Int. J. Devl Neuroscience 37 (2014) 58–64

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Autism-relevant social abnormalities in mice exposed perinatally to extremely low frequency electromagnetic fields

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ARTICLE INFO

Article history:
Received 21 April 2014
Received in revised form 16 June 2014
Accepted 16 June 2014

Keywords:
Autism spectrum disorder
Electromagnetic field
Perinatal exposure
Anxiety
Social novelty
Motor coordination

ABSTRACT

The incidence of autism spectrum disorders (ASD) has been rising, but the causes of ASD remain largely unidentified. Collective data have implicated the increased human exposure to electromagnetic fields (EMF) in the increasing incidence of ASD. There are established biological effects of extremely low-frequency (ELF) EMF, but the relation to ASD is not investigated enough. In this study we examined the effects of perinatal exposure to ELF EMF on some ASD-relevant behavioral parameters in mice. The EMF was delivered via a Helmholtz coil pair. Male BALB/C mice were used and divided into exposed and control groups ($n=8$ and $n=9$, respectively). Tests were used to assess sociability, preference for social novelty, locomotion, anxiety, exploratory behavior, motor coordination, and olfaction. The examined mice were all males and exposed to EMF during the last week of gestation and for 7 days after delivery. The exposed mice demonstrated a lack of normal sociability and preference for social novelty while maintaining normal anxiety-like behavior, locomotion, motor coordination, and olfaction. Exposed mice also demonstrated decreased exploratory activity. We concluded that these results are supportive of the hypothesis of a causal link between exposure to ELF–EMF and ASD; however, replications of the study with further tests are recommended.

1. Introduction

The incidence of autism spectrum disorder (ASD) has significantly and progressively increased during the last few decades (Centers for Disease Control and Prevention, 2014). Some studies have shown that there might be a component of an actual rise in the incidence of ASD rather than being entirely a result of changes in the diagnostic practices (Fombonne, 2003; Grether et al., 2009; Newschaffer et al., 2005). This potentially implicates an environmental factor, which remains unidentified. Many injurious events during gestation and perinatal periods have been recognized as risk factors for developing ASD (Lathe, 2008).

The rise in the incidence of ASD can be seen to coincide with increased human exposure to extremely low frequency electromagnetic fields (ELF EMF) from electrical appliances and cables (Thornton, 2006). The increased exposure to EMF is possibly one of the most dramatic and progressively escalating changes humans have had, in terms of environmental exposure, in the last few decades. Several papers have been published to demonstrate the plausibility of a causal link between the increased exposure to electromagnetism and the rise in the incidence of ASD (Kane, 2004; Lathe, 2008; Mariea and Carlo, 2007). A large number of studies have demonstrated that ELF EMF can have some biological (Giudice et al., 2007; Salehi et al., 2012; Tasset et al., 2012) and biochemical effects (He et al., 2013; Sadeghipour et al., 2012; Ulku et al., 2011), as well as effects on the behavior (Al-Maliki and Al-Rawi, 1992; Lai et al., 1998; Seppia et al., 2003; Sienkiewicz et al., 1998) and brain function (Choleris et al., 2001; Reyes-Guerrero et al., 2006; Sakhnini et al., 2012; Vázquez-Garcı′a et al., 2004) of rodents. Some studies also examined the effects of EMF on human behavior and brain (Cook et al., 2002; Legros and Beuter, 2005; Legros et al., 2006; Marino et al., 2004); however, there has been no experimental study addressing the possible causal link between EMF and ASD.

In this study, we investigated the effects of exposure to ELF EMF during the perinatal period on some measures of social behavior attributed to ASD.

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http://dx.doi.org/10.1016/j.ijdevneu.2014.06.010
0736-5748/

2. Methodology and materials

2.1. EMF exposure

The EMF was delivered via a Helmholtz coil pair. Each of the coils is 40 cm in diameter, has 154 turns, and the coils are separated at a distance of 20 cm. The coils were energized with a generator of standard signals, providing stability of voltage and frequency with a precision of significant digits. A sinusoidal current (50 Hz) was passed through the magnet, producing relatively homogenous alternating magnetic field with an average (B) of 1 mT (measured by a PHYWE Tesla meter probe, PHYWE System GmbH & Co. KG, Germany). Magnetic force lines were parallel to the horizontal component of the local geomagnetic field. The background magnetic field of 50 Hz did not exceed 1 nT as measured using a Multidetector II (Gewerbegebiet Aaronia AG, Germany) with a sensitivity of 1 nT.

2.2. Animals

Male BALB/C mice were used. The animals were divided into two groups. The exposed group of mice ($n = 8$, from 4 different mothers) were exposed continuously to the ELF EMF from the last week of gestation and continued for one week after birth. The control group ($n = 9$, from 4 different mothers) were placed in the same boxes and experimental setup, but no EMF was applied. All behavioral tests were performed when the animals were 8–11 weeks of age. All experimental procedures abided by animal care ethics of Arabian Gulf University/Manama/Bahrain.

2.3. The tests

Behavioral tests were conducted between 9:00 and 18:00. All behavioral tests were conducted and scored blindly. All sessions, except the rotarod and olfaction test, were videotaped and scored accordingly. Each parameter was scored by one observer for all mice to eliminate any potential inter-observer variability. Each mouse was tested a maximum of one trial per day.

2.3.1. Three-chambers social apparatus (Crawley's sociability and preference for social novelty test)

Sociability and preference for social novelty was assessed using the Three-chambers social apparatus, as described previously (Kaidanovich-Beilin et al., 2011; Nadler et al., 2004). The apparatus comprised a rectangular, three-chambered box. Each chamber was 20 cm × 40 cm × 22 cm. The walls were made from clear Plexiglas. The dividing walls (made from the same material) had small square openings (5 cm × 3 cm) allowing access into each chamber. Each chamber contained a circular wire cage which was 11 cm high, with a bottom diameter of 9 cm and bars spaced 0.5 cm apart. The subject mouse was first placed in the middle chamber to habituate for 5 min. Session 1 was started when an unfamiliar male (stranger 1) that had no prior contact with the subject mouse was placed inside the wire cage in one of the side chambers while the subject mouse was allowed to explore the entire apparatus freely. The placement of stranger 1 in the left or right side chambers was systematically alternated between trials. Session 1 continued for 10 min, and the time spent in each chamber as well as the number of chamber entries were recorded. Immediately after the 10 min of session 1 finished, session 2 started with a second unfamiliar mouse being placed in the wire cage inside the chamber that had been empty during the first session. The test mouse had a choice between the chamber containing the already investigated mouse (stranger 1), and the one containing the novel unfamiliar mouse (stranger 2). The same parameters recorded for session 1 were recorded for session 2. The apparatus was cleaned with 70% ethanol and water between subjects. Three trials were done for each mouse. Session 1 tests for sociability (or social motivation/affiliation), which is spending significantly more time in the chamber containing a mouse than in the empty chamber. Session 2 tests for preference for social novelty, which is spending significantly more time in the chamber containing the novel mouse than the one containing the already investigated mouse.

2.3.2. Open field test

Locomotor activity and anxiety-like behavior were measured using an open field test, as mentioned previously (Gould et al., 2009). Each mouse was placed in next to the wall of the open field apparatus (44 cm × 44 cm × 32 cm), facing away from the experimenter. The chamber of the test was illuminated at 150 lux (measured in the center of the apparatus). The arena was divided into 16 even squares. Distance traveled (number of squares crossed) and time spent in the central zone (the central four squares) were recorded. Distance traveled was the measure for locomotor activity, and reduced time spent in the central zone was indicative of elevated level of anxiety. Test duration was 10 min. Three trials were done for each mouse. Test chambers were cleaned with 70% ethanol between subjects.

2.3.3. Elevated plus maze test

The elevated plus maze test is for anxiety-like behavior (Komada et al., 2008); it consisted of two open arms (25 cm × 5 cm) and two enclosed arms of the same size with 15-cm-high walls. The arms were elevated 55 cm above the floor. To minimize the likelihood of animals falling from the apparatus, 3-mm-high walls surrounded the sides of the open arms. Arms of the same type were located opposite from each other. Each mouse was individually placed in the central square of the maze (5 cm × 5 cm), facing one of the closed arms and allowed to freely explore the apparatus. Mouse behavior was recorded during a 10-min test period. The number of entries into an arm and the time spent in the open and enclosed arms were recorded. Percentage of entries into open arms, time spent in open arms (s), and total number of entries were analyzed. Entering the open arms less frequently and spending less time in them were indicative of anxiety-like behavior. The apparatus was cleaned with 70% ethanol between subjects. An open- or closed-arm entry was defined as all four paws in an arm. The numbers of open- and closed-arm entries were combined to yield a measure of total entries, which reflected general activity during the10 min test.

2.3.4. Hole-board

Exploratory activity was estimated by a hole-board test. The apparatus consisted of a wooden board measuring 40 × 40 with 16 evenly spaced holes (3 cm in diameter). The board was surrounded by 25-cm-high wooden wall and was raised 10 cm above the floor. Each subject mouse was individually placed in the center of the board, facing away from the experimenter, and left to move freely for 10 min. The experiments were videotaped, and the videos were watched by a blind observer recording the number of times the mice dipped their heads into the holes to the level of their eyes. Head dipping was thought to reflect exploratory behavior.

2.3.5. Olfactory habituation/dishabituation

Olfactory abilities were assessed using the olfactory habituation/dishabituation assay (Yang and Crawley, 2009). Prior to the start of testing, each mouse was placed into a clean standard cage containing fresh bedding and a plain cotton swab tip suspended from the cage lid. After a 45 min acclimation period, olfactory testing began. Subjects were tested for time spent sniffing cotton swab tips saturated with familiar and unfamiliar odors, with and without social valence. Sequences of three identical swab tips assayed