Maternal stress-induced behavioral changes diminished by maternal exposure to an electromagnetic field (50 Hz, 100 µTesla) in male rat offspring

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Abstract

Prenatal exposure to stress predisposes offspring to mental health problems in adulthood. However, the underlying mechanisms remain obscure. The prefrontal cortex's (PFC) role is vital in regulating sleep and mood. Cryptochrome type 2 (CRY2), as a magnetoreceptor and an important part of the circadian system, has been linked to depression and anxiety. We aimed to determine CRY2 role in prenatal stress and extremely low-frequency electromagnetic fields (ELF-EMF) on the PFC of rat offspring and its relationship with behavior. Female Wistar rats were exposed to chronic mild stress (CMS) or electromagnetic field (EMF) (50 Hz, 100 μT, 4 h/day) for 21 days before and during pregnancy. Behavioral tests, including the elevated plus maze, open field, and forced swimming test, were conducted on the male offspring at postnatal day (PND) 80, 81, 90. The expression of CRY2 in the PFC and levels of serum corticosterone (CORT) were also measured. The results showed that maternal stress exposure caused anxiety- and depression-like behaviors in the male offspring, accompanied by decreased prefrontal CRY2 protein expression and increased serum CORT levels. In addition, maternal EMF had no significant effect on CRY2 expression in the male offspring. However, parallel ELF-EMF and stress exposure significantly attenuated anxiety and depression-like behaviors and decreased serum CORT levels.

1. Introduction

Stress is a physical, mental, or emotional factor that causes bodily or mental tension [Weinstock, 2017]. Prenatal stress has adverse impacts on the health of both mother and offspring [Weinstock, 2005]. Stress during pregnancy causes impairments in the cognition, behavior, and brain development of offspring [Huang et al., 2010]. Maternal stress exposure predisposes offspring to depression and anxiety [Iturra-Mena et al., 2018; Miyagawa et al., 2011]. Possible mechanisms behind these effects are not precisely known, but the critical role of the hypothalamic-pituitary-adrenal (HPA) axis and its final products (cortisol in humans and CORT in animals) are indicated previously [Tollenaar et al., 2011]. Cortisol is crucial in many aspects of fetal development, but its high concentration has various adverse effects [Peña et al., 2012].

Exposure to extremely low-frequency electromagnetic fields (ELF-EMF) is inevitable in daily life, raising public health concerns due to its potential adverse health impacts (diabetes, obesity, cancer, cognitive impairments, reduced fertility, miscarriage, and developmental impairment in fetus) [Behari and Rajamani, 2012; Bellieni and Pinto, 2012]. The EMF is generated by power lines and household electric devices, implicated in behavior modulation [Djordjevic et al., 2017; Kitaoka et al., 2013]. Additionally, ELF-EMF is considered a mild stressor that activates the HPA axis and increases CORT levels [Martínez-Sámano et al., 2018]. Although some studies have reported the harmful effects of EMF, its application has received considerable attention as a therapeutic or diagnostic tool in medicine [Rohan et al., 2004; Vadalà et al., 2016]. There is a substantial ambiguity about the effects of ELF-EMF on mood and behavior. One week of ELF-EMF exposure induces anxiety-like behavior through oxidative stress in the hypothalamus of rats [Djordjevic et al., 2017]. On the other hand, ELF-EMF exposure for 30 days attenuated depression-like behaviors while increased anxiety-like behaviors in mice [Ebrahimi et al., 2019].
The antidepressant effect of low field magnetic stimulation in depressed patients has previously been reported [Rohan et al., 2004]. Meanwhile, another study demonstrated that ELF-EMF exposure does not affect anxiety-like behaviors in adult rats [Rostami et al., 2016].

The prefrontal cortex (PFC), as an essential center for planning complex cognitive behaviors [Swearingen, 2016], is implicated in stress-related disorders, namely depression [Treadway et al., 2015] and anxiety [Park and Moghaddam, 2017]. The PFC expresses a high density of glucocorticoid (GC) receptors, increasing its vulnerability to the stress exposure's harmful effects [Otsuka et al., 2020]. Prenatal and postnatal stress exposure has been shown to influence the PFC development and PFC-dependent behavioral responses [Barfield and Gourley, 2018].

Circadian rhythm coordinates the brain and physiological functions across different phases of light/dark cycles; hence abnormalities in this system accelerate behavioral disorders [McEwen and Morrison, 2013]. Clock genes regulate circadian rhythms, and deficiency in clock genes is associated with mood disorders such as anxiety and depression. Exposure to stress also initiates the HPA axis activation resulting in disruption of circadian rhythm by modifying clock gene expression, which may increase the risk of mood disorders [Salgado-Delgado et al., 2011; Swearingen, 2016]. One crucial component of the circadian clock machinery is cryptochrome (CRY) [Otsuka et al., 2020], which is considered as a magnetoreceptor in birds and helps them to sense magnetic direction and orientation in different positions [Liedvogel and Mouritsen, 2010; Wiltschko and Wiltschko, 2014]. Moreover, recent evidence elucidates the role of PFC in coordinating mood states with the function of the circadian clock [Karatsoreos et al., 2011; Woodruff et al., 2018]. Furthermore, some alternations in the physiological processes induced by EMF exposure might be mediated by modifying biological clock machinery and disturbance of circadian rhythms [Manzella et al., 2015].

This study aimed to determine the effect of maternal stress and EMF exposure before and during pregnancy on postnatal anxiety- and depression-like behaviors, and prefrontal cortex CRY2 protein expression and serum CORT levels of male rat offspring.

2. Materials And Methods

2.1 Animals

Adult female (200–250 g) and male (250–300 g) Wistar rats were provided by the animal house of Tabriz University of Medical Sciences (Tabriz, Iran). Animals were transferred to the standard animal rooms and acclimatized for 7 days. All animals were maintained in a 12/12-h light/dark cycle (7:00 am to 7:00 pm), at 25°C temperature, six per cage with ad libitum access to water and food. All procedures and experiments were approved by Ethical Committee of Tabriz University of Medical Sciences for the protection and use of animals in research (IR.TBZMED.VCR.REC.1397.230), and was according to the guidelines of the National Institutes of Health.

2.2 Experimental design
Female Wistar rats (200–250 g) were randomly divided into four groups (n = 8): control (C), electromagnetic field (EMF), stress (S), and S-EMF groups. Dams in the control group were placed in a device like other animals, but the generator was off. (Four animals were placed in a thin plastic box (25*30*20 Cm) inside the EMF device simultaneously). The EMF group dams were exposed to ELF-MF (50 Hz, 100 µT) for 4 h each day from 10:00 a.m. to 2:00 p.m. Dams in the S group were subjected to different types of stressors (Table. 1). The S-EMF group dams were exposed to ELF-EMF and stressors at the same time. Except the control group, all experimental groups were exposed to ELF-EMF or chronic mild stress (CMS) for 21 days before mating and during pregnancy.

After pre-gestational experiments, all groups were mated with intact Wistar male (2:1) rats overnight, and a positive vaginal smear confirmed the pregnancy on the following morning.

2.3 Offspring

After delivery, (C = 29 M, 33 F), (EMF = 31 M, 26 F), (S = 28 M, 17 F), (S-EMF = 25 M, 31 F), each mother was kept with their pups in a separate cage for the lactation period of 21 days. The day after weaning, six male pups were randomly selected from different dams in each group, placed in a cage, and kept in the standard room until postnatal day (PND) 80. The elevated plus maze (EPM) and the open field test (OFT) were carried out on PND 80 and 81, respectively, to evaluate anxiety-like behaviors. Depression-like behaviors were evaluated using a forced swimming test (FST) on PND 90. The study design is summarized in Fig. 1.

2.4 Chronic mild stress

The CMS protocol was a slightly modified version of the procedure reported by Lewitus et al. [2009]. Briefly, CMS consists of eight various stressors every week as following: two-course of stroboscopic shining (300 flashes per min), one course of cage soiling, two-course of white noise (80 dB), two-course of 45 angle slop, one course of paired dwelling, food or water deprivation followed by a vacant bottle or limited food. The stress protocol was repeated for 6 weeks, 21 days before mating, and during pregnancy [Lewitus et al., 2009]. Details of the CMS procedure are shown in Table. 1.

2.5 Electromagnetic field

The ELF-EMF was produced by an apparatus made according to Helmholtz's design. The electromagnetic field was generated by a Helmholtz coil made of copper wire (30 cm radius and each coil has 154 turns) separated by a 30 cm distance. The coil carrier ring was made of wood without any metal and was placed at a fixed distance between them using a wooden tripod located on a wooden board (Fig. 2). No metal piece was used for the production of the electromagnetic field in this system. The uniform electric field was produced in the space between the two rings (radius = 30 cm and height = 30 cm). In this study, electromagnetic field intensity was 100 µT with a frequency of 50 Hz in the period of 4180 triple-axis gauss meter. Animals (4 dams in each cage) were located inside the coil system in the cage that had the same size used for the housing of the rat (2 h/day: 10 a.m. to 2 p.m. for 21 days before mating and 21 days during pregnancy). Electromagnetic field intensity was measured by a digital Tesla Meter (Lutron...
828, Taipei, Taiwan) before exposure in different space of the device to confirm its heterogeneous distribution [Podaru et al., 2015]. The heat generated by the coil seems to be dissipated due to good ventilation in the lab and in the exposure area.

2.6 Behavioral evaluation

2.6.1 Elevated plus-maze

The apparatus consists of a wooden structure with two open arms (50×10 cm) and two closed arms (50×10×20 cm) raised 50 cm above the floor. All arms were joined to a square center and made a plus shape. The animals were brought to the experiment room for 30 min before the test to habituate. Then, the animal was placed in the apparatus's core square facing an open arm and allowed to freely explore the maze for 5 min, and the session was recorded. Between each test, the maze was cleaned with 70% ethanol. At the end of the experiments, the percentage of time spent in the open arms (%OAT) and the percentage of open arm entries (%OAE) were analyzed by a blinded researcher to the experimental groups. An arm entry means the entrance of the animal to the arm with all four feet. The more time spent in the open arms and higher open arm entry number reflects low levels of anxiety. The number of total arm entries (TAE) into the four arms was also determined [Farajdokht et al., 2017].

2.6.2 Open field test

The apparatus was a large square chamber (50×50×40 cm) without a roof, and the floor region was divided into 25 squares (5×5 cm). The animal was placed in the central region that had 9 squares and signed by the black line, and the behaviors of the rat were recorded for 5 min. For the final analysis, the number of grooming, number of rearing, number of the entrance to the central square, and time spent in the center arena were recorded by a blinded researcher to the experimental design [Pardon et al., 2000].

2.6.3 Forced swimming test

To study depression-like behaviors, each rat was individually placed in a cylindrical container (40 cm in diameter × 80 cm in height) at a water depth of 45 cm (24 ± 1°C) and then forced to swim for 5 min, and a blinded researcher recorded the behaviors of the animal. Immobility behaviors were described as hanging in a vertical state without any movement or doing only those movements necessary to keep the head above the water and floating without struggling [Farzin et al., 2013; López-Rubalcava and Lucki, 2000].

2.7 Sampling

After performing the behavioral tests, the blood samples were collected on PND 91 under deep anesthesia with ketamine and xylazine (90 mg/kg and 10 mg/kg, respectively) [Hajipour et al., 2016] from the heart, and animals were decapitated. Then the brain tissues were immediately taken out, and the PFC was excised and frozen in liquid nitrogen. To obtain serum, the blood samples were centrifuged at 1500 g for 15 min. Finally, both serum and PFC were stored at -70°C until CORT and CRY2 measurements.
2.7.1 Serum concentration of CORT

CORT was measured in serum samples using an enzyme-linked immunosorbent assay (ELISA) kit (Catalog Number KGE009; R&D Systems, Shanghai, China) based on the manufacturer’s recommendations.

2.7.2 Western blotting

The PFC content of the CRY2 protein level was measured by the Western blotting method. Briefly, The PFC tissue samples were lysed and homogenated in RIPA lysis buffer supplemented with protease inhibitor cocktail and centrifuged at 12,000 × g for 15 min at 4 °C to obtain a supernatant. Following the protein concentration measurement in the supernatant using the Bradford method, an equal amount of protein (20 µg) was separated by 12.5% SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride (PVDF) membrane (Roche, Norwich, United Kingdom). After blocking non-specific binding, the membranes were incubated with primary rabbit antibodies (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) against anti-CRY2 (sc-293263) and anti-β-actin (sc-47778), as internal control, overnight at 4 °C. Subsequently, the membrane was washed three times with PBS then incubated for 2 h at room temperature with horseradish peroxidase-conjugated (HRP) goat anti-rabbit IgG secondary antibody (sc-2004, 1:5000). Finally, the membrane was soaked in the enhanced chemiluminescence (ECL) reagents (Amersham Pharmacia Biotech Ltd., Amersham, UK) and then exposed to X-ray film (Kodak, Rochester, NY) to visualize the protein bands. Image J software (National Institutes of Health, Bethesda, Maryland, USA) was used to quantify obtained protein bands [Mohammadi et al., 2019].

3. Data Analysis

Data are shown as a means ± SEM. Results were statistically analyzed by Graph Pad Prism 8 software (Graph Pad Software Inc., La Jolla, CA). All comparisons were conducted using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Results were considered significant when p < 0.05.

4. Results

4.1 EMF exposure in maternal CMS diminished stress-induced offspring anxiety-like behaviors in the EPM task

The results of the EPM are shown in Fig. 3A. One-way ANOVA demonstrated a significant [F (3, 20) = 96.93, p < 0.0001] difference in %OAT among groups (Fig. 3A). Post-hoc analysis revealed that %OAT was significantly decreased in the S (p < 0.001), EMF (p < 0.05), and S-EMF (p < 0.01) groups compared to the control group. However, the EMF (p < 0.001) and S-EMF (p < 0.001) groups demonstrated a significant increase in %OAT compared to the S group.

Moreover, the one-way ANOVA demonstrated a significant difference in %OAE [F (3, 20) = 17.11, p < 0.0001] among groups (Fig. 3B). Post-hoc analysis showed that maternal stress exposure significantly (p
< 0.001) decreased %OAE in the offspring compared to the control group. Nevertheless, EMF exposure in the non-stress and stress-exposed dams significantly (p < 0.01 for both) increased %OAE compared to the S group.

Besides, there was no significant [F (3, 20) = 0.1448, p = 0.9318] difference in TAE among groups (Fig. 3C).

4.2 EMF exposure in maternal CMS diminished stress-induced offspring anxiety-like behaviors in the OF task

The results of one-way ANOVA for the number of rearing in the male offspring demonstrated a significant [F (3, 20) = 5.38, p = 0.0070] difference among groups (Fig. 4A). Post-hoc analysis showed that maternal stress exposure significantly (p < 0.05) increased the number of rearing in the offspring compared to the control animals. In contrast, animals in the S-EMF group showed lower rearing than the S group (p < 0.05).

The results of one-way ANOVA also showed a significant difference in the number of grooming among groups [F (3, 20) = 8.03, p = 0.0010] in the OFT (Fig. 4B). Post-hoc analysis indicated that maternal stress exposure significantly (p < 0.01) increased grooming in the S group compared to the control group. Moreover, maternal EMF exposure significantly decreased the number of grooming in the EMF (p < 0.01) and S-EMF (p < 0.05) groups compared to the S group.

One-way ANOVA for the number of center entries in the OFT (Fig. 4C) also demonstrated a significant difference among groups [F (3, 20) = 15.25, p < 0.0001]. Post-hoc analysis indicated that stress, EMF, and combined stress and EMF exposure significantly decreased the number of center entries in the OFT compared to the control group (C vs. S group, p < 0.001; C vs. EMF group, p < 0.001; C vs. S-EMF group, p < 0.01).

Additionally, one-way ANOVA for the center time in the OFT (Fig. 4D) demonstrated a significant difference among groups [F (3, 20) = 22.21, p < 0.0001]. Post-hoc analysis showed that stress and concomitant stress and EMF exposure significantly decreased the center time in the male offspring compared to the control group (p < 0.001 for both). However, EMF exposure significantly (p < 0.001) increased time spent in the center compared to the S group. Furthermore, there was a significant (p < 0.05) difference between the EMF and S-MF groups.

However, we found no significant [F (3, 20) = 0.2197, p = 0.8816] difference in locomotor activity in the OFT among the experimental groups (Fig. 4E).

4.3 EMF exposure in maternal CMS diminished stress-induced offspring depressive-like behaviors in the FST task

The results of FST are shown in Fig. 5. The results of one-way ANOVA of immobility time in the FST indicated a significant difference among groups [F (3, 20) = 46.89, p < 0.0001]. Post-hoc analysis
demonstrated that maternal stress and concomitant stress and EMF exposure significantly increased immobility time in the offspring compared to the control group (p < 0.001 and p < 0.05, respectively). Nevertheless, maternal EMF exposure markedly (p < 0.001 for both) decreased immobility time in the EMF and S-EMF groups compared to the S group. Interestingly, offspring of the EMF group showed lower immobility time (p < 0.001) than the S-EMF group.

However, EMF exposure in the stress-subjected dams markedly decreased immobility time in the S-EMF group compared to the S group (p < 0.001).

### 4.4 EMF exposure in maternal CMS diminished serum CORT levels of the offspring

As shown in Fig. 6, one-way ANOVA for serum concentration of CORT revealed a significant difference among groups [F (3, 16) = 15.65, p < 0.001]. Post-hoc analysis indicated that maternal stress exposure significantly increased CORT in the serum of the offspring compared to the control (p < 0.001). Besides, EMF exposure in the non-stress and the stress-subjected dams significantly decreased serum CORT levels in the offspring compared to the S group (p < 0.001 for both).

### 4.5 EMF exposure in maternal CMS decreased protein expression of CRY2 in the PFC of the offspring

Figure 7 shows the protein expression of CRY2 in the PFC. The results of one-way ANOVA demonstrated a significant [F (3, 16) = 15.55, p < 0.001] difference among groups. Post-hoc analysis indicated that maternal stress exposure and stress concomitant EMF exposure significantly decreased CRY2 protein expression in the PFC of male offspring compared to the control group (p < 0.01, p < 0.05, respectively). However, maternal EMF had no significant effect on CRY2 expression in the male offspring compared to the control group. Besides, EMF exposure significantly (p < 0.001) increased CY2 protein expression in the PFC compared to the S group. Furthermore, offspring of the S-EMF group had lower CRY2 protein levels than the offspring of the EMF group (p < 0.001).

### Discussion

This study demonstrated that prenatal stress decreased %OAT and %OAE in the EPM test and increased immobility time in the FST, confirming increased anxiety- and depression-like behaviors in adult male offspring. Moreover, prenatal stress increased serum CORT levels and decreased CRY2 protein levels in the PFC of offspring. The EMF exposure did not cause any anxiety- and depression-like behaviors or change in serum CORT levels and content of CRY2 in the PFC of EMF offspring. However, exposure to the ELF-EMF in the S group decreased anxiety- and depression-like behaviors and serum CORT levels compared with the S group. Moreover, CRY2 levels did not significantly change in the S-EMF offspring.

Evidence shows that prenatal stress has an extended effect on the birth outcome [Vollmayr and Henn, 2003]. Stress during pregnancy impacts the developing fetus and eventually the adult offspring [Fatima et al., 2017]. Social deficit stress prior to pregnancy induces depression-like behaviors in the adult male
offspring rats [Wei et al., 2018]. Exposure to prenatal stress has also been shown to increase stress-related behaviors and the HPA axis activity in the adult male guinea pig offspring [Kapoor and Matthews, 2005]. In addition, CMS induction increased the function of the HPA axis and CORT hypersecretion in rats [Challis et al., 2001]. Fetal GC concentration is associated with maternal GC concentration [Challis et al., 2001], and changes in the maternal HPA axis function during pregnancy increases GC transfer to the fetus [Duthie and Reynolds, 2013]. Besides, high CORT levels result in behavioral changes similar to anxiety and depression [Bakshi and Kalin, 2000; Gregus et al., 2005a; Stenzel-Poore et al., 1994].

This study indicated that maternal EMF exposure did not induce anxiety-and depressive-like behaviors in the male offspring compared to the control animals. However, the EMF group offspring demonstrated lower anxiety- or depression-like behaviors, lower serum CORT, and higher PFC CRY2 protein levels than the offspring of the S group. In line with our findings, a previous study showed that long-term (24 weeks) exposure to ELF-EMF (50 Hz and 100 μT) did not cause any anxiety- and depression-like behaviors in rats [Lai et al., 2016]. However, another study reported that exposure to ELF-EMF (50 Hz,100 μT, 2 h daily for 60 days) developed anxiety-like behaviors in Wistar rats [Karimi et al., 2019]. It has also been demonstrated that long-term (4-6 weeks, 24 h daily) exposure to high-intensity EMF (50 Hz, 0.5 mT) developed depression-like behaviors and increased blood glucose and pro-opiomelanocortin mRNA levels in the anterior lobe of the pituitary gland, whilst it did not induce anxiety-like behaviors [Szemerszky et al., 2010]. Moreover, prenatal (Days 3.5-18) exposure to microwave (9.417-GHz) has been shown to increase anxiety-like behaviors in the offspring [Zhang et al., 2015]. In contrast to our findings, a previous study demonstrated that exposure to ELF-EMF (60 Hz and 2.4 mT2 h/day) for 21 days increased CORT levels in male Wistar rats [Martínez-Sámano et al., 2018]. These discrepancies are possibly due to the EMF exposure severity and duration, type of animal and strain, and the animal’s life period.

Prenatal simultaneous exposure to stress and EMF decreased anxiety- and depression-like behaviors and serum CORT levels in the adult offspring compared to the S group. It has been indicated that long-term exposure of either ELF-MF or low-frequency pulsed EMF improved depression-like behaviors in rodents [Ansari et al., 2016; Yang et al., 2019]. Besides, pulsed EMF exposure has been shown to reduce anxiety-like behaviors in animals and humans [Pawluk, 2019]. Moreover, radiofrequency exposure in pregnant dams had an anxiolytic effect on the offspring [Aldad et al., 2012]. These studies are in line with our findings. Conversely, exposure of dams to restraint stress (2 h/day along gestation) and Wi-Fi signals (2.45 GHz) increased anxiety-like behaviors in the offspring’s juvenile and adult age [Othman et al., 2017]. Different experimental periods and durations and severities of the electromagnetic field in these studies may lead to discrepancies. Moreover, similar to our study, ELF-EMF exposure has been shown to decrease CRH and proopiomelanocortin gene expression, hence CORT synthesis in mice [de Kleijn et al., 2016]. It is suggested that decreased anxiety- and depression-like behaviors in the S-EMF offspring are probably due to decreased serum CORT levels [Gregus et al., 2005b].

CRY1 and CRY2 modulate circadian rhythms and have vital roles in regulating mood and emotion [Kovanen et al., 2017; Partonen, 2015]. Disruption of circadian proteins is associated with behavioral disorders [Lavebratt et al., 2010]. Evidence shows that CRY deficiency causes behavioral
abnormalities such as depression, bipolar disorder, and seasonal affective disorder [Bakshi and Kalin, 2000; Partonen, 2012]. CRY2 especially has a vital role in the core symptoms of depressive disorders. It has also been indicated that CMS exposure reduces clock genes expression, namely CRY and PER, in the PFC of adult rats [Calabrese et al., 2016]. However, short-term restraint stress for 1 h failed to change the expression of CRY1 and CRY2 in the peripheral tissues of mice [Yamamoto et al., 2005]. Moreover, evidence shows that CRY knockout increases anxiety [De Bundel et al., 2013] and depression [Kripke et al., 2009] in rodents. Similarly, we found that maternal CMS exposure increased anxiety-like behaviors accompanied by decreased CRY2 protein levels in the PFC of the S and S-EMF offspring groups. In contrast to our finding, Schnell et al. [2015] reported that CRY2 deficiency reduced anxiety behavior [Schnell et al., 2015]. Though EMF exposure in the S-EMF group improved depression- and anxiety-like behaviors compared to the S group, there was no significant difference in CRY2 protein expression between the S and S-EMF groups. However, prenatal EMF exposure in non-stress dams markedly increased CRY2 levels in the PFC. From a mechanistic point of view, exposure to chronic stress leads to the down-regulation of GC receptors and impairs the transcription of different genes, such as CRY [Calabrese et al., 2016]. Therefore, it seems that prenatal CMS exposure reduced CRY2 protein levels in the PFC of offspring by a similar mechanism.

Different mechanisms are proposed for the beneficial effects of the low-frequency EMF in treating depression, such as improved brain plasticity, neuronal connectivity, and brain metabolism [Van Belkum et al., 2016]. Neurogeneration and neural protective effects of rTMS by the involvement of BDNF in the hippocampus and other brain regions have also been indicated previously [Müller et al., 2000]. In addition, it is well established that decreased BDNF and VEGF-B levels in the brain impair behavior [Duman and Monteggia, 2006], and exposure to chronic stress reduces BDNF levels in the hippocampus, resulting in dysregulation of the HPA axis and increases serum CORT levels [Nowacka and Obuchowicz, 2013]. On the other hand, genetic deficits in CRY expression can cause a decrease in BDNF and VEGF-B [Savalli et al., 2015]. Therefore, EMF exposure in the S group has possibly increased brain BDNF levels, prevented HPA axis hyperactivation, and reduced CORT levels and depression-like behaviors.

**Conclusion**

This study indicated that decreased CRY2 protein in the PFC and increased serum CORT levels are possibly contributing factors to the anxiety- and depression-like behaviors in the offspring of stressed dams. EMF exposure in the stress-exposed dams attenuated anxiety- and depression-like behaviors in the offspring, probably related to decreased serum CORT levels. However, EMF exposure in the stress-subjected dams had no significant effect on CRY2 expression in the male offspring. Further studies are needed to elucidate the exact mechanisms through which maternal EMF exposure decreased anxiety- and depression-like behaviors in offspring.

**Declarations**

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Conflict of interest: none.

References


Table

Table 1 Schedule of chronic mild stress (CMS).
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<td></td>
<td>Add food ad libitum</td>
<td>12:00</td>
</tr>
<tr>
<td>Fri</td>
<td></td>
<td>Start food and water deprivation</td>
<td>19:00</td>
</tr>
<tr>
<td>Sat</td>
<td></td>
<td>Restore food and water</td>
<td>14:30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tilt cages</td>
<td></td>
</tr>
</tbody>
</table>
**Figures**

**Figure 1**

The experimental plan of the study. EMF: electromagnetic field, PND: postnatal day, EPM: elevated plus maze, OFT: open field test, FST: forced swimming test.

**Figure 2**

Helmholtz-coil apparatus for the EMF exposure.
Figure 3

The effects of maternal stress and electromagnetic field exposure on the A) percentage of open arm time (%OAT), B) percentage of open arm entries (%OAE), and C) total arms entries (TAE) in the offspring. Data are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post-hoc test. *p<0.05 **p<0.01, ***p<0.001 vs. C group; ##p<0.01 ###p<0.001 vs. S group. [C: control (N= 6), S: stress (N= 6), EMF: electromagnetic (N= 6), S-EMF: stress+ electromagnetic (N= 6)].
Figure 4

The effects of maternal stress and electromagnetic field exposure on the numbers of A) rearing, B) grooming, C) center entries, D) center time, and E) locomotor activity in the offspring in the open field test (OFT). Data are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post-hoc test.

*p<0.05, **p<0.01, ***p<0.001 vs. C group; #p<0.05, ## p<0.01, ###p<0.001 vs. S group; +p<0.05 vs. EMF
group. [C: control (N= 6), S: stress (N= 6), EMF: electromagnetic (N= 6), S-EMF: stress+ electromagnetic (N= 6)].

Figure 5

The effects of maternal stress and electromagnetic field exposure on forced swimming test immobility time in the offspring. Data are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post-hoc test. *p<0.05, ***p<0.001 vs. C group. ###p<0.001 vs. S group. +++ p<0.001 vs. EMF group. [C: control (N= 6), S: stress (N= 6), EMF: electromagnetic (N= 6), S-EMF: stress+ electromagnetic (N= 6)].
Figure 6

The effects of maternal stress and electromagnetic field exposure on serum concentration of corticosterone (CORT) in the offspring. Data are expressed as mean± SEM (n=5). One-way ANOVA followed by Tukey's post-hoc test. ***p<0.001 vs. C group; ###p<0.001 vs. S vs. group. [C: control (N= 5), S: stress (N= 5), EMF: electromagnetic (N= 5), S-EMF: stress+ electromagnetic (N= 5)].
Figure 7

The effects of maternal stress and electromagnetic exposure on CRY2 protein expression in the PFC of the offspring. A) Immunoblotting images of CRY2 and β-actin proteins. B) Quantitative densitometry analysis of the CRY2 protein levels in the PFC. Data are expressed as mean± SEM (n=5). One-way ANOVA followed by Tukey’s post-hoc test. *p<0.05, **p<0.01 vs. C group. ###p<0.001 vs. S group. +++p<0.001 vs. EMF group. [C: control (N= 5), S: stress (N= 5), EMF: electromagnetic (N= 5), S-EMF: stress+ electromagnetic (N= 5)].