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Effects of pre- and postnatal exposure to 1880–1900 MHz DECT base radiation on development in the rat

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ABSTRACT

In the present study, to evaluate the effects of wireless 1880–1900 MHz Digital Enhanced Communication Telephony (DECT) base radiation on fetal and postnatal development, Wistar rats were exposed at an average electric field intensity of 3.7 V/m, 12 h/day, during pregnancy. After parturition, a group of dams and offspring were similarly exposed for another 22 days. Controls were sham-exposed. The data showed that DECT base radiation exposure caused heart rate increase in the embryos on the 17th day of pregnancy. Moreover, significant changes on the newborns' somatometric characteristics were noticed. Pyramidal cell loss and glia fibrilliary acidic protein (GFAP) over-expression were detected in the CA4 region of the hippocampus of the 22-day old pups that were irradiated either during prenatal life or both pre- and postnatally. Changes in the integrity of the brain in the 22-day old pups could potentially be related to developmental behavioral changes during the fetal period.

1. Introduction

In the last few decades, environmental level of non-ionizing electromagnetic fields (EMF) has increased significantly and their possible effects on living organisms have become a major health concern. Electromagnetic fields are generated mainly from the uses of electric energy and wireless communication devices, such as mobile phones, cordless phones, wireless routers (Wi-Fi), tablets, i-Pads, *etc*. Of great importance is the possibility that adverse effects of EMF could be greater among various vulnerable groups of people, such as pregnant women, children, elders, and electrohypersensitive people [1,2].

Since the development of an organism from the early fetal stages throughout childhood is a very sensitive process, concerns have been raised as to whether EMF exposure of pregnant females could be harmful to their offspring. The reproductive and teratogenetic effects of EMF were investigated very early from 1961 to 1991. The majority of these experimental studies dealt with exposure of animals to 2450-MHz EMF, while few studies investigated the effects of 915- and 970-MHz EMF on embryonic development [3,4], and have been reviewed by Verschaeve and Maes in terms of the genetic, carcinogenic, and teratogenic effects of radiofrequency (RF) fields (300 MHz–300 GHz) [5].

Later on, other researchers have shown that RF fields were teratogenic when the specific absorption rate (SAR) level was high enough to raise considerably maternal body temperature (cf review [6]), but only a few have found effects on animal reproduction (cf reviews [7,8]), as well as on growth and development [6,9,10] after exposure of animals to SAR values below the ICNIRP limit, *i.e.*, at a whole body SAR of 0.08 W/kg [11].

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Researchers have reported effects on the reproductive system and fertility of female [12] and male [13,14] animals following RF field exposure. Skeletal abnormalities of fetuses [15], alterations on levels of endocrine hormones [16,17], as well as stress response, and subsequent suppression of implantation in female mice and deformity of embryos [18] have been reported after exposure to RF fields. On the other hand, no significant effect was found after lifetime exposure over four generations of mice to an UMTS1966-MHz field (SAR 0.08, 0.4, and 1.3 W/kg) on reproduction and development [19], or after exposure to a 2140-MHz (Downlink) W-CDMA signal for 20 h/day during gestation and lactation period over two and three generations in the rat [20,21]. Absence of effects of Wireless Fidelity (Wi-Fi) frequency band exposure (2450 MHz, 1 h/day, 6 days/week for 5 or 6 weeks) was reported on fertility of male and female rats even at a very high whole-body SAR value of 4 W/kg [10]. We could comment however that the absence of exposure for 1 day per week in this study might have allowed repair to occur.

Many studies have also focused on the effects of *in utero* RF field exposure [GSM 900 or 1800 MHz emitted by generators or Transverse electromagnetic transmission line chambers (TEM-cells)] on a very sensitive and complex organ, the brain of the offspring [22]. Loss of pyramidal cells in the hippocampus [23], decrease of granule cells in the dentate gyrus [24], as well as appearance of apoptotic neuronal, meningeal, and glial cells [25] have been reported. Moreover, deficits on cognitive functions have been detected after *in utero* exposure of animals to RF field [26]. On the other hand, no significant effect on operant-behavior performances of adult rats was observed following prenatal exposure to a 900-MHz cell-phone EMF [27]. A recent study revealed a dose-dependent impairment in glutaminergic synaptic transmission of prefrontal cortex layer V pyramidal neurons in mice following *in-utero* exposure to mobile phone radiation [28].

One of the wireless devices that has been used for decades is the "wireless telephone" or currently Digital Enhanced Cordless Telephone (DECT), which consists of two parts, a base and a handset and has specific characteristics (see Methods section). Only several studies have dealt with the potential biological effects of exposure to DECT radiation. At the epidemiological level, Hardell et al. reported an increased brain tumor risk after 10 years in heavy users (more than 500 h of cumulative life time use) [29]. There are also studies at the clinical level showing an increase in transthyretin (TTR), an indicator of malfunction of the blood-cerebrospinal fluid barrier, in the blood of users [30]. Finally, four experimental studies on DECT radiation have been published, with three of them from our research group (using similar exposure conditions as in the current study), showing brain protein expression changes in the mouse [31], reduced fertility and apoptosis induction [1] and reactive oxygen species increase in Drosophila [32]. The other was from Canada [33] showing heart rate and heart rate variability changes in human volunteers exposed for 3-min intervals to radiation from 3.4 to 4.3 V/m, generated by a cordless phone at 2.4 GHz. To our knowledge, no study has investigated so far the effects of DECT radiation *in utero* and during development in the rat.

The objective of this study was to investigate the risk of exposure to DECT base EMF during prenatal and postnatal life on development in the rat. More specifically, the effects on pregnancy capacity, embryo development, newborn growth, and brain integrity in rat pups were investigated.

2. Materials and methods

The study was performed in the Animal Facility of the Center of Clinical, Experimental Surgery and Translational Research of the Biomedical Research Foundation of the Academy of Athens (BRFAA) and in the Department of Cell Biology and Biophysics of the University of Athens.

2.1. Animals

Animal experimentation was performed in BRFAA and protocols were evaluated and approved by the Veterinary Service of the Prefecture of Athens (permit number K/4215/24.11.11), as required by the Greek legal requirements for animal experimentation. All experiments were performed in accordance with the approved guidelines. The Facility in BFRAA is registered as a "breeding" and "experimental" facility according to the Greek Presidential Decree 56/2013, which harmonizes national legislation with the European Community Directive 63/2010 on the Protection of Animals Used for Scientific Purposes.

Eighty 16–20 weeks old female Wistar rats (HsdOla:WI) were obtained from the breeding colony of the Animal Facility of the Foundation. All animals (mothers "m") showed normal 4-days oestrous cycles as determined by taking vaginal smears for four consecutive days using the swab smear technique. They were randomly divided into two groups (Am and Bm) of 40 animals each. After mating animals in Group Am and their embryos (Ae) (if any, dependent of whether the mother got pregnant) were sham-exposed, whereas those in Group Bm and their embryos (Be) (if any, dependent of whether the mother got pregnant) were exposed to DECT base radiation. At the start of the experiment, the body weights (mean ± SD) of Groups Am and Bm were 216.59 ± 21.09 gm and 220.03 ± 27.96 gm, respectively. Ultrasonographic examination was performed on the 17th day of gestation in representative embryos from groups Ae and Be. At birth (postnatal day 1), all pups (newborns, n) from groups Ae and Be were measured in order to analyze their somatometric characteristics and these groups are named as An and Bn for sham-exposed and exposed newborns respectively. After parturition, Group Bn was subdivided in two further groups (B1p, B2p; p = pups). Groups B1p was then sham-exposed until PND22, whereas B2p were continuously being exposed until PND22. The initial sham-exposed (Ae) group and then An group was not further divided, since all pups (groups Ap, B1p, B2p) were sacrificed on PND22 and both B1p and B2p were compared to group Ap (for a schematic illustration of the experimental protocol please refer to Fig. 1I).

Animals were housed in pairs in H-Temp^{TM} polysulfone type III open top cages [425 mm (L) × 266 mm (W) × 185 mm (H), Tecniplast, Milan, Italy]. All cages were kept in the same animal room with HEPA filtered air supply, 15 ACH (air changes per hour), at a room temperature of 24 ± 2 °C, relative humidity of 55 ± 10%, 12-h light/dark cycle (light on between 07:00-19:00 h), light intensity of 300 lx (measured one meter above the floor in the middle of the room), and positive air pressure of 0.6 Pa within the room.

The rats were maintained according to the Guide for the Care and Use of Laboratory Animals and the relevant recommendations of the European Commission on the care and use of laboratory animals. All animals of the Facility were regularly screened using a health monitoring program, in accordance to the Federation of European Laboratory Animal Science Association recommendations and were free from a wide range of tested pathogens.

All rats had *ad libitum* access to filtered tap water in drinking bottles and to pellet chow that contained 18.5% protein, 5.5% fat, 4.5% fiber, 6% ash (Teklad 2918, Harlan, Italy). The bedding in each cage comprised of ~240 gm of corncob bedding (Rehofix MK 2000, J. Rettenmaier & Söhne, Rosenberg, Germany). The cages were cleaned once a week.