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EFFECTS OF EXTREMELY LOW-FREQUENCY ELECTRIC FIELDS AT DIFFERENT INTENSITIES AND EXPOSURE DURATIONS ON MISMATCH NEGATIVITY

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Abstract—The effects of extremely low-frequency electric fields (ELF-EFs, 3–300 Hz) on lipid peroxidation levels and antioxidant enzyme activities have been shown in many tissues and plasma after exposure to 50-Hz alternating current (AC) electric fields. However, similar studies investigating brain lipid peroxidation status are limited. Moreover and as far as we know, no study has been conducted to examine mismatch negativity (MMN) response in rats following exposure to a 50-Hz AC electric field. Therefore, the purpose of the study was to investigate different intensities and exposure durations of ELF-EFs on MMN component of event-related potentials (ERPs) as well as apoptosis and oxidative brain damage in rats. Ninety male rats, aged 3 months were used in our study. A total of six groups, composed of 15 animals each, was formed as follows: sham-exposed rats for 2 weeks (C2), sham-exposed rats for 4 weeks (C4), rats exposed to 12-kV/m and 18-kV/m electric fields for 2 weeks (E12-2 and E18-2), rats exposed to 12- and 18-kV/m electric fields for 4 weeks (E12-4 and E18-4). At the end of the experimental period, MMN responses were recorded in urethane-anesthetized rats by electrodes positioned stereotaxically to the surface of the dura. After MMN recordings, animals were killed by exsanguination and their brain tissues were removed for 4-hydroxy-2-nonenal (4-HNE), protein carbonyl and TUNEL analysis. In the current study, different change patterns in ERP parameters were observed dependent on the intensity and exposure duration of ELF-EFs. There were differences in the amplitudes of ERP between the responses to the standard and the deviant tones in all groups. When peak-to-peak amplitude of the difference curves was evaluated, MMN amplitude was significantly decreased in the E18-4 group compared with the C4 group. Additionally, the amount of 4-HNE was increased in all experimental groups compared with the control group. Consequently, it could be concluded that electric field decreased MMN amplitudes possibly induced by lipid peroxidation.

Key words: electric field, mismatch negativity, 4-hydroxy-2-nonenal, protein carbonyl, apoptosis.

INTRODUCTION

During the last half century, people have been constantly exposed to extremely low-frequency (3–300 Hz) electric (EF) and magnetic fields (MF), which have dramatically increased in our environment. Extremely low-frequency electric fields (ELF-EFs) come from electrical appliances, alternating current (AC) transmission and distribution lines. One of the greatest sources of ELF-EFs exposure is transformers and power lines, which produce higher levels of field strength as high as 12-kV/m around AC transmission lines and 16-kV/m around electricity-generating stations in comparison with environmental field strength (Valberg et al., 1997; Repacholi and Greenebaum, 1999; Kheifets et al., 2010). Therefore, due to increased electricity together with distorted urbanization in developing countries, power lines cause greater risk for people living around passing transmission lines and electricity-generating stations. So, this has raised public health concerns and accelerated research to identify possible biological effects associated with exposure to high-level ELF-EFs, which are produced by power lines (Repacholi and Greenebaum, 1999). On the other hand, it is still uncertain not only whether power lines but also current pollution levels of ELF-EFs may constitute a risk to human health. Previous studies suggest that there is a possible association between ELF exposure and increased risk of cardiovascular disease, cancers and neurodegenerative disorders (Guler et al., 2006, 2007). Hence, biological effects of ELF-EFs are receiving increasing scientific interest since humans are chronically exposed to ELF-EFs in varying degrees (Repacholi and Greenebaum, 1999).

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Abbreviations: 4-HNE, 4-hydroxy-2-nonenal; AC, alternating current; ANOVA, analysis of variance; ELF-EFs, extremely low-frequency electric fields; ERPs, event-related potentials; MMN, mismatch negativity; PBS, phosphate-buffered saline; SOD, synthesis of superoxide dismutase; StbD, standard before deviant; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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The brain and central nervous system are considered as the most likely sites of interaction between biological systems and ELF-EFs. Some controlled laboratory studies have demonstrated subtle effects of ELF-EFs on human cerebral functioning, such as modifications of performances, alterations in the latency and amplitude of event-related potentials (ERPs) (Graham et al., 1987, 1994, 1999; Cook et al., 1992). It is well known that ERPs reflect the reception and processing of sensory information as well as higher level processing that involves selective attention, memory updating, semantic comprehension, and other types of cognitive activity (Picton, 1992; Demiralp et al., 1999; Polish, 1999).

The mismatch negativity (MMN) component of ERPs is regarded as a bioelectric correlate of a result of the mismatch between a sensory memory trace and an incoming stimulus (Naatanen et al., 1978; Naatanen, 1990). MMN is elicited using the so-called oddball stimulus paradigm, in which a series of repeated stimuli (standards) are interrupted by an occasional, slightly different stimulus (the deviant). It can be recorded in the condition only if the distinguishing feature(s) of the standard relative to the deviant are successfully represented neurally, stored in transient auditory memory, and compared to the auditory input by the deviant (Naatanen et al., 1978; Naatanen, 1990; Jacobsen and Schroger, 2001). This component is thought to reflect an automatic process, since it occurs in the absence of attention and even during sleep or under anesthesia, and which makes it useful in the assessment of very young or impaired participants (Duncan et al., 2009). MMN, higher amplitude responses to deviants than standards have been reported several times in animals (Csépe et al., 1987, 1989, 1994; Javitt et al., 1992; Kraus et al., 1994; Ruusuvirta et al., 1996, 1998, 2007; Astikainen et al., 2005, 2006; Eriksson and Villa, 2005; Umbricht et al., 2005; Tikhonravov et al., 2008). Animal studies have been conducted in order to understand the neural mechanisms involved in generating MMN. It has been recorded cortically and/or subcortically in several animal species including rats (Ruusuvirta et al., 1998; Tikhonravov et al., 2008), primates (Javitt et al., 1992, 1994), rabbits (Ruusuvirta et al., 1996), cats (Csépe et al., 1989, 1994), mice (Umbricht et al., 2005) and guinea pigs (Kraus et al., 1994). In awake and anesthetized rats, the MMN has been found to occur in the latency range of 30–250 ms (Ruusuvirta et al., 1998; Astikainen et al., 2006; Nakamura et al., 2011; Tikhonravov et al., 2008; Ahmed et al., 2011).

Many researches have been focused on adverse health effects in biological systems resulting from exposure to 50-Hz ELF-EFs. Consistent with this point, we also showed that when the intensity of the ELF-EFs was increased, lipid peroxidation increased proportionally (Akpinar et al., 2012). Hence, based on these data (Akpinar et al., 2012) and previous investigations (Marino et al., 1986; Cossarizza et al., 1993; Margonato et al., 1993; Benov et al., 1994) indicating that an increment of strength and duration of ELF-EFs had greater effects on living organisms, EFs at 12- and 18-kV/m strengths were used in the present study to investigate biochemical and electrophysiological alterations in the central nervous system. To date, there have been no studies investigating the MMN changes in rats, which were exposed to 50-Hz AC electric fields. Therefore, the purpose of the study was to examine MMN alterations as well as oxidative brain damage and apoptosis in rats exposed to 50-Hz ELF-EFs in different strengths and periods. In order to evaluate the relationship between oxidative cell injury induced by ELF-EFs and differences in ERP parameters, 4-hydroxy-2-nonenal (4-HNE) levels and protein carbonyl values of the brain tissue were determined in the present research. Additionally, apoptotic cells in the brain tissue of control and experimental groups were analyzed by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. On the other hand, the effects of magnetic component of ELF electromagnetic fields on biological systems have been reported in many researches, but there are limited data on brain tissues related to the effect of pure ELF-EFs without magnetic field. Hence, particular field strengths (Harakawa et al., 2005; Guler et al., 2008) and exposure durations (Aydin et al., 2006) were selected in this study.

EXPERIMENTAL PROCEDURES

Animals

All experiments were approved by the Akdeniz University Animal Care and Use Committee and were performed in accordance with the European Community directive. All effort was taken to minimize the number of animals used and their suffering. Animals were maintained at 12-h light–dark cycles and a constant temperature of 23 ± 1 °C at all times. In our study, Male albino Wistar rats aged 3 months, weighing 300–350 g were housed in stainless steel cages in groups of four rats per cage and given food and water *ad libitum*. Rats were divided into six groups of 15 animals each: Group 1: sham-exposed rats for 2 weeks (C2); Group 2: sham-exposed rats for 4 weeks (C4); Group 3: rats exposed to 12-kV/m EF for 2 weeks (E12-2); Group 4: rats exposed to 18-kV/m EF for 2 weeks (E18-2); Group 5: rats exposed to 12-kV/m EF for 4 weeks (E12-4); Group 6: rats exposed to 18-kV/m EF for 4 weeks (E18-4). Experimental groups were exposed to 50-Hz EF at a given intensity for 1 h per day. Animals of C2 and C4 groups were kept under the same experimental conditions without being exposed to any EF for 2 and 4 weeks, respectively. Experiments were performed between 09:00 am and 05:00 pm.

Electric field exposure system

The exposure system is presented in Fig. 1. Parallel plate capacitor was used to generate EF. Custom-made parallel copper plates (50 × 80 cm) were plated with zinc (2-mm thickness). In order to produce uniform EF, the corners of parallel plates were rounded, plates were placed upright on wooden stands and positioned parallel to each other. Cables were connected to the center of the plates on their outer surfaces to preserve EF homogeneity. A plastic cage was placed between plates. Plastic cage was suitable for free movement of