to the sham pups for both males and females, at PND 3 or 7.

Figures 6a and 6b presented gripping for females at PND 3 and PND 7, respectively. Figures 6c and 6d presented gripping for males at PND 3 and PND 7, respectively. The latency to fall was significantly longer at PND 7 compared to PND 3 ( $p < 0.0001$ ). The log-rank test indicated that the 3500 MHz exposed pups gripping reflexes were not modified compared to the sham pups for both males and females, at PND 3 or 7. There was no sex difference for neonatal tests ( $p < 0.05$ ).

Data reported in Figure 7 presents the percentage of successful pups to perform the negative geotaxis at PND 7. The contingency test (Fisher's exact test) performed in both males and females indicated no difference in their reflex to turn  $180^\circ$  between the 3500 MHz-exposed and the sham-exposed pups (males:  $p = 0.16$ ; females:  $p = 0.75$ ; all pups:  $p = 0.17$ ).

## 3. Open field tests

Data reported in Figure 8a presents the total interruptions of the infrared beams in the arena of OF for the pups (males and females, at PND 21 and 43) and their mothers (at about PND 85). The three-way ANOVA performed on total activity indicated no effect of RF or sex but did show significant sex by RF interaction ( $F_{1,48} = 15.71$ ,  $p = 0.0002$ , Table 2) with increased total activity. With Sidak test, sex effect was not significant for sham group but significant in RF exposed group ( $p = 0.0067$ ). When looking at the RF effect in male or female pups, no effect of exposure was found in male pups, but an effect was found with a decrease in total activity for female pups ( $p = 0.028$ ).

Data reported in Figure 8b presents the horizontal interruptions of the infrared beams in the arena of OF for the pups (males and females, at PND 21 and 43) and their mothers (at about PND 85). The three-way ANOVA performed on horizontal activity indicated no effect of RF, sex or interaction but did show significant effects of age ( $F_{1,48} = 25.51$ ,  $p < 0.0001$ , Table 2).

Data reported in Figure 8c presents the fast and repetitive interruptions of the infrared beams in the arena of OF for the pups (males and females, at PND 21 and 43) and their mothers (at about PND 85) (stereotypic activity). The three-way ANOVA performed on stereotyped movements indicated effect of RF ( $F_{1,48} = 8.58$ ,  $p = 0.0052$ , Table 2), sex ( $F_{1,48} = 6.86$ ,  $p = 0.012$ , Table 2), and interaction between sex and RF ( $F_{1,48} = 63.85$ ,  $p < 0.0001$ , Table 2). Two-way ANOVA test with sex and RF factors showed an effect for RF ( $F_{1,52} = 6.445$ ,  $p = 0.0142$ , Table 2), sex ( $F_{1,52} = 5.156$ ,  $p = 0.0273$ ) and a significant interaction between sex x RF ( $F_{1,52} = 47.97$ ,  $p < 0.0001$ ). The multiple comparison with Sidak test for adolescent males showed that the RF-exposed group had more stereotyped behaviors ( $p = 0.0062$ ). In females, it showed that they had less ( $p < 0.0001$ ). There was also an effect of sex in both sham ( $p = 0.0036$ ) and exposed groups ( $p < 0.0001$ ).

Following an adult period exposure, the independent t-test suggested no effect of 3500 MHz exposure. This data suggested that the perinatal period is likely to be more critical than the adult period for the possible 3500 MHz exposure induced stereotypy modification.

## DISCUSSIONS

The present study explored the potential effects of 3500 MHz 5G exposure at reglementary threshold during the rat neurodevelopment. We did not observe any effect on maternal or pup morphology regarding their weight, length, or viability. Incisor eruption was postponed in juvenile males and females of the 3500 MHz exposed group. At adolescence, stereotyped activity was higher in males (+50%) and lower in females (-70%) of the 3500 MHz exposed group.

The RF exposure applied in the chamber was set to correspond to the 5G frequency of 3500 MHz CW with a whole-adult body averaged SAR of  $0.07 \pm 0.008$  W/kg and 62.2 V/m. Our goal by setting the exposure to the authorized threshold was to assess the limits that has been set to prevent adverse effects from EMF exposures.

In previous modelling performed in perinatal RF exposures in rodents, fetuses received a SAR about 10 to 14% lower than the mothers. In mice, the fetuses SAR was 14% of the mother's, which was 4 W/kg at 900 MHz (McIntolsh et al [2010](#)). In rats, the SAR of the fetuses was about  $0.7 \pm 1.5$  W/kg when the local belly SAR of the mothers was 6 W/kg at 900 MHz (Petitdant et al [2018](#)). We may hypothesis that in the present protocol the fetuses and pups whole body SAR were lower than the mother's, i.e. than 0.07 W/kg.

The actual environmental exposure in Europe in 2013 was estimated to reach between 0.08 V/m and 1.8 V/m with less than 1% above 6 V/m and less than 0.1% above 20 V/m ([Gajsek et al. 2013](#)). A more recent paper published in 2022 estimated between 0.67 to 1.51 V/m ([Iakovidis et al. 2022](#)). Recent measures performed in South Korea by our Institution indicated that the 5G 3500 MHz frequency deployment did not increase these environmental levels (data not shown).

Peak brain growth spurt occurs between PND 7 and PND 10 for rats and in the last gestational weeks in human, around the 36<sup>th</sup> to the 40<sup>th</sup> weeks of gestation ([Semple BD et al. 2013](#)). Cerebral cortex maturation in rats at PND 12 to 13 is considered equivalent to the one of termed human newborn (PND 0) (Romijn et al. [1991](#)).

Here, our results showed that perinatal exposure did not affect the mothers on weight, water intake and locomotor activity. Litters were not affected either on size, percent of pups born alive or sex ratio. Our findings are consistent with results found at similar SAR levels at different frequencies (with similar levels of penetration into the body or deeper). The study of Poullietier de Gannes et al. ([2012](#)) has reported no evidence of teratology during pre- or post-natal development following the *in-utero* exposure to a 2450 MHz Wi-Fi signal at SAR of 0.08 W/kg or 0.4 W/kg. Similarly, according to Shirai et al. ([2017](#)), gestational exposure to multiple frequency (two of 800 MHz band, two of 2000 MHz band, one of 2400 MHz band, two of 2500 MHz band and one of 5200 MHz band) at SAR of 0.4 W/kg did not induce embryotoxicity or effect on physical development in the offspring's.

Some effects were observed with similar protocols but lower RF frequencies. Global System for Mobile Communications (GSM) signal has shown an effect on pre- and post-natal development in rats with a reduction in the number of live embryos ([Alchalabi et al. 2016](#)). In mouse, GSM exposure showed an increase in the rate of supernumerary fingers of fetuses ([Yang et al. 2013](#)). To date, in the same protocol using 900 MHz, we showed reduced male pup's weight with SAR of 0.4 W/kg (Bodin et al. In preparation). Interestingly, Bosquillon de Jenlis et al. ([2020](#)) showed that the body mass was increased in juvenile rats exposed PND 25 to PND 61 at 900 MHz with SAR of 0.03 W/kg. Several studies showed that exposure to 900 MHz induced increase in mice body mass and length ([Alimohammadi et al. 2018](#)). Shahin et al. ([2013](#)) and Sangun et al. ([2014](#)) reported that prenatal exposure to 2450 MHz signal reduced fetal growth (during 1 to 6 days of gestation) or post-natal (2<sup>nd</sup> week to 4<sup>th</sup> week postnatal) in rats. After GSM signal exposure, Alchalabi et al. ([2016](#)) showed a reduction of body mass in fetus at GD 20.

According to the EMF physical properties, at 1 MHz the body tissue penetration depth is 70 cm, 3.4 cm for 1000 MHz and 0.27 cm for 10 000 MHz in specific biological tissue ([Adair et al. 2002](#)). According to the Anses report, at 3500 MHz, the typical penetration depth in human body reaches about 1.5 cm in dry skins ([anses report, 2021](#)). If we assume a direct-action mechanism, we expect more effect with more penetration in the body of the RF waves.

Thus, we cannot exclude that 900 MHz frequency may elicit more effects than the 3500 MHz frequency because of its deeper penetration in the body. Moreover, the developmental window of exposure (pre, neo or post-natal or both) may also impact the RF exposure effects. Beside these two parameters, the previous experimental protocols used different power density, source of radiation, time of exposure per day. These differences lead to conflicts in concluding about the potential effects of perinatal exposure to RF on pups' growth.

Here, pups showed ear detachment at PND3, incisor eruption at PND9 and eye opening at PND14. The 3500 MHz exposed male and female pups showed a 1-day postponed incisor eruption but the same timeline of physical development for ear detachment at PND3 and eyes opening at PND14 as the sham-exposed group. Here, the superficial EMF energy penetration into the ears did not show direct macroscopic impact on detachment which occurred at PND3. We cannot exclude that only 3 days postnatal exposure was a too short duration to elicit an impact. In the same way, the superficial EMF energy penetration into the eyes did not show direct macroscopic impact on opening which occurred at PND14.

The 14-day prenatal and 9-day postnatal exposure may have impacted incisor eruption through modification of its neuronal circuitry ontogeny and/or gum or tooth germ maturation through a superficial EMF energy penetration. Indeed, a 1.5 cm penetration depth of the 3500 MHz frequency in rodents may have induced energy absorption in the fetuses and energy absorption in the whole brain of the newborn with the highest dose in the superficial organs.

Delay in incisor eruption was also observed after pharmacological treatments with methamphetamine (acting on dopaminergic, noradrenergic and serotonergic transporters) in pregnant rats ([Rüedi-Bettschen et al.2017](#)). A recent study conducted by Lozano et al. ([2021](#)) also showed that exposure of pregnant rats to stress and/or sertraline (a serotonin reuptake inhibitor) induced a delay in incisor eruption in male offspring. Serotonin and dopamine are known to play a role in the tooth germ maturation ([Fujino et al. 2020](#), [Moiseiwitsch et al. 1996](#)) and in neurodevelopment ([Hanswijk et al. 2020](#) , [Areal et al. 2020](#)). We hypothesize that RFs could induce alterations in serotonergic or/and dopaminergic components which may explain the postponed incisor eruption from PND9 to PND10.

This effect occurred without any impact on the neonatal reflexes at PND 3 and 7 on righting, gripping and negative geotaxis. This data may be interpreted as a harmonious cortical development during fetal and newborn growth ([Nguyen et al. 2017](#)) without any impact of the perinatal 3500 MHz exposure. We cannot exclude that the 7 days postnatal exposure was a too short exposure duration and/or that the pre- and post-natal whole body maternal SAR (0.07 W/kg) was a too low SAR to modify measurably the PND 7 neonatal reflexes.

Activity in the OF test gives evaluation of horizontal, vertical, and stereotyped activity ([Prut et al. 2003](#)). In our study, the adolescent rats which received the perinatal exposure at 3500 MHz showed the same horizontal activity as the sham exposed group. The previous studies conducted on the behavioural effects of perinatal or adult exposures to RF at non-thermal levels showed conflicting results which were not conclusive. A transient adverse effect on emotional and motor behaviour of rats exposed to 2 450 MHz RF perinatally was also observed ([Othman et al. 2017](#)). However, recent studies have described behavioural disturbance with a reduced activity in the OF test when exposed to 900 MHz ([Petitdant et al. 2018](#)). No effect was found for multiple frequency RF exposures at low SAR levels (0.4 W/kg) ([Shirai et al. 2017](#)).

Regarding stereotyped behaviour, our data indicated that the 3500 MHz exposed adolescent females showed a reduction of stereotyped movements whereas adolescent males an increase of stereotyped movements. Stereotyped movements can be defined as repetitive motor responses that are invariant and have no purpose or goal. Studies performed in rats have shown a reduction of these responses when the administration of amphetamines was combined with 2 hours of exposure to extremely low frequency field ([Prolic et al. 2005](#) , [Janac et al. 2005](#) ). These responses can model symptoms of mental disorders like autism or of drug (as amphetamine) consumption. Studies in a mice model of autism showed that mutation in shank3 gene, playing a major role in the



development of neuronal connectivity, induce the appearance of stereotyped movements ([Wang et al. 2011](#) ; [Peca et al. 2011](#)).

The sex dependant effects of perinatal 3500 MHz on pup's stereotypies showed in our study have also been shown in several RF studies ([Danker-Hopfe et al. 2020](#) ; [Zhang et al. 2015](#) ; [Yan et al. 2022](#)). The behavioural difference between male and female adolescents may be linked to all the brain sexual differentiation which occurred from conception until adolescence ([Seney et al. 2013](#)). Interestingly, it has been previously described that dopamine ([Woodcock et al. 2020](#); [Zachry et al. 2021](#) ; [Williams et al. 2021](#)) and serotonin ([Songtachalert et al. 2018](#) ; [Spies et al. 2020](#)) are differentially expressed in males and females.

An effect of RF was previously shown on serotonin metabolites in adult rats exposed to a 915 MHz signal at 2 W/kg for 8 h/day, 5 days/week for 2 weeks ([Kim et al. 2021](#)). An effect of RF exposure was also shown to decrease dopamine levels in the striatum in mice exposed to 835 MHz at 4W/kg for 5h/day, for 12 weeks ([Kim et al. 2019](#)). If it is possible that serotonin and dopamine may be involved in the sex dependant 3500 MHz effect on stereotyped behavior ([Lee et al. 2021](#); [Mandic-Maravic et al. 2022](#)), horizontal activity which is also dependent on these neuromodulators was not modified in the 3500 MHz-exposed groups in the present study.

The dosimetry work of Petitdant et al (2018) highlighted that the SAR varied up to 10 times in the fetuses depending on their position in the uterine horns. Here, prenatal SAR assessment would have required to identify the position of each descendant in the uterine horn early during pregnancy. According to Bibin et al (2009), fetal exposure in human also depends on the fetal position. Thus, one limitation in the present study is the difficulty to give an accurate prenatal SAR – response relationship.

## CONCLUSIONS

This paper explored the potential effects of 3500 MHz 5G exposure at reglementary threshold during the rat neurodevelopment. We did not observe any effect on maternal or pup morphology regarding their weight, length, or viability. Incisor eruption in juvenile males and females showed a postponed. This group also showed a main behavioral modification with an increased activity in adolescent males and a decreased activity in adolescent females in the OF. Our data support the hypothesis of an impact of the 3500 MHz 5G exposure in the developing rat at SAR below the authorized environmental threshold. It is worth noticing that if we consider a penetration of 1.5 cm of this frequency, energy is likely to be absorbed in the fetus rat and the newborn rat brain, due to their small size (inferior to 1.5 cm). If one transpose this data to human, penetration will not reach the brain, but energy will meanly be absorbed in the superficial sensorial organs in the 1.5 cm depth as the skin, the eyes, the ear. Thus, one may consider probable the transposition of the effect of 3500 MHz to delay the rat incisor eruption to human. This point may need to be further assessed in human. Meanwhile, further studies are needed to assess whether exposure to 5G during neurodevelopment could have long-term effects on behavior and to identify the mechanism by which these effects are induced.

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### **Ethical Approval**

Protocols complied with the decree on vertebrate animal experiments (French State Council, 1987) and were approved by the Regional Ethical Committee N. 96 (CREMEAP) and the French Ministry of Research.

### **Consent to participate**

All individual participants gave their oral and written informed consent in this work.

### **Consent for publication**

All authors agreed to publish this manuscript.

### **Author Contributions**

[Raphaël Bodin], [Stéphane Delanaud], [Amandine Pelletier] and [Anne-Sophie Villegier] contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Raphaël Bodin], [Chandreshwar Seewooruttun], [Aurélié Corona], [Stéphane Delanaud], [Amandine Pelletier] and [Anne-Sophie Villegier]. The first draft of the manuscript was written by [Raphaël Bodin]. [Chandreshwar Seewooruttun], [Aurélié Corona], [Stéphane Delanaud], [Amandine Pelletier] and [Anne-Sophie Villegier] read, commented and approved the manuscript.

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### **Competing Interests**

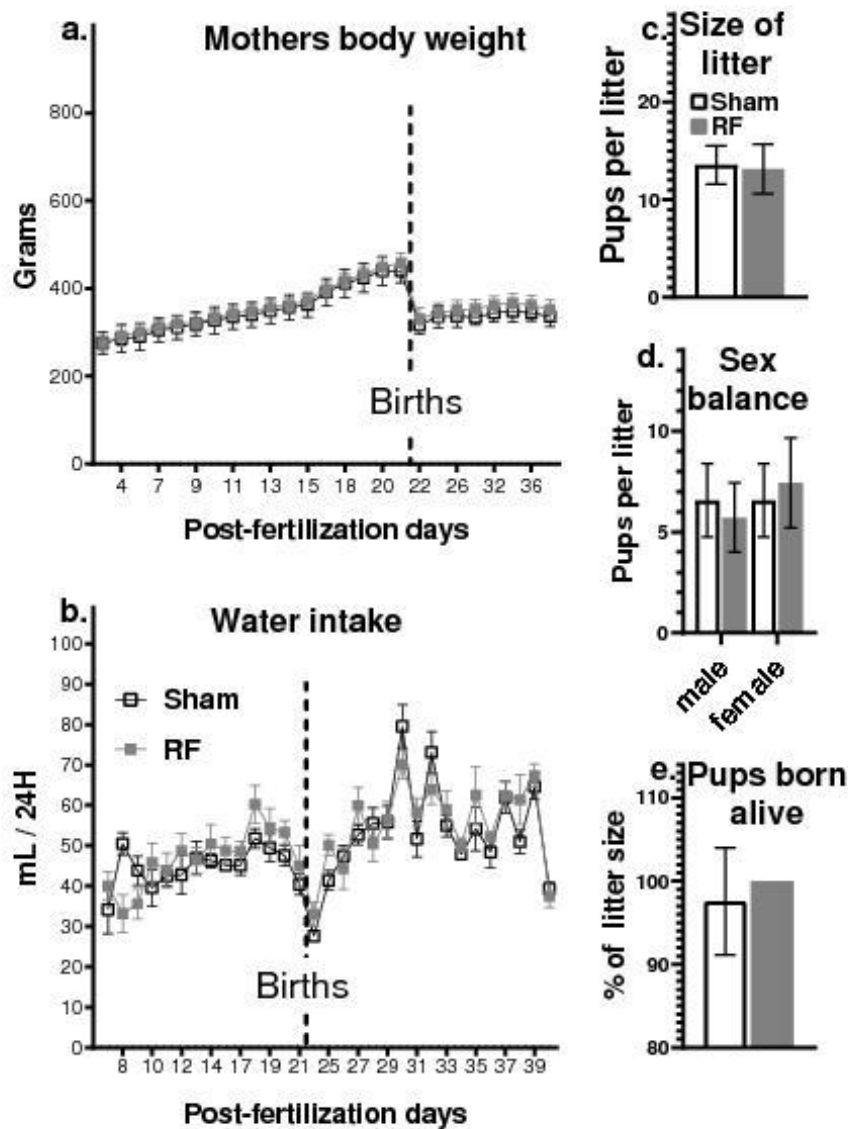
The authors have no relevant financial or non-financial interests to disclose.

## FIGURE

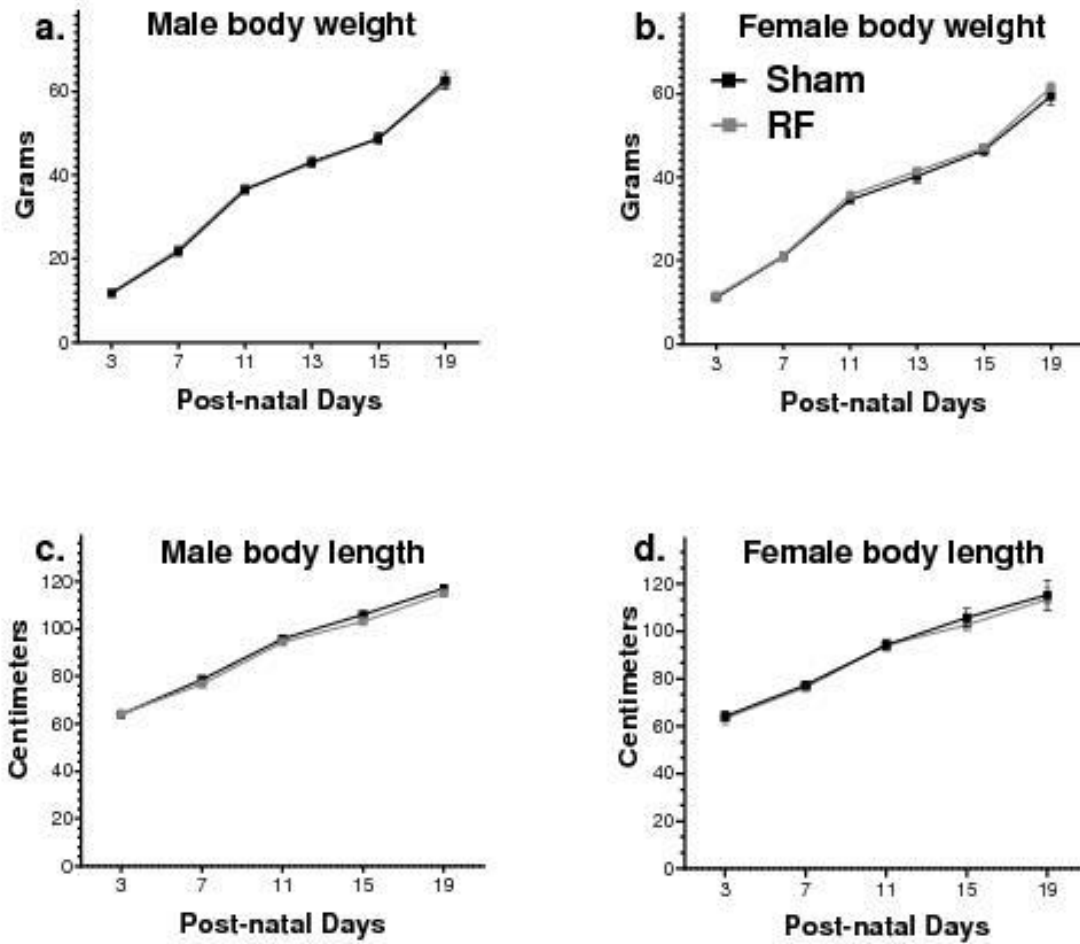
**Fig. 1** Gestational and post-natal RF exposure and post-natal test experimental design. From GD 8 until PND 21, the rats were daily exposed to 3500 MHz radiofrequency (RF) from 6:00 p.m. to 4:00 p.m. the next day (at a SAR of 0 (sham-exposed group) or 0.07 W/kg (RF-exposed group). Physical development was evaluated every 4 days from birth. Neonatal tests were performed at PND 3 and 7. Open field (OF) test was performed in independent groups of juveniles (PND 21) and adolescents (PND 43).

RF exposure									
Gestation		Lactation					Withdraw		
Prenatal		Neonatal			Adolescence				
GD 8	2	PND 0	3	7	9	21	23	42	43
<b>Behavioral tests :</b>									
		Reflex	Reflex	Sacrifice	Open field and sacrifice	Dams sacrifice	Open field	Sacrifice	

**Fig. 2** Absence of effect of perinatal 3500 MHz exposure on the mothers and the litters at birth. There was no difference between the 3500 MHz and sham-exposed groups regarding the dam's body weight (a), the mothers water intake (b), the total number of pups per litter (litter size) (c), the proportion of males and females per litters (d) and death pups at birth (percentage of pups born alive) (e) ( $p > 0.05$ ).  $N = 7$  / group.



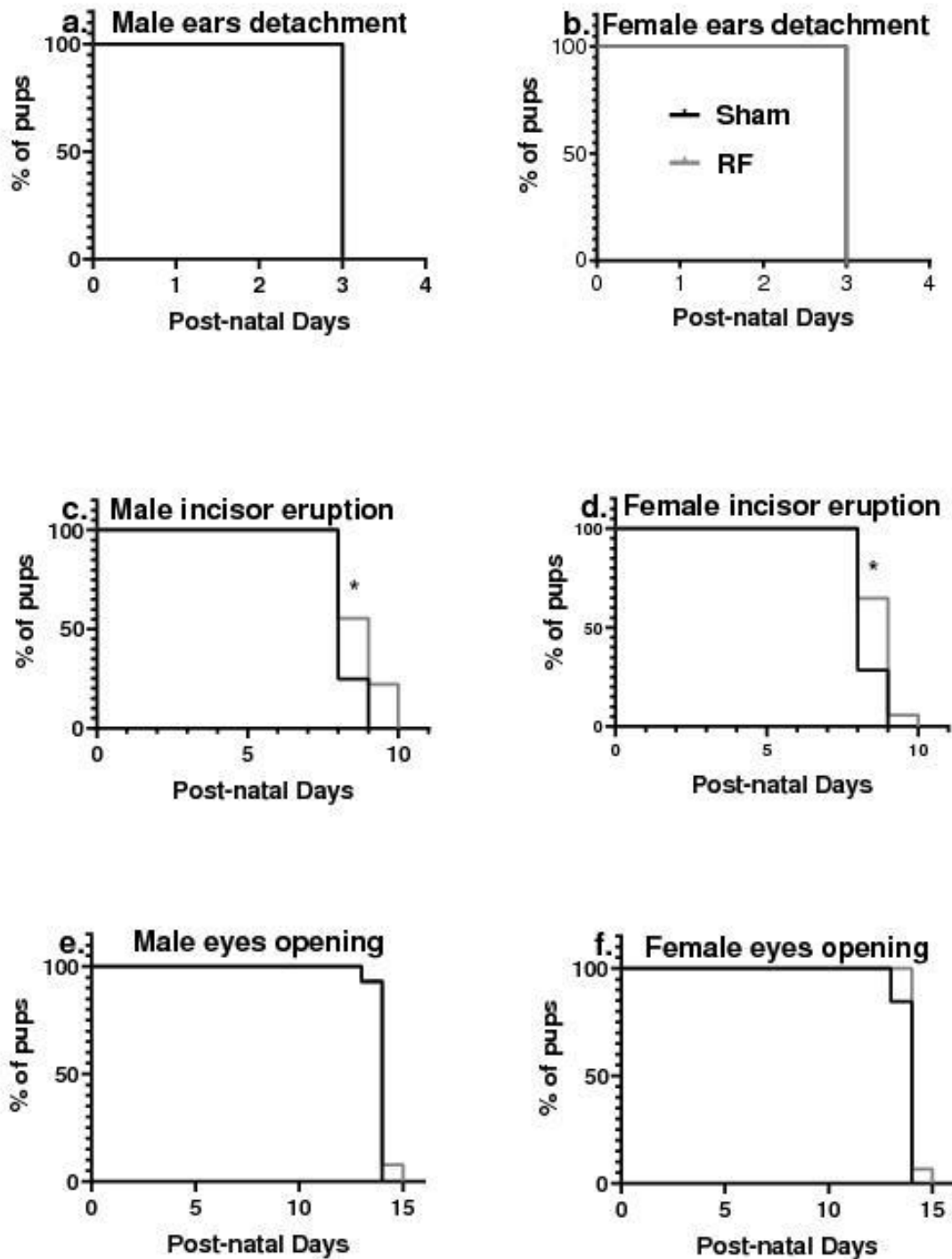
**Fig. 3** Absence of effect of perinatal 3500 MHz exposure on the growth of the descendance. There was no difference between the 3500 MHz and sham-exposed groups regarding the body weight in males (a) and females (b) and the body length in males (c) and females (d) ( $p > 0.05$ ).  $N = 7$  / group.



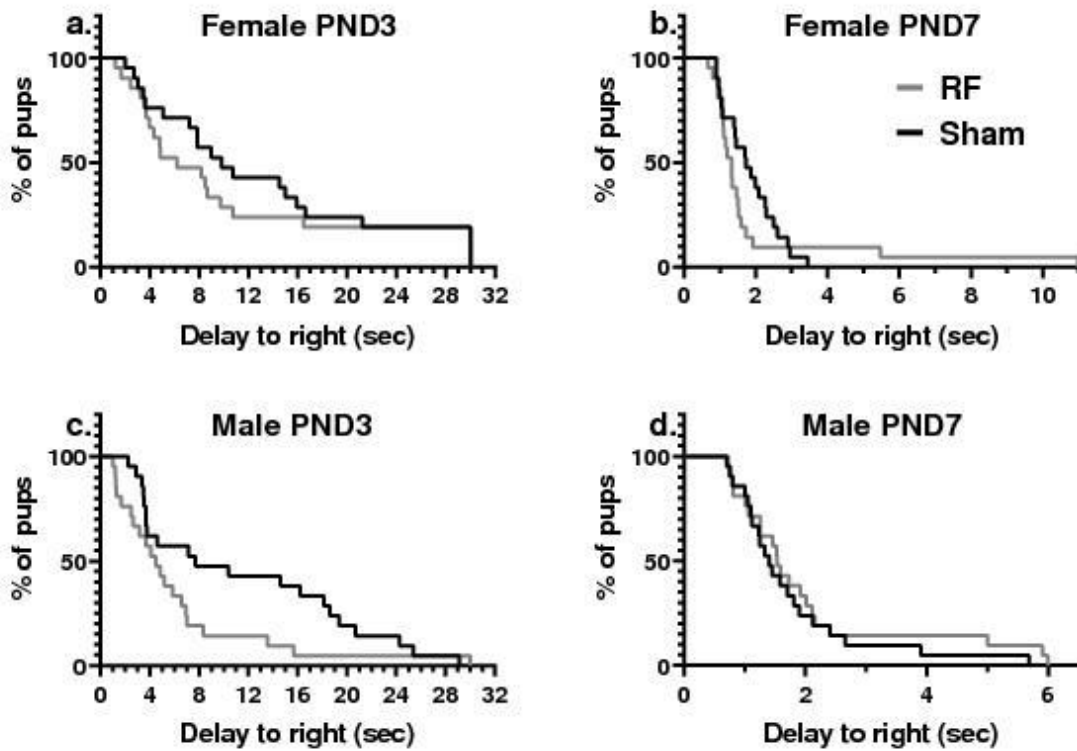


**Fig. 4** The perinatal 3500 MHz exposure postponed incisor eruption.

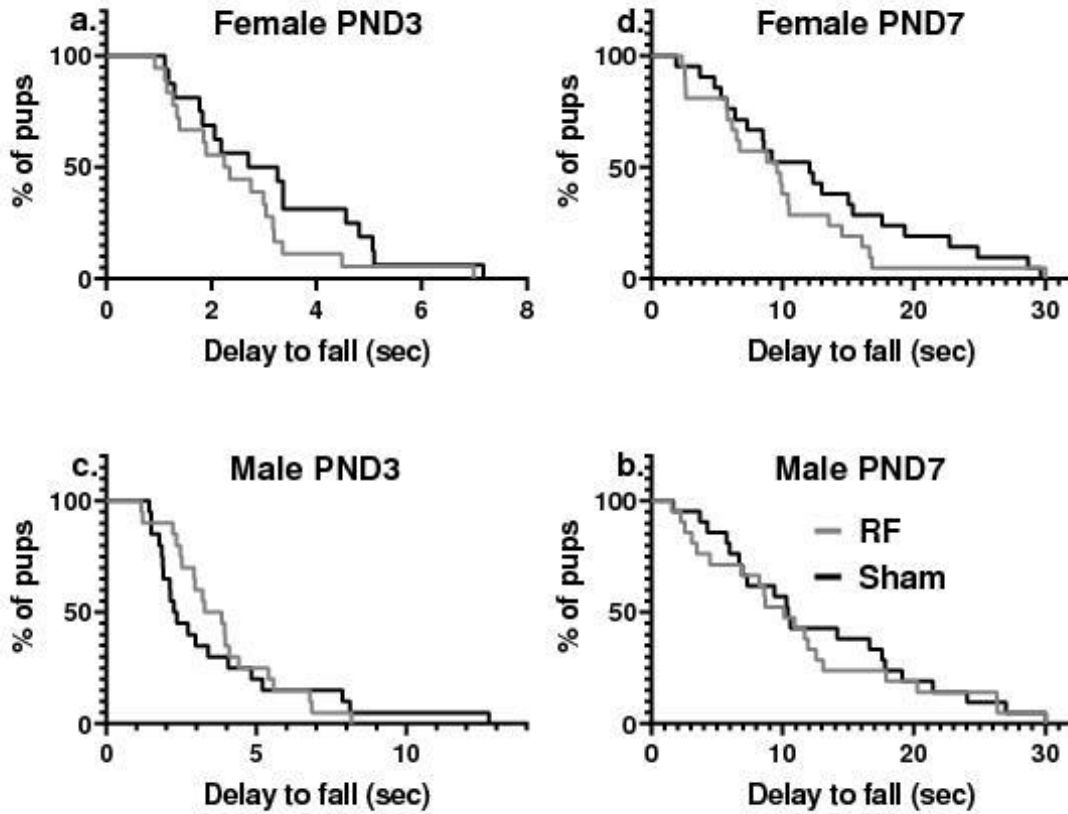
There was no difference between the 3500 MHz and sham-exposed groups regarding ear detachment for males (a) and females (b) at PND 3 ( $p > 0.05$ ). Incisor eruption was postponed in the 3500 MHz-exposed males (c) and females (c) at PND 10 compared to the sham-exposed groups (at PND 9) ( $p < 0.05$ ). There was no difference between the 3500 MHz and sham-exposed groups regarding eye opening for males (e) and females (f) at PND 14 ( $p > 0.05$ ).  $N = 7$  / group.



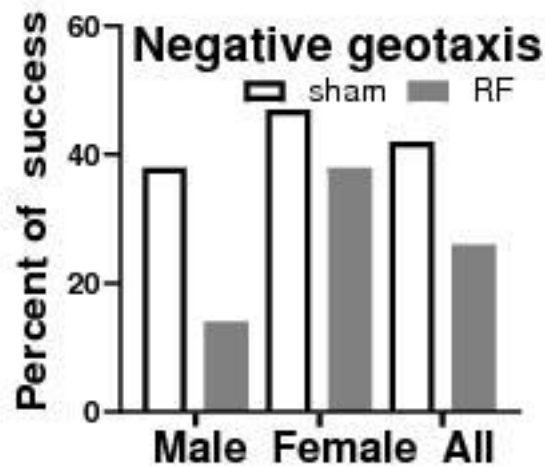
**Fig. 5** Absence of effect of perinatal 3500 MHz exposure on the neonatal righting reflex of the descendance. There was no difference between the 3500 MHz and sham-exposed groups regarding the latency to right in females at PND3 (a) at PND 7 (b) and in males at PND3 (c) and at PND7 (d) ( $p > 0.05$ ).  $N = 7$  / group.



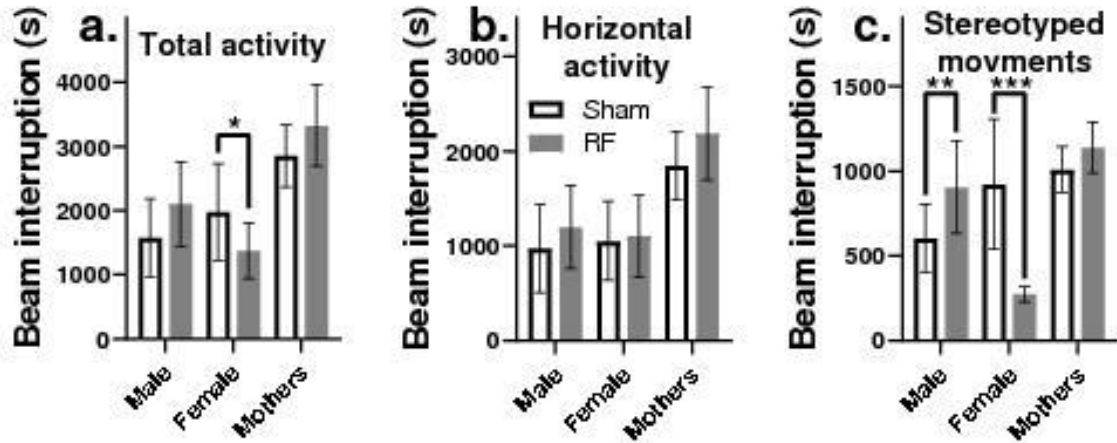
**Fig. 6** Absence of effect of perinatal 3500 MHz exposure on the neonatal gripping reflex of the descendance. There was no difference between the 3500 MHz and sham-exposed groups regarding the latency to fall in females at PND3 (a) at PND 7 (b) and in males at PND3 (c) and at PND7 (d) ( $p > 0.05$ ).  $N = 7 / \text{group}$ .



**Fig. 7** Absence of effect of perinatal 3500 MHz exposure on the neonatal negative geotaxis reflex of the descendance. There was no difference between the 3500 MHz and sham-exposed groups regarding the negative geotaxis in males, females, or all (males + females) at PND 7 ( $p > 0.05$ ).  $N = 7 / \text{group}$ .



**Fig. 8** The perinatal 3500 MHz exposure showed sex-dependant impairment of stereotyped behavior in the descendance. (a) Total activity was not different between the 3500 MHz and sham-exposed groups in adolescent males and adult mothers ( $p > 0.05$ ) but was reduced in adolescent females (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). (b) Horizontal activity was not different between the 3500 MHz and sham-exposed groups in adolescent males and females and in adult mothers ( $p > 0.05$ ). (c) Stereotyped behavior was not different between the 3500 MHz and sham-exposed groups in adult mothers ( $p > 0.05$ ) but was reduced in adolescent females (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ) and increased in adolescent males (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ).  $N = 7$  / group.



**Table 1.** Results of the analysis of variance for physical development

Variables		Source of Interaction	dF	MS	F	p-value
Two-way Anova	Mother's body weight	<b>Age<sup>a</sup></b>	<b>24</b>	<b>28550</b>	<b>265.1</b>	<b>&lt;0.0001</b>
		RF exposure	1	9099	0.7692	0.3977
		Age <sup>a</sup> * RF exposure	24	101.1	0.9388	0.5488
Mixed-effects	Mother's water intake	<b>Age<sup>a</sup></b>	<b>6.945</b>	/	<b>17.84</b>	<b>&lt;0.0001</b>
		RF exposure	1	/	0.4541	0.5132
		<b>Age<sup>a</sup> * RF exposure</b>	<b>30</b>	/	<b>1.878</b>	<b>0.0043</b>
Three-way Anova	Pup's body weight	<b>Age<sup>b</sup></b>	<b>5</b>	<b>22256</b>	<b>1529</b>	<b>&lt;0.0001</b>
		<b>Sex</b>	<b>1</b>	<b>197.9</b>	<b>13.60</b>	<b>0.0003</b>
		RF exposure	1	17.78	1.221	0.2698
		Age <sup>b</sup> * Sex	5	6.447	0.4429	0.8184
		Age <sup>b</sup> * RF exposure	5	0.5244	0.03603	0.9993
		Sex* RF exposure	1	20.97	1.441	0.2308
		Age <sup>b</sup> * Sex * RF exposure	5	5.769	0.3963	0.8513
	Pup's body length	<b>Age<sup>b</sup></b>	<b>4</b>	<b>29283</b>	<b>2741</b>	<b>&lt;0.0001</b>
		<b>Sex</b>	<b>1</b>	<b>56.78</b>	<b>5.315</b>	<b>0.0218</b>
		<b>RF exposure</b>	<b>1</b>	<b>166.8</b>	<b>15.61</b>	<b>&lt;0.0001</b>
		Age <sup>b</sup> * Sex	4	3.642	0.3409	0.8502
		Age <sup>b</sup> * RF exposure	4	17.78	1.665	0.1580
		Sex* RF exposure	1	1.931	0.1807	0.6711
		Age <sup>b</sup> * Sex * RF exposure	4	4.543	0.4252	0.7904

Legend: dF - degrees of freedom; MS - mean square; F - MS factor/MS residual; P-value - statistical significance; Age<sup>a</sup> - age of mother; Age<sup>b</sup> - age of the pups

**Table 2.** Results of the analysis of variance for the open field test

Variables		Source of Interaction		dF	MS	F	p-value
Three-way Anova	Pup's total activity	Age <sup>b</sup>		1	<b>6649539</b>	<b>23.64</b>	<b>&lt;0.0001</b>
		Sex		1	375069	1.333	0.2539
		RF exposure		1	18180	0.06462	0.8004
		Age <sup>b</sup> * Sex		1	38012	0.1351	0.7148
		Age <sup>b</sup> * RF exposure		1	33174	0.1179	0.7328
		<b>Sex* RF exposure</b>		<b>1</b>	<b>4419006</b>	<b>15.71</b>	<b>0.0002</b>
		Age <sup>b</sup> * Sex * RF exposure		1	43736	0.1555	0.6951
	Pup's horizontal activity	Age <sup>b</sup>		1	<b>3397758</b>	<b>25.51</b>	<b>&lt;0.0001</b>
		Sex		1	928.3	0.006970	0.9338
		RF exposure		1	266064	1.998	0.1640
		Age <sup>b</sup> * Sex		1	10920	0.08199	0.7759
		Age <sup>b</sup> * RF exposure		1	2829	0.02124	0.8847
		Sex* RF exposure		1	107013	0.8035	0.3745
		Age <sup>b</sup> * Sex * RF exposure		1	208132	1.563	0.2173
	Pup's stereotyped activity	Age <sup>b</sup>		1	<b>540768</b>	<b>10.96</b>	<b>0.0018</b>
		Sex		1	<b>338679</b>	<b>6.864</b>	<b>0.0117</b>
		RF exposure		1	<b>423342</b>	<b>8.580</b>	<b>0.0052</b>
		Age <sup>b</sup> * Sex		1	8184	0.1659	0.6856
		Age <sup>b</sup> * RF exposure		1	55377	1.122	0.2947
		<b>Sex* RF exposure</b>		<b>1</b>	<b>3150680</b>	<b>63.85</b>	<b>&lt;0.0001</b>
		<b>Age<sup>b</sup> * Sex * RF exposure</b>		<b>1</b>	<b>442686</b>	<b>8.972</b>	<b>0.0043</b>
Variables		Source of Interaction		dF	SE	t ratio	p-value
t-test	Total activity mothers	RF exposure	12	301.7	1.566	0.143263	
	Horizontal activity mothers	RF exposure	12	230.3	1.484	0.163685	
	Stereotyped activity mothers	RF exposure	12	76.26	1.716	0.111869	

Legend: dF - degrees of freedom ; MS - mean square; F - MS factor/MS residual; SE – standard error of the difference ; t-ratio – ratio t test ; P-value - statistical significance; Age<sup>a</sup> - age of mother; Age<sup>b</sup> - age of the pups

