Journal of Radiation Research, Vol. 57, No. 2, 2016, pp. 127–132
doi: 10.1093/jrr/rrv093
Advance Access Publication: 24 January 2016

The effect of continuous ELF-MFs on the level of 5-HIAA in the raphe nucleus of the rat

Daryoush SHAHBAZI-GAHROUEI[1]*, Leila SHIRI[1], Hojjatollah ALAEI[2] and Naser NAGHDI[3]

[1]Department of Medical Physics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Department of Physiology, Pasteur Institute, Tehran, Iran
*Corresponding author. Department of Medical Physics, Isfahan University of Medical Sciences, Isfahan, Iran. Tel: : +98-31-37929095;
Email: shahbazi24@yahoo.com or shahbazi@med.mui.ac.ir
Received August 10, 2015; Revised October 3, 2015; Accepted October 19, 2015

ABSTRACT

The aim of this study was to investigate the effect of continuous extremely low frequency magnetic fields (ELF-MFs) with a frequency of 10 Hz and an intensity of 690–720 μT on the level of 5-hydroxyindolacetic acid (5-HIAA) in adult male Wistar rats. A total of 24 adult Wistar male rats were used, and after exposure with an ELF-MF for 15 successive days, all rats in each test were anesthetized with chloral hydrate. Then, they were placed in a stereotaxic frame for surgery and a microdialysis process. Dialysate samples were analyzed to measure the amount of 5-HIAA by high performance liquid chromatography (HPLC) using electrochemical detection. Results showed that ELF-MF exposure for 15 days, 1 h daily, was not effective in altering the level of 5-HIAA. However, ELF-MF exposure for 15 days, 3 h daily, decreased the level of the 5-HIAA in the raphe nucleus. It can be concluded that ELF-MFs affect the serotonergic system and may be used to treat nervous system diseases. This study is an initial step towards helping cure depression using ELF-MFs.

KEYWORDS: ELF-MF, 5-hydroxyindoleacetic acid, microdialysis, HPLC, raphe nuclei

INTRODUCTION

It has long been known that depression is an illness that diminishes the quality of people's life around the world [1, 2]. In the past two decades, considerable evidence has come to light indicating that changes in serotonergic function in the central nervous system of patients causes acute depression [3–5]. In other evidence, extremely low frequency magnetic fields (ELF-MFs) have been shown to influence the nervous and neurotransmitter systems in the brain [1]. Several experimental studies have indicated that ELF-MFs cause changes in or regeneration of cells (particularly, nerve cells) [6–9]. In view of this, researchers have investigated the effects of ELF-MFs on brain activity and electroencephalograms [10–12]. Chronic exposure to ELF-MFs (for 1 or 2 weeks) causes a significant increase in the level of corticosterone in the blood plasma and also has effects on memory [13]. Continuous exposure to a 50-Hz, 0.5-mT magnetic field for 7 days causes the conjunction of serotonin 5-hydroxytryptamine 2 A (5-HT2A) receptor decrease and the density of these receptors to increase in the prefrontal cortex of the rat [14]. Exposure to ELF-MFs can influence monoamine metabolism in some areas [15]. It has been demonstrated that levels of pineal serotonin increase with 1 h of exposure to a 40-μT magnetic field (activated and deactivated at 5 min) in mice and rats [16]. This field increases pineal 5-hydroxyindole acetic acid (5-HIAA) and decreases the activity of the pineal enzyme serotonin-N-acetyltransferase in the rat. Based on a recent in-depth review of the literature, we concluded that there is insufficient data available regarding the effects of ELF-MFs on the level of serotonin and its metabolite. Sieron *et al.* demonstrated that ELF-MFs (10 Hz, 1.8–3.8 mT) does not influence the level of any examined biogenic amines or metabolites, such as 5-hydroxytryptamine (5-HT) and 5-HIAA, in the corpus striatum or the frontal cortex. The ELF-MF increases the rate of synthesis (turn-over) of dopamine (DA) and 5-HT in the rat frontal cortex [17]. In another study [18], it has been reported that pulsating ELF-MFs (max 0.005 T, 12 Hz to 4 Hz) increases the release of serotonin in the raphe nuclei. Exposure to an ELF-MF (55.6 Hz, 8.1 mT) for four successive days increases the concentrations of hypothalamus β-endorphin, substance P, and brainstem serotonin (5-HT) [19].

As mentioned earlier, the serotonergic system has an important role in the abnormality of the nervous system, and there is not enough evidence regarding the application of ELF-MFs for treating abnormalities. There are a few published papers and some data available concerning the effects of ELF-MFs on the level of serotonin and its metabolite in animals. In these limited studies, there are doubtful reports on the effects of ELF-MFs on serotonin and its metabolite concentrations. Therefore, the aim of this study was to investigate the influence of continuous ELF-MFs with different times of exposure on the level of 5-HIAA and to clarify the influence of exposure time on the level of this neurotransmitter. Our main reason for choosing this receptor (5-HIAA) was the previously unpublished pilot study by authors, which showed that measurement of 5-HIAA is a good indicator to use in assessment of the serotonergic system.

MATERIALS AND METHODS

Animals

Twenty-four adult Wistar male rats, 200–250 g, were housed in the Physiology Department's Animals House, three animals per cage, at a temperature of 20–26°C, under a 12-h light–dark cycle (light on at 07:00 h). All rats were fed sterilized standard mouse chow and water *ad libitum*. The Ethics Committee for Animal Experiments at Isfahan University of Medical Sciences approved the study, and all experiments were conducted in accordance with the international guiding principles for biomedical research involving animals (revised 1985).

Electromagnetic field exposure system

The ELF-MF exposure apparatus used consists of a Faraday cage, a coil, a function generator (LEYBOLD DIDACTIC GMBH Model S12, Huerth, Germany), an AC amplifier (LEYBOLD DIDACTIC GMBH Model 522 61, Huerth, Germany) and a plastic box for placing the rats inside the coil (Fig. 1). The length and diameter of the coil were 355 mm and 250 mm, respectively. Copper wire with a diameter of 1 mm and a coil of 2500 turns was used. The exposure apparatus could generate continuous semi sine wave magnetic field signals of 10 Hz, 0.69 ± 0.01 mT Root Mean Square (RMS) in the center of the coil. The magnetic field intensity was non-uniform inside the coil. The magnetic flux density in the coil was measured with a Gauss/Tesla meter (LEYBOLD DIDACTIC GMBH model 516 62, Huerth, Germany). Five points inside the plastic box were measured. The range of magnetic intensity was 0.54 ± 0.01 to 0.72 ± 0.01 mT RMS. The maximum magnetic intensity (0.72 ± 0.01 mT RMS) was near the edge of the coil, and the minimum value (0.54 ± 0.01 mT RMS) was in the head and bottom of the plastic box inside the coil (Fig. 1). The coil was placed on the ground in a Faraday cage, horizontally. The temperature inside the solenoid was kept similar to the temperature of the animal house.

Experimental design and magnetic field exposure

Twenty-four rats were divided into four groups of six, two controls (CO.1 h.C (Control 1 h continuous) and CO.3 h.C (Control 3 h continuous)) and two exposed (PR.1 h.C (Primary 1 h continuous) and PR.3 h.C (Primary 3 h continuous)). The group named PR.1 h.C were exposed to a 10 Hz and 0.69 ± 0.01–0.72 ± 0.01 mT continuous semi-sinusoidal magnetic field generated in the coil for 1 h for 15 successive days. This 1 h was between 11.00 a.m. and 2.00 p.m. The whole body of each animal was exposed. The box placed in the coil had the capacity for three rats each time. In each session, three animals were exposed in the coil. Each group of rates was divided into two lots of three rats, and each group of three was under the exposure for 15 successive days. The group named PR.3 h.C was exposed to the same sinusoidal magnetic field for 3 h for 15 successive days. These 3 h were between 8.00 a.m. and 1.00 p.m. The groups named CO.1 h.C and CO.3 h.C were sham groups for groups PR.1 h.C and PR.3 h.C, respectively. The rats in the groups named CO.1 h.C and CO.3 h.C were subjected to a sham exposure in which the coil

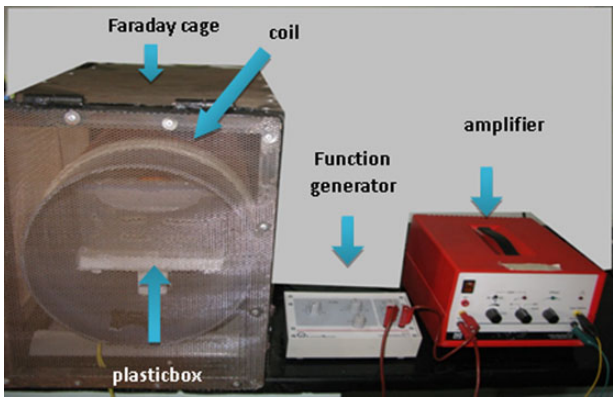

Fig. 1. Continuous field set-up, consisting of coil, function generator and amplifier. This set-up generates a quasi-sine wave with 10 Hz frequency and 690 micro-Tesla intensity in the center of the coil.

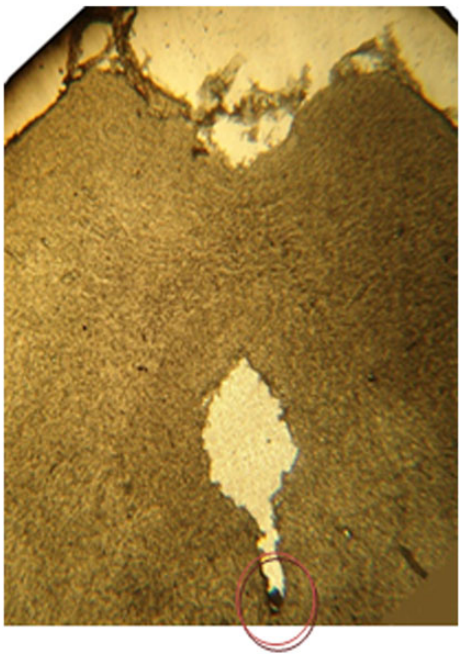

Fig. 2. Schematic of slide preparation of coronal section of the rat brain; raphe nucleus torn by probe is shown with red oval (bregma –8.04 mm).

received no voltage and, therefore, the coil did not generate a magnetic field. In all other respects, the situations were the same as for groups PR.1 h.C and PR.3 h.C.

Microdialysis

After exposing rats to an ELF-MF for 15 successive days, all three rats in each test were anesthetized with chloral hydrate (450 mg/kg intraperitoneally (i.p)) and doses were administered if needed. Then, they were placed in a stereotaxic frame for the surgery and microdialysis process.

A microdialysis probe (A-1-12-01 M, Eicom, Kyoto, Japan) was inserted through a guide cannula (AG-12, Eicom, Kyoto, Japan), then was implanted into the raphe nuclei (anterior–posterior (AP) –8.02 mm, lambda (L) ± 0.2 mm, dorsal–ventral (DV) –6.4 mm) [20].

The probe was continuously perfused with artificial cerebro-spinal fluid (composition in mm: Na_2HPO_4 20; $MgCl_2$, 10; $CaCl_2$ 12; KCl 27; NaCl 1.45; pH 7.4) at a flow rate of 2 µl/min, using a microinjection pump (Stoelting, Holliston, MA, USA). Dialysate samples ($n = 6$) were collected every 20 min in small micro tubes [21]. The micro tubes were placed in iced boxes during collection. Samples were collected for 2 h. The dialysate samples were stored at −70°C until analyzed.

Histology

After microdilysis, the rats were sacrificed and their brains removed and stored in 10% formalin for 7–10 days. The rat brains were sectioned coronally with a freezing microtome (Leica, Nussloch, Germany). Each slice was floated in saline and then in 0.5% gelatin, then placed on a slide (Fig. 2).

The tracks left by the probes were identified using a light microscope (Erma, Tokyo, Japan), and their exact positions were checked by using a rat brain atlas [20]. The data from rats in which the position of the probe was outside the raphe nucleus were omitted.

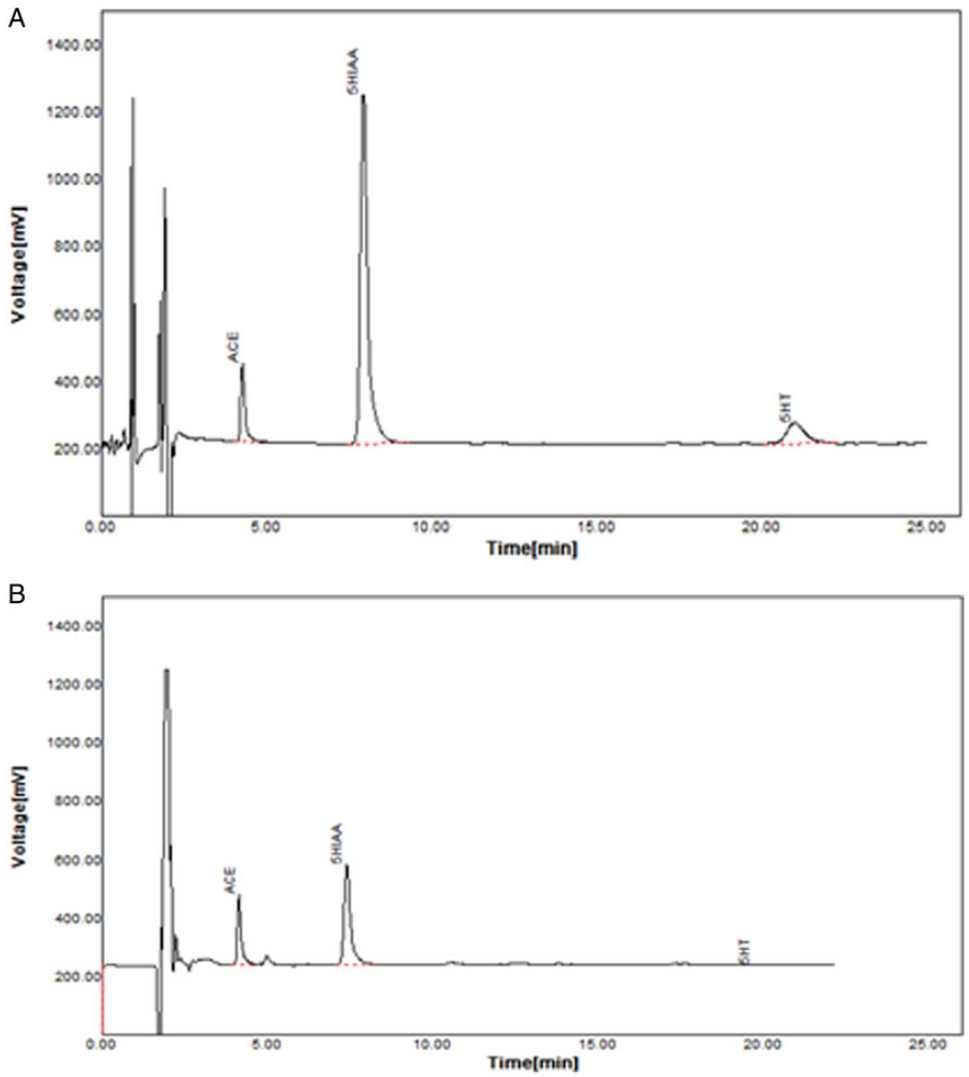

Fig. 3. (A) Peaks from standard solution injected into the machine HPLC-ECD system. (B) Peaks of sample injected into the HPLC-ECD system. ACE = Acetaminophen (internal standard), 5-HT = 5-hydroxytryptamine, 5-HIAA = 5-hydroxyindolacetic acid.

5-HIAA assay

Dialysate samples were analyzed for 5-HIAA by high performance liquid chromatography (HPLC) with electrochemical detection. All dialysate samples were injected into an HPLC column (Teknokroma, 120 ODSA, 150 × 4.6 mm, Barcelona, Spain) that was coupled to an electrochemical detector (Pharmacia LKB, type-2143 RPE, USA). A glassy carbon electrode was set at a potential of +750 mV relative to an Ag/AgCl reference. The mobile phase consisted of sodium phosphate (8.4 g), 1-octane-sulfonic acid (360 mg), ethylenediaminetetraacetic acid (EDTA) 30 mg and 12% methanol per liter of water (final pH = 3.5). The mobile phase was pumped (YONGLIN SD930D, Anyang-si, Gyeonggi-do, Korea) at a rate of 1.0 ml/min. Chromatography data were acquired on-line and exported to a software system (Autochro 2000, USA) for peak amplification, integration and analysis (Fig. 3).

Statistical analysis

A computer program (SPSS version 20, Chicago, IL, USA) was used for statistical analysis. The normalization of data was done by the Kolmogorov–Smirnov test. To compare the two groups, the independent *t*-test was used. All data analysis was considered statistically significant using a criterion level of $P < 0.05$.

RESULTS

Results were obtained only for rats in which the position of the probe was exactly inside the raphe nucleus. As indicated in Fig. 4, the 15-day exposure of rats to continuous ELF-MFs for 1 h a day (group PR.1 h.C) did not significantly affect the level of the 5-HIAA concentration in the raphe nucleus of rats compared with the control group (group CO.1 h.C). In the raphe nucleus, in 1-h ELF-MF –exposed rats, the mean values of 5-HIAA were insignificantly low ($P > 0.05$).

The 15-day exposure of rats to continuous ELF-MFs for 3 h a day (group PR.3 h.C) did have an effect on the level of the 5-HIAA concentration in the raphe nucleus of rats compared with the control group, as shown in Fig. 5 (group CO.3 h.C). Figure 6 showed that in the raphe nucleus, in 3-h ELF-MF –exposed rats, the mean values of 5-HIAA were also significantly low ($P < 0.05$). Also, this figure showed that the 5-HIAA concentration in the raphe nucleus for 3-hours exposed rat was significantly lower than that of 1-h–exposed rats ($P < 0.01$).

DISCUSSION

Recent research on the effects of electromagnetic fields on animal (rat) cells and on human cells is of great interest [5, 22]. However, there are only a few inconclusive published papers and limited data available on the effects of ELF-MFs on the level of serotonin and its metabolite concentrations in animals.

The comparison between the rat groups PR.1 h.C and PR.3 h.C studied here indicates that time has an important role in the reduction of serotonin metabolite levels. These levels can be an indicator of the rate of release of serotonin into the synaptic space and the pre-synaptic re-uptake by the cells [23] or of the activity of monoamine oxidase [24].

The method used in the present study (including the time of exposure, frequency and waveforms) used for group PR.1 h.C is similar to that used by Sieron's *et al.* [17]. Sireon and colleagues reported that a 1 h daily for 14 days exposure of rats to 10 Hz ELF-MFs with an intensity of 1.8–3.8 mT did not influence the endogenous level of 5-HT or its metabolite in the corpus striatum or the frontal cortex [17]. Differences between the present work and the Seiron study include differences between the intensity of the ELF-MF and the nucleus of the rat. In both mentioned study results showed, ELF-MFs did not influence the level of 5-HIAA significantly. As our results indicate, increase in the exposure time decreased the level of 5-HIAA in the raphe nucleus of the rat (group PR.3 h.C).

In the Zhang *et al.* study, exposure to 4–12 Hz ELF-MFs of 5-mT intensity for 45 min affected the release of serotonin, followed by increase in the level of serotonin in the raphe nucleus [18].

Bao *et al.* reported that exposure to ELF-MFs of 55.6 Hz and 8.1 mT for an hour a day over four consecutive days caused a significant increase in brain serotonin in the rat hypothalamus, which is different from the results of this study. This differences arise from differences in the pulse intensities and the duration of the exposures. However, exposure to the same ELF-MF after 14 days did not affect serotonin [25]. The study by Wilson *et al.* suggested that continuous exposure to ELF-MFs reduces the level of serotonin and may cause some

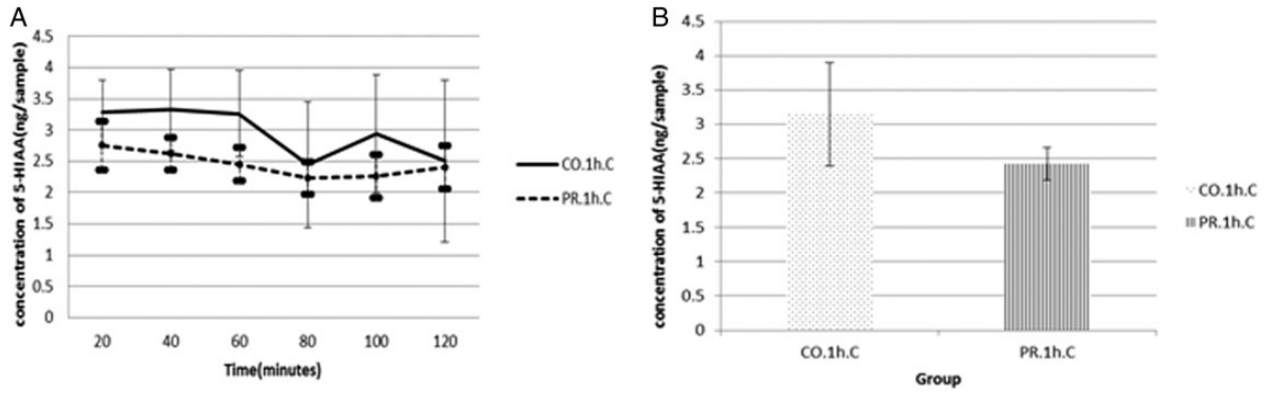

Fig. 4. Comparison of metabolites of serotonin concentrations in the raphe nucleus between group PR.1 h.C (under the continuous ELF field for 1 h per day for 15 consecutive days) compared with the control group CO.1 h.C. (A) Line Chart and (B) column chart. Error bars indicated the standard error of the mean (SEM) (Mean ± SEM, $P > 0.05$, $n = 3$ rats).

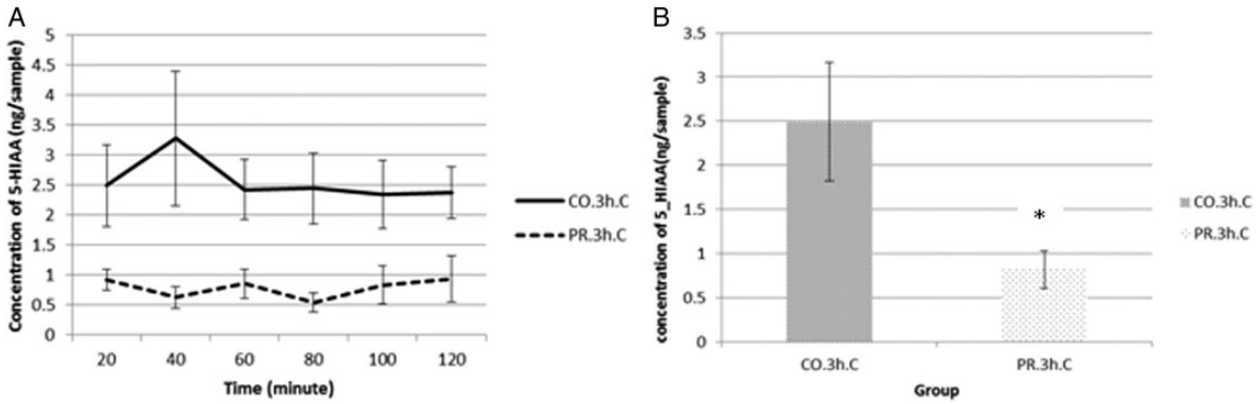

Fig. 5. Comparison of metabolites of serotonin concentrations in the raphe nucleus between group PR.3 h.C (under the continuous ELF field for 3 h per day for 15 consecutive days) compared with the control group CO.3 h.C. (A) Line chart and (B) column chart. Error bars indicated the standard error of the mean (SEM) (Mean ± SEM, *$P < 0.05$, $n = 4$ rats for group PR.3 h.C and $n = 3$ rats for group CO.3 h.C).

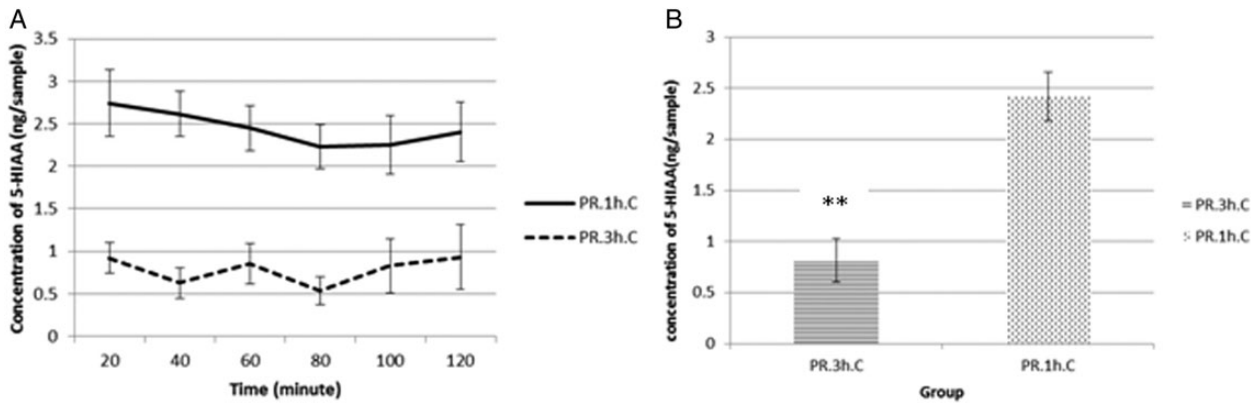

Fig. 6. Comparison of metabolites of serotonin concentrations in the raphe nucleus between group PR.1 h.C (under the continuous ELF field for 1 h per day for 15 consecutive days) compared with the group PR.3 h.C. (A) Line chart and (B) column chart. Error bars indicate the standard error of the mean (SEM) (Mean ± SEM, **$P < 0.001$, $n = 4$ rats for group PR.3 h.C and $n = 3$ rats for group PR.1 h.C).

neurological disorders, which is in good agreement with the results of the present study [26].

Concentration of the serotonin metabolite, in fact, an index of serotonergic activity. As mentioned above, a decreased level of the serotonin metabolite can occur for the following reasons: reduced monoamine oxidase (MAO) activity, reduced release of serotonin into the synaptic space, and increased pre-synaptic re-uptake of serotonin by neurons.

Based on the first reason, decreased activity of MAO is a key trigger for increasing the level of serotonin in the synaptic space. Reduction of serotonin in the synaptic space can cause or exacerbate depression [27], so this is an important avenue for curing depression.

It is known that the time of exposure and its duration play an important role in the reaction of living systems to ELF-MFs. In support of this, our study showed that ELF-MF exposure for 15 days at 1 h daily was not sufficient to alter the level of 5-HIAA. However, ELF-MF exposure for 15 days at 3 h daily decreased the level of 5-HIAA in raphe nucleus. Also, the outcome of such experiments depends on the characteristics of the applied ELF-MF (such as frequency, intensity and impulse shape). The actual cause for the decrease in serotonin metabolites is unknown and is thought to be a complex process.

Further studies are needed to clarify the effects of ELF-MF exposure in altering the concentration or activity of serotonin and MAO. This study is an initial step towards helping cure depression with the use of ELF-MFs.

FUNDING

This work was financially supported by Isfahan University of Medical Sciences, Isfahan, Iran.

REFERENCES

1. Kato M. *Electromagnetics in Biology*, 1st edn. Hicom, Japan: Springer, 2006, 63–113.

2. Shahbazi-Gahrouei D, Karbalae M, Moradi H, et al. Health effects of living near mobile phone base transceiver station (BTS) antennae: a report from Isfahan, Iran. *Electromagn Biol Med* 2014;33:206–10.
3. Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. *Clin Chem* 1994;40:288–95.
4. Chu LY, Lee JH, Nam YS, et al. Extremely low frequency magnetic field induces oxidative stress in mouse cerebellum. *Gen Physiol Biophys* 2011;30:415–21.
5. Manikonda PK, Rajendra P, Devendranath D, et al. Extremely low frequency magnetic fields induce oxidative stress in rat brain. *Gen Physiol Biophys* 2014;33:81–90.
6. Borgens RB, Roederer E, Cohen MJ. Enhanced spinal cord regeneration in lamprey by applied electric fields. *Science* 1981;213:611–7.
7. Dubey N, Letourneau PC, Tranquillo RT. Guided neurite elongation and Schwann cell invasion into magnetically aligned collagen in simulated peripheral nerve regeneration. *Exp Neurol* 1999;158:338–50.
8. Macias MY, Battocletti JH, Sutton CH, et al. Directed and enhanced neurite growth with pulsed magnetic field stimulation. *Bioelectromagnetics* 2000;21:272–86.
9. Song B, Zhao M, Forrester J, et al. Nerve regeneration and wound healing are stimulated and directed by an endogenous electrical field *in vivo*. *J Cell Sci* 2004;117:4681–90.
10. Bell GB, Marino AA, Chesson AL. Alterations in brain electrical activity caused by magnetic fields: detecting the detection process. *Electroencephalogr Clin Neurophysiol* 1992;83:389–97.
11. Bell GB, Marino AA, Chesson AL. Frequency-specific responses in the human brain caused by electromagnetic fields. *J Neurol Sci* 1994;123:26–32.
12. Marino AA, Nilsen E, Chessonet AL, et al. Effect of low-frequency magnetic fields on brain electrical activity in human subjects. *Clin Neurophysiol* 2004;115:1195–201.
13. Mostafa RM, Mostafa YM, Ennaceur A. Effects of exposure to extremely low-frequency magnetic field of 2G intensity on memory and corticosterone level in rats. *Physiol Behav* 2002;76:589–95.
14. Janac B, Tovilovic G, Tomic M, et al. Effect of continuous exposure to alternating magnetic field (50 Hz, 0.5 mT) on serotonin and dopamine receptors activity in rat brain. *Gen Physiol Biophys* 2009;28:41–6.
15. Kabuto H, Yokoi I, Mori A, et al. Effects of an *in vivo* 60 Hz magnetic field on monoamine levels in mouse brain. *Pathophysiology* 2000;7:115–9.
16. Lerchl A, Nonaka KO, Stokkan KA, et al. Marked rapid alterations in nocturnal pineal serotonin metabolism in mice and rats exposed to weak intermittent magnetic fields. *Biochem Biophys Res Commun* 1990;169:102–8.
17. Sieron AL, Nowak P, Cieślar G, et al. Alternating extremely low frequency magnetic field increases turnover of dopamine and serotonin in rat frontal cortex. *Bioelectromagnetics* 2004;25:426–30.
18. Zhang J, Wang X, Wang M. Influence of time-varying magnetic field on the release of neurotransmitters in raphe nuclei of rats. *Conf Proc IEEE Eng Med Biol Soc* 2005;6:6214–6.
19. Xu F, Gao M, Wang L, et al. Study on the effect of electromagnetic impulse on neurotransmitter metabolism in nerve cells by high-performance liquid chromatography–electrochemical detection coupled with microdialysis. *Anal Biochem* 2002;307:33–9.
20. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*, 6th edn. Amsterdam, Netherlands: Elsevier/Academic Press, 2006.
21. Zapata A, Chefer VI, Shippenberg TS. Microdialysis in rodents. *Curr Protoc Neurosci* 2009;Suppl 47:7.2.
22. Razavi S, Salimi M, Shahbazi-Gahrouei D, et al. Extremely low-frequency electromagnetic field influences the survival and proliferation effect of human adipose derived stem cells. *Adv Biomed Res* 2014;3:25.
23. Reinhard JF Jr, Wurtman RJ. Relation between brain 5-HIAA levels and the release of serotonin into brain synapses. *Life Sci* 1977;21:1741–6.
24. Wolf WA, Youdim MB, Kuhn DM. Does brain 5-HIAA indicate serotonin release or monoamine oxidase activity? *Eur J Pharmacol* 1985;109:381–7.
25. Bao X, Shi Y, Huo X, et al. A possible involvement of β-endorphin, substance P, and serotonin in rat analgesia induced by extremely low frequency magnetic field. *Bioelectromagnetics* 2006;27:467–72.
26. Wilson BW, Anderson LE, Hilton DI, et al. Chronic exposure to 60-Hz electric fields: effects on pineal function in the rat. *Bioelectromagnetics* 1981;2:371–80.
27. Zhang Y, Li Zi, Gao Y, et al. Effects of fetal microwave radiation exposure on offspring behavior in mice. *J Radiat Res* 2015;56:261–8.