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Research Article

LOW FREQUENCY MAGNETIC FIELD INDUCES DEPRESSION BY RISING NITRIC OXIDE LEVELS IN THE MOUSE BRAIN

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ABSTRACT

The present study was designed to investigate the influence of Extremely low frequency magnetic field (ELF MF) on depression related behavior, and mechanism involved therein. Mice were exposed to ELF MF (50Hz, 10G) 8h/day for 7, 30, 60, 90 and 120 days. Depression was assessed using forced swim test (FST), in which no significant effect was observed on 7th, 30th, 60th, 90th exposure day. However, depression was observed on 120th exposure day. It is evident that ELF MF exposure modulates level of nitric oxide (NO); therefore, the level of NO was measured in the regions of brain viz; cortex, striatum, hippocampus and hypothalamus. Results established that ELF MF elevated NO levels in the regions of brain. Furthermore, the implication of NO was assessed by nitric oxide synthase (NOS) inhibitors suggesting the involvement of NO in ELF MF induced depression.
Keywords: Extremely low frequency magnetic field; Nitric oxide; Brain; Depression.

Abbreviations: ELF MF, extremely low frequency magnetic field; FST, forced swim test; NO, nitric oxide; NOS, nitric oxide synthase; L-NAME, N^G-nitro-L-arginine methyl ester; 7- NI, 7-nitroindazole; L-ARG, L-arginine; EMF, electromagnetic field; rTMS, trans cranial magnetic stimulation; CNS, central nervous system; ANOVA, analyses of variance.

INTRODUCTION

Natural electromagnetic environment is necessary for life on the earth and, this environment has sharply changed because of introduction of the enormous and rising spectrum of manmade electromagnetic field (EMF) (Adey, 1993). Today, intensity of artificial EMF is ten to hundred times higher than of natural, and hence danger from EMF is a problem that necessitates awareness. Extremely low frequency electromagnetic field (ELF MF) are electromagnetic oscillating fields defined as having frequencies below 300 Hz (Lyon and IARC., 2002). Further, EMF produced by electrical or electronic power systems have been added to the list of environmental agents that are a likely threat to public health. Since long time, magnetic and electrostatic fields have been considered as a beneficial therapeutic application, this exposure to magnetic fields had opened up a new area of magnetic therapy, subsequently the range of scientific applications to facilitate bone growth and speed the healing of fractures. In addition, newly trans cranial magnetic stimulation (rTMS) has been suggested as a therapeutic means for the treatment of depression (Baeken et al., 2010; Miniussi et al., 2005). Even if such kinds of EMF have remedial application in human being (Dragasevic et al., 2002), other can be detrimental, e.g. ELF MF. The ELF MF generated by power lines including domestic and industrialized devices which operates on electricity, is not

easy to shield as they are induced with the current flow, and therefore cannot be avoided by public.

The effect of EMF on human health is of prime importance since humans are the creators of artificial EMF; therefore, taking into consideration potential benefits and harmful effects of EMF exposure, this area needs substantial focus. Previous study have attempted to light on the subject line and suggest a relationship between the increased incidence of depressive and neurotic symptoms in humans (Poole et al., 1993; Wilson, 1988). In an subsequent investigation conducted by Savitz and coworkers on severe depression in relation to magnetic field found a pattern of amplified hazard for depression among electricians (Savitz et al., 1994). It is suggested that ELF MF exposure affects brain electrical activity or cognitive function at field strengths similar to that in the proximity of some household and industrial electrical devices (Crasson M, 2003). Also, EMF has been suggested as a potential risk of depression by causing pineal dysfunction, and the danger of severe depression was reported to be increased among subjects living inside 100m of a high voltage power line (Verkasalo et al., 1997). Moreover, it is evident that ELF MF could increase trait of anxiety in residential females (P. Boscolo et al., 2006) and also in animals (Liu et al., 2008). Experimental data have shown that exposure to ELF MF affects spatial learning memory in rodents (Sienkiewicz et al., 1998). Furthermore, rTMS, a therapeutic tool for the treatment of depression influences mood in healthy volunteers (Schaller et al., 2011) is reported to produce anxiety in human subjects (George M et al., 2000) and also in experimental animals (Isogawa et al., 2005; K. Isogawa et al., 2003). Overabundance of continual evidences has suggested that ELF MF produced effects on the function of nervous system and the brain activity (Jelenkovic et al., 2006; Sienkiewicz et al., 2005). Collectively, all these reports suggesting the influence of ELF MF exposure on the central nervous system (CNS).

NO is one of the important neurotransmitters, which is known to be implicated in behaviors like learning, pain perception, aggression, depression, anxiety and obsessive compulsive disorder. Evidence established increased levels of nitrates in the brain of depressed patients (Suzuki et al., 2001) indicating the implication of NO in this condition. Further, EMF is known to affect release and metabolism of certain kind of neurotransmitters like serotonin, dopamine in the mouse brain (Kabuto et al., 2000). Additionally, it is evident that acute exposure of rats to power frequency magnetic field decreases the activity of cholinergic pathways in the frontal cortex and the hippocampus (Lai et al., 1993). Moreover, it is reported that EMF has pronounced effect on the glutamate turnover in the mice hippocampal tissue (Wieraszko et al., 2005), including NOS activity and therefore responsible for the surplus generation of NO (Kavaliers M and Ossenkopp, 1991; Nathan C and Xie, 1994). In addition, experimental data showed that ELF MF exposure enhances the rate of NO generation (Yoshikawa et al., 2000). Incidentally, in recent past it has been also demonstrated that ELF MF exposure modulates the level of NO in the brain and spinal cord (Jeong et al., 2006).

In view of above understanding, it appears that exposure to ELF MF produce promising effects on the behavioral pattern in humans as well as in animals and modulated the levels of different neurotransmitters including NO in the CNS, which is responsible for regulating certain kind of behaviors, and therefore may be correlated with depression. Thus, the present investigation was designed to find the association between ELF MF exposure, brain levels of NO and propensity towards depression, and attempt was made to make out the mechanism involved therein.'

MATERIAL AND METHODS

Animals

All experimental procedures were approved by the Institutional Animal Ethics Committee, and the experiments were carried out in strict accordance with the guidelines approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) by Ministry of Environment and Forests, Government of India, New Delhi. The investigations were carried out in young healthy adult male albino Swiss mice (22–26 g) which were naive to drug treatments and experimentation. Mice were housed in groups of twelve (in cages measuring 40×28×14cm) under standard laboratory conditions (12h light:12h dark cycle with lights on at 07:00 a.m., 22-25 ◦C, and 45–55% relative humidity). They received standard rodent chow (Trimurti Feeds, Nagpur, India) and water *ad libitum*. The mice were randomly assigned into treatment groups as: (1) no ELF MF exposure (control group); (2) exposure to ELF MF

8 h/day (MF 8h group); (3) ELF MF 8 h/day+L-ARG; (4) ELF MF 8 h/day+L-NAME; (5) ELF MF 8 h/day+7-NI; (6) saline; (7) saline+L-ARG; (8) saline +L-NAME; (9) saline +7-NI

Materials

L-Arginine, L-NAME, 7-NI were purchased from Sigma–Aldrich, MO, USA. Except 7-NI, all the drugs were dissolved in normal saline, whereas 7-NI was dissolved first in few drops of Tween 80 and then the volume was made with normal saline. Potassium iodide, copper, cadmium, potassium nitrite, and sulphuric acid were obtained from SRL (Sisco Research Laboratory Ltd., India).

Experimental design

Device for electromagnetic field exposure

EMF exposure has been carried out as described earlier (Liu et al., 2008), with slight modification. In brief, the ELF MF (50 Hz, 10G) was generated from a pair of round Helmholtz coils, spaced apart at a distance equal to their radii (45cm), which were constructed of glaze insulated copper wire (SWG#18, d=1.2mm) and had 100 turns on wooden frame to achieve shielding against emission of electric field. The coils were then attached to regulated AC power supply through step down transformer (12V) which acts as an isolator. The generated strength of magnetic field was measured by digital gauss meter (SEA-20, OSAW Industrial Products Pvt. Ltd., India) with Hall Effect Probe. Dimmer stat (2D-1P, Automatic electric Ltd., Mumbai, India) was function as a variable transformer, used to adjust the strength of magnetic field (10G). During the exposure, all mice were housed in their home cages with grid plastic covers. Mice in ELF MF exposure groups were placed on a platform settled in the center of the coils; control mice were exposed to only geomagnetic field in the local area (0.01G). The ELF MF exposure was conducted in every morning (8:00 a.m.-4:00 p.m.) for 7, 30, 60, 90 and 120 days. Exposure was carried out at ambient room temperature and no significant temperature change was detected between the two activated Helmholtz coils (25±0.5°C). Depression was assessed in the FST at the morning hours (8:00 a.m.-12:00 p.m.) on the 7th, 30th, 60th, 90th and 120th exposure day, respectively. For each test, control and exposure groups were assessed on the same day. All mice were naive and tested only once.

Assessment of depression and locomotor activity:

Depression

Depression was assessed in terms of immobility time in a well known animal model of FST, which was carried out by a method described earlier (Porsolt et al., 1977). Mice were placed for 6 min in a glass cylinder (height:40cm; diameter:17cm) filled with water (25±1°C). The deepness of water was adjusted so that the mouse must swim or float without touching the hind limbs or tail to the bottom. Individual mouse was placed in the cylinder for 6 min, and the duration of immobility (time during which mouse made only small movements necessary to keep the head above water) was scored. As suggested by Porsolt, time of immobility during the last 4 min were analyzed and presented. After each individual mouse testing, water in the glass cylinder was replaced with fresh water of same temperature. Mice were gently dried after each individual assessment and were returned to their home cages.

Locomotor activity

Locomotor test was performed in separate group of mice using Actophotometer (VJ Instruments, Washim, Maharashtra, India), having a circular arena of 40 cm, equipped with infrared beams and photo cells associated with digital counter. The activity was expressed in terms of total number of counts of infrared beams interruptions by individual mouse in 30 min.

Estimation of NO levels in the brain tissue

Following decapitation, the brains were removed and dissected rapidly over the ice cooled slab into the cortex, striatum, hippocampus and hypothalamus as per Glowinski and Iversen (Glowinski and Iversen, 1966). Each region was identified according to the mouse brain atlas of Paxinos and Franklin (Konsman, 2003), and NO levels were determined in each identified brain region according to previously published procedure (Umathe et al., 2009). The NO levels in the brain tissue were measured in terms of evolved NO & nitrites levels (NOx) with minor modification. In brief, 0.25 ml of brain tissue homogenate (10% in phosphate buffered saline, PH 7.4) was incubated with 150 mg of copper cadmium alloy (1:10) for 1h at room temperature with intermittent shaking, for converting nitrate to nitrite. It was then centrifuged for 10 min at 10,000g and 10 µL of the supernatant was added to bath (1 mL capacity) containing a freshly prepared mixture of 20 mM potassium iodide in 0.1

M sulphuric acid to evolve NO. The evolved NO was detected by NO specific sensor (AmiNO 700) connected to NO measuring system equipped with inNO II software (version 3.2, Innovative Instruments Inc., Tempa, FL, USA). The conversion efficiency of the alloy was checked by using potassium nitrite standards. The NOx levels were calculated from standard linearity of potassium nitrite solution.

Statistical analysis

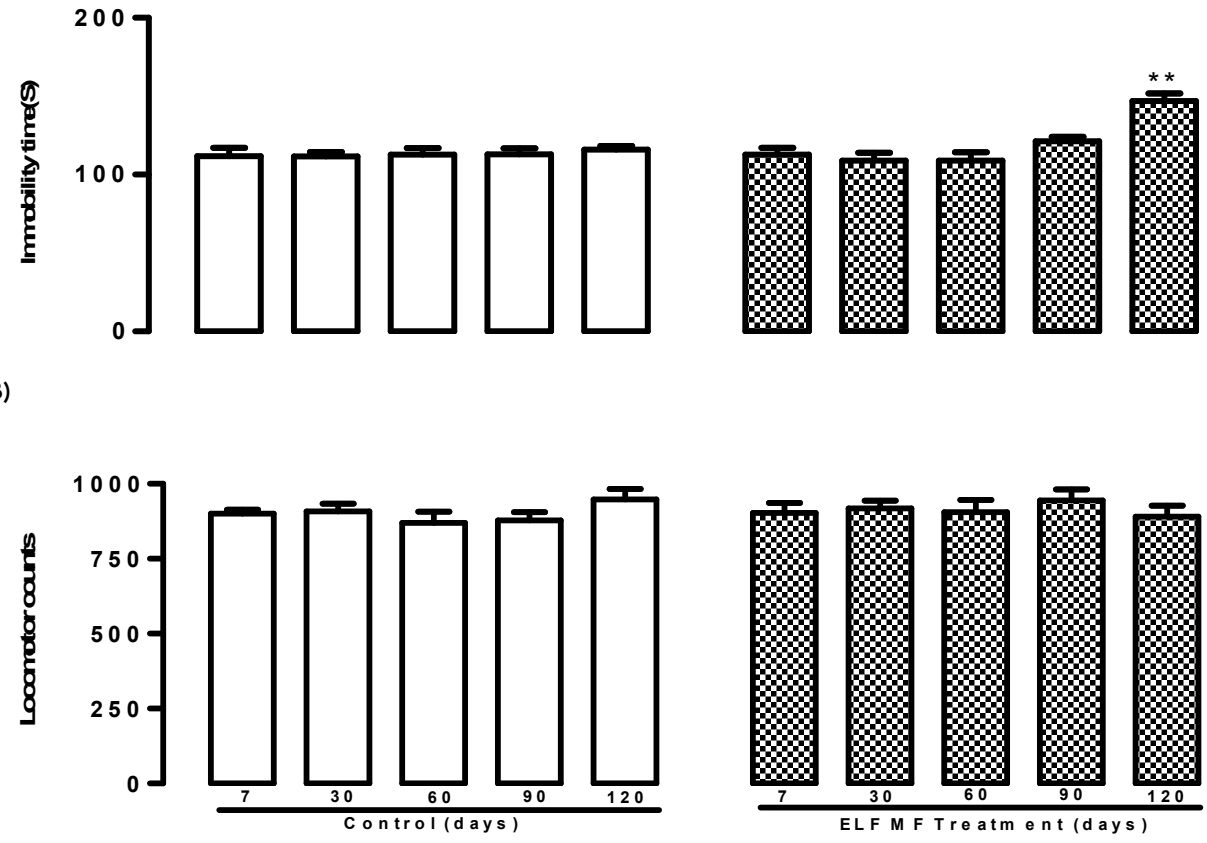
Data were analyzed by Two-way ANOVA with repeated measures followed by Bonferroni post hoc test, One-way ANOVA followed by Dunnett's post hoc test, and t-tests using Graph Pad Prism Software, version 4.0 (Inc, San Diego, CA). The results are expressed as means±S.E.M. of six to eight observations. $P<0.05$ was considered statistically significant in all the comparisons.

RESULTS

Influence of ELF MF exposure in FST, locomotor test

Two-way ANOVA with repeat measures followed by Bonferroni post hoc test revealed a significant effect of ELF-MF exposure on immobility time [factor 'ELF MF exposure' $F(1,48)= 5.821$, $P=0.0328$, factor 'duration of exposure' $F(4,48)=8.836$, $P<0.0001$, interaction 'ELF MF exposure×duration of exposure' $F(4,48)= 5.859$, $P=0.0006$] (Fig.1A);without significant influence on locomotor counts (Fig.1B). However, post hoc test showed no significant influence on immobility time in FST on 7th, 30th, 60th, 90th exposure day. Significant influence on immobility time was observed on 120th day of exposure ($P<0.01$). Data are shown in Fig.1.

Fig. 1. Influence of ELF MF exposure in FST, locomotor test: **(A) immobility time (B) locomotor counts**. Mice were exposed to ELF MF (10G) for 8h (8.00am to 4.00pm) daily in Helmholtz coil or exposed to geomagnetic field, upto 120 days. Thereafter separate group of mice were subjected to FST, and locomotor test on 7th, 30th, 60th, 90th & 120th exposure day, respectively; immobility time & locomotor counts were recorded. Each bar represents separate group of mice. All data were presented as means±S.E.M., n =6-8 in each group. **P<0.01, vs. respective control group [Two way ANOVA with repeat measures followed by Bonferroni post hoc test].



Dose dependent effect of L-arginine (L-ARG), N^G-nitro-L-arginine methyl ester (L-NAME), and 7-nitroindazole (7-NI) in FST, locomotor test

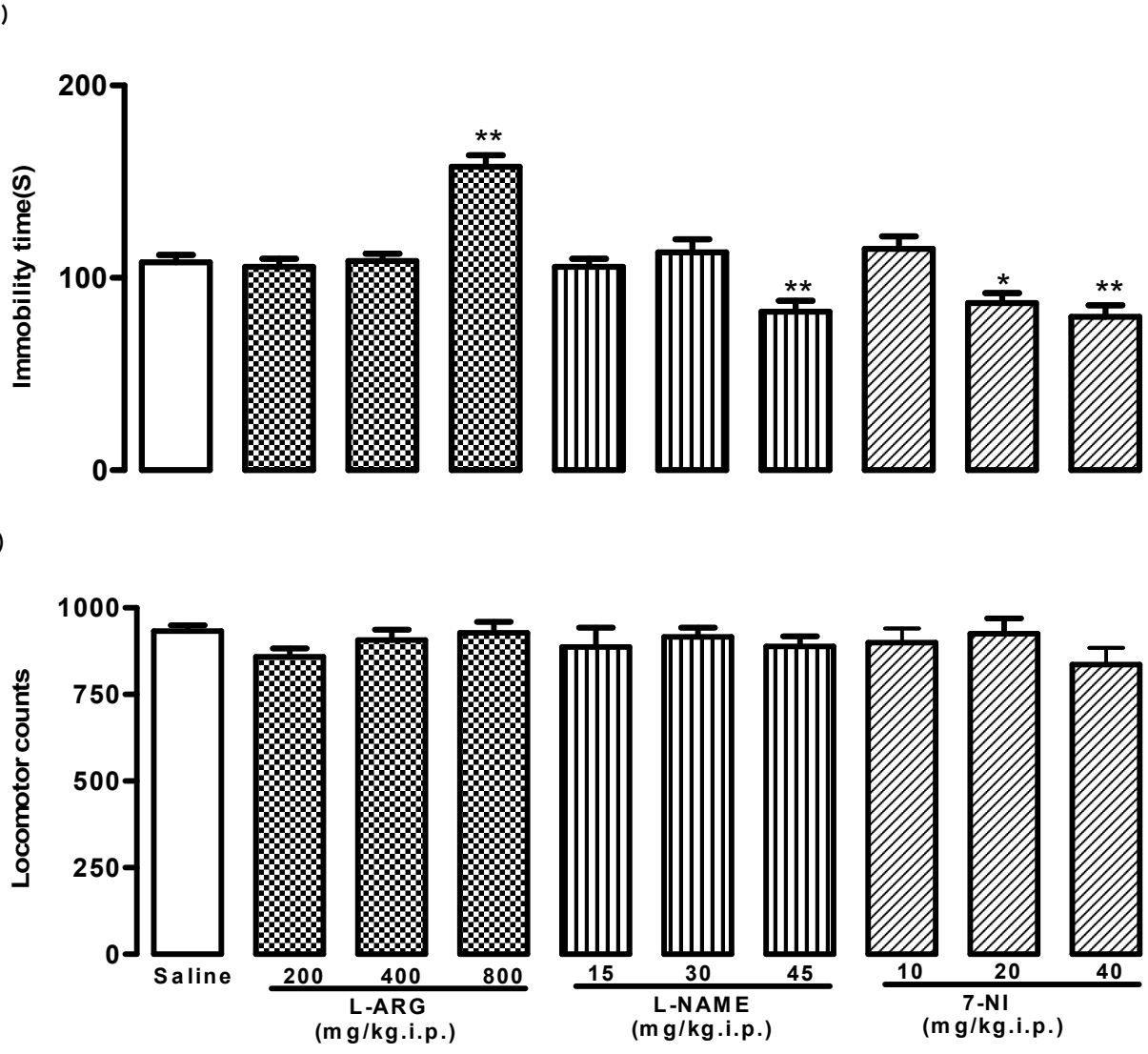
L-ARG, L-NAME and 7-NI significantly influenced immobility time (Fig.2A) [$F(9,69)= 17.38$, $P<0.0001$]. However, above drug treatments failed to influence locomotor activity at tested doses (Fig.2B). Post-hoc test indicated that L-ARG (800mg/kg) significantly ($P<0.01$) increased immobility time. Whereas, L-NAME (45mg/kg) ($P<0.01$), and 7-NI (20, 40mg/kg) treatments significantly ($P<0.05$, $P<0.01$) decreased the immobility time. Data are shown in Fig.2.

Effect of L-ARG, L-NAME and 7-NI in FST, locomotor test on 120th ELF MF exposure day

One way ANOVA revealed that NOS modulators, L-ARG, L-NAME, and 7-NI treatments had significant effect on 120th ELF MF exposure day as indicated by immobility time in FST (Fig.3A) [$F(3,28)=28.00$, $P<0.0001$]. However, none of the drug treatments influenced the locomotor counts (Fig.3B).

Post hoc test indicated that subeffective treatments of L-ARG (400mg/kg), a NO precursor, significantly ($P<0.05$) increased, while L-NAME (30mg/kg) or 7-NI (10mg/kg), NOS inhibitors, significantly ($P<0.01$) decreased immobility

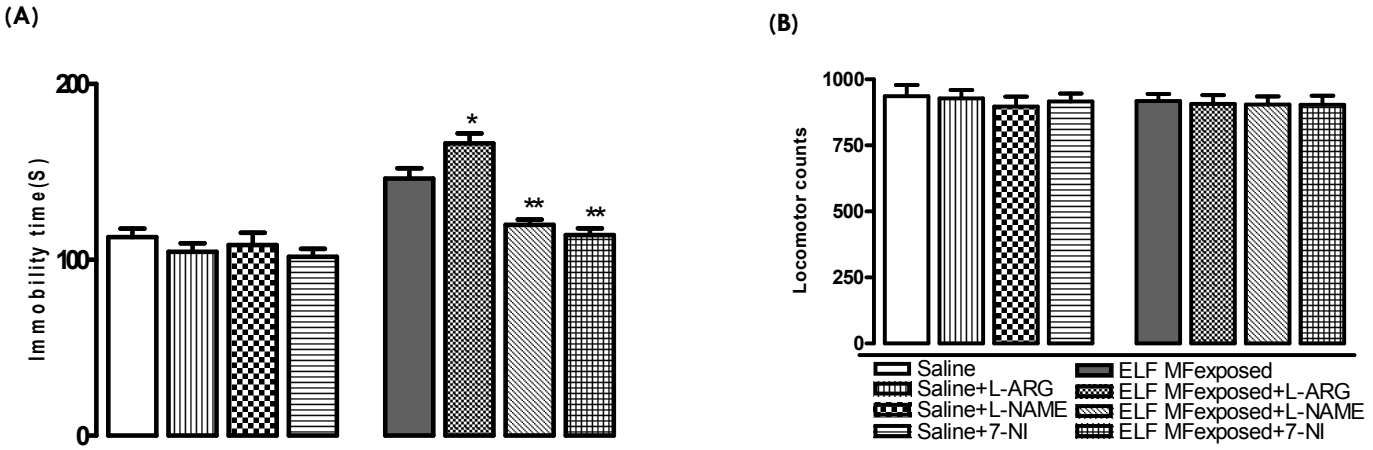
Fig.2. Dose dependant effect of L-ARG, L-NAME, and 7-NI in FST, locomotor test: **(A) immobility time (B) locomotor counts.** Separate group of mice were employed for each dose and 30 min after i.p. injection with vehicle (saline, 10 ml/kg) or L-ARG (200, 400, 800 mg/kg) or L-NAME (15, 30, 45 mg/kg) or 7-NI (10, 20, 40 mg/kg) mice were subjected for FST. All data were presented as means±S.E.M., n =6-8 in each group. *P<0.05, **P<0.01 vs. vehicle group [One-way ANOVA followed by Dunnett's test].



time. However, at employed doses NO modulators did not produce significant effects *per se* in above parameters. Data are shown in Fig.3.

cortex [t=4.120 df=10, P=0.0021], hippocampus [t=3.361 df=11, P=0.0064], and hypothalamus [t=3.955 df=10, P=0.0027] except striatum [t=0.1365 df=11, P=0.8939].

Fig.3. Effect of L-ARG, L-NAME, and 7-NI in FST, locomotor test on 120th ELF MF exposure day: **(A) immobility time, (B) locomotor counts**. Separate group of control or ELF MF exposed mice were treated with subeffective doses of L-ARG (400mg/kg) or L-NAME (30mg/kg) or 7-NI (10mg/kg), and 30 min after i.p. administration mice were subjected to FST, and locomotor test. Each bar represents separate group of mice. All data were presented as means±S.E.M., n =6-8 in each group. *P<0.05, **P<0.01vs. ELF MF exposed group [One-way ANOVA followed by Dunnett's test].



Influence of ELF MF after 120th exposure day on NO levels in the regions of the brain

When dealing with a directional hypothesis, one sided statistic test is indicated. According to a one sided statistics, t-test revealed significant effect of ELF MF exposure on brain NOx levels in the specified areas. The NOx levels were significantly increased in the cortex [t=4.930 df=11, P=0.0004], hippocampus [t=4.881 df=11, P=0.0005], and hypothalamus [t=6.899 df=10, P<0.0001], but not in striatum [t=0.05999 df=11, P=0.9532] indicating the significant effect of ELF MF exposure on NO levels in mice brain. Data are shown in Fig.4.

Effect of L-ARG, L-NAME, and 7-NI treatment on NO level in the regions of the brain of control and ELF MF exposed mice

The t-tests revealed no significant effect of L-ARG or L-NAME or 7-NI treatments on brain NOx levels in control mice, in their site specific areas, like cortex, striatum, hippocampus and hypothalamus (Fig.5A). However, t-tests showed significant effects of subeffectvie doses of NO modulators on brain NOx levels in ELF MF exposed mice. L-ARG treatment significantly raised the NOx levels in the

In contrast, L-NAME or 7-NI has significantly attenuated the ELF MF induced increase in NOx levels in cortex [t=2.937 df=10, P=0.0149] or [t=3.894 df=11, P=0.0025], hippocampus [t=5.811 df=10, P=0.0002] or [t=9.990 df=10, P<0.0001], and hypothalamus [t=6.197 df=10, P=0.0001] or [t=5.646 df=10, P=0.0002], respectively. However, L-NAME [t=0.0004458 df=11, P= 0.9997] or 7-NI [t=0.9940 df=11, P= 0.3416] treatment did not shown significant effect in striatum (Fig.5B). Data are shown in Fig.5.

DISCUSSION

The present study investigates the influence of ELF MF exposure on depression related behavior in mice and mechanism involved therein. The statistical data revealed that ELF MF (10G) failed to show significant effect in FST on exposure day 7, 30, 60 and 90. However, ELF MF significantly induced depression on 120th exposure day assessed through FST as indicated by increased immobility time. But, ELF MF did not significantly affect locomotor activity on 7th, 30th, 60th, 90th, and, 120th exposure day as indicated by locomotor counts suggesting effect is centrally based. ELF MF generated from residentially power lines,

Fig.4. Influence of ELF MF after 120th exposure day on NOx levels in the regions of the brain: Separate group of ELF MF exposed, 120 days (8 h/day) mice were decapitated for determination of NO levels in the regions of the brain. Each bar represents separate group of mice. All data were presented as means±S.E.M., n =6-8 in each group. ***P<0.001vs. control group [t-tests].

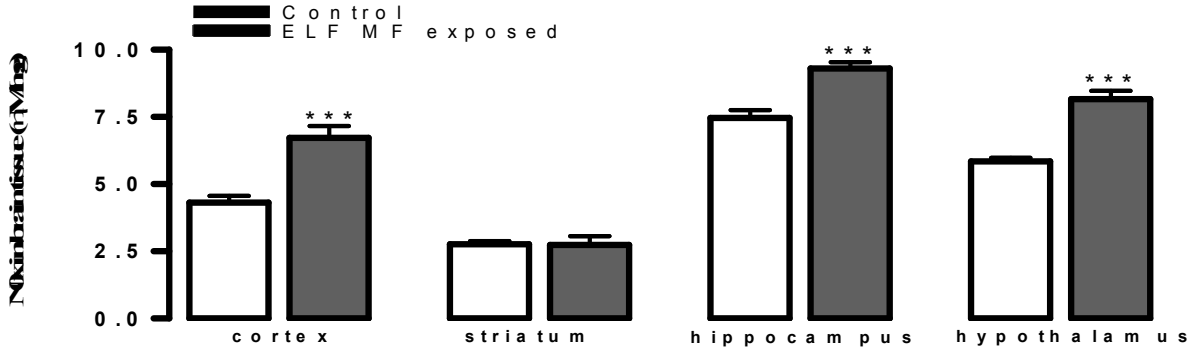
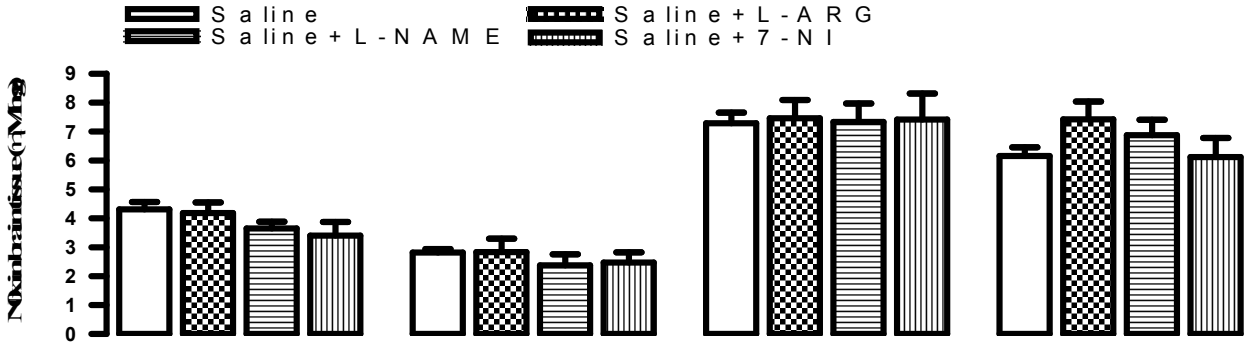


Fig.5. Effect of L-ARG, L-NAME, and 7-NI treatment on NOx level in the regions of the brain of control and ELF MF exposed mice: (A) control (B) ELF MF exposed. Separate group of control or ELF MF exposed (120 days, 8 h/day), mice were treated with L-ARG (400mg/kg) or L-NAME (30mg/kg) or 7-NI (10mg/kg), and then after 30 min of i.p. administration mice were decapitated for determination of NO level in the regions of the brain. Each bar represents mean ± SEM of 6-8 animals, P>0.05 vs. saline or *P<0.05, **P<0.01, ***P<0.001 vs. ELF MF exposed groups (t-tests).

(A)



(B)

household appliances, cell phones and remedial devices used in medical practice, have been reported to produce a variety of biological effects, including effect on the activity of the brain (Eulitz et al., 1998; Freude et al., 2000; Jenrow et al., 1998; Krause et al., 2000) and, also shown behavioral and cognitive disturbances (Hladky et al., 1999;

Lai et al., 1998; Preece et al., 1998; Sienkiewicz et al., 1998). The results obtained also suggesting effect on behavioral pattern, and are inline with previous published report which concluded that chronic exposure to ELF MF induces depression (Wilson, 1988). Plenty of evidence suggested a strong association between depression and suicide on exposure to power frequency electric and magnetic fields (Baris and Armstrong, 1990; Baris et al., 1996; Dowson et al., 1988; Perry et al., 1981; Reichmanis et al., 1979). Accompanying to this, recently it has been demonstrated that chronic stress induces depression in rodents (Mizoguchi et al., 2008; Willner, 2005), and enhanced depression like behavior, more particularly longer floating time and a tendency of shorter struggling time in FST on exposure to 50 Hz electromagnetic fields (Szemerszky et al., 2010). In accordance with this, depression like behavior was detected in the present study. Therefore, the observed behavioral effect can be correlated with the action of ELF MF on CNS and particularly on the activity of neurochemicals involved.

NOS inhibitors have been shown to possess antidepressant like (Harkin et al., 2003), and antipsychotic like (Klamer et al., 2004) actions in animal models. In addition, L-NAME is reported to have anxiolytic effect in EPM (Faria et al., 1997; Volke et al., 1995). Moreover, L-NAME and 7-NI have been reported to be effective in the FST in rodents (da Silva et al., 2000; Harkin et al., 2003; Jefferys and Funder, 1996), and the effects have been appeared to be centrally based. Interestingly in CNS, it has been identified that an increase in Ca^{2+} leads to activation of NOS, which generates NO and thereby increases the intracellular content of cGMP, resulting in depression (Joca and Guimaraes, 2006; Joca et al., 2007).

Therefore, in view of above understanding, to establish neurological pathway by which ELF MF causes depression, we studied the effect of NO precursor and inhibitors, since these are of having recent relevance in the treatment of depression. The investigations demonstrated that acute subeffective treatments of L-NAME (30.0mg/kg, i.p.) and 7 NI (10.0mg/kg, i.p.), NOS inhibitors, antagonized the ELF MF induced depression like effect. Moreover, L-NAME and 7-NI alone did not produce a significant antidepressant effect per se at tested doses. However, the treatment with NO precursor, L-ARG (400mg/kg, i.p.) potentiated the ELF MF induced depression. At tested dose, L-ARG did not produce a significant effect on depression per se. None of the above treatments significantly affected locomotor counts. The results indicated the potential involvement of NOS in ELF MF induced behavioral effect, and hence can be attributed towards the overactivity of NOS and subsequently surplus generation of NO in the regions of the brain, as previous published report also suggest the prominent role of NO in the pathogenesis of major depression (Dhir and Kulkarni, 2011). L-ARG-NO-cGMP is an important signaling pathway that is reported to be involved in depression (Mantovani et al., 2003). Secondly, exposure to ELF MF results in altered Ca^{2+} signalling events, contributing to aberrant NMDA receptor activity and NO-cGMP signalling pathway in hippocampal regions (Manikonda et al., 2007) could be the one of the reason for surplus generation of NO in the hippocampus, a region critically implicated in the modulation of several anxiety related behaviors (McNaughton and Gray, 2000) including bipolar disorder, depression (Marchetti et al., 2010; Ng et al., 2009; Sheline et al., 2002). Moreover, it is postulate that calcium influx via activation of NMDA receptors is a key trigger for NO production and suggested the role of NO in pathophysiology (Vincent, 2010), and nNOS which is responsible for generation of NO has been implicated in modulating physiological functions such as learning, memory, neurogenesis, and human diseases (Zhou and Zhu, 2009).

Substantial investigations suggest the role of dopamine, serotonin, noradrenalin, and glutamate but no one determined the level of classical neurotransmitter NO, in ELF MF induced depression. Consequently, this prompted us to determine the level of this wonder molecule. Therefore, to corroborate the above observations, corresponding level of NO was determined in the site specific brain areas viz; cortex, striatum, hippocampus, and hypothalamus. As per our knowledge, this is first study which demonstrates influence of ELF MF on brain levels of NO in concern with depression.

The estimation data revealed that the level of NO in the cortex, hippocampus and hypothalamus of mice brain was significantly increased but not in striatum. These results are in agreement with previous published report that showed the exposure to EMF rises NO level in the brain and spine of

mice (Jeong et al., 2006), and increased production of NO in some brain structures, like frontal cortex, basal forebrain, hippocampus, and brainstem (Jelenkovic et al., 2006) . Further, we tested the effect of NO precursor and NOS inhibitors on ELF MF induced increase in NO level. These treatments had significant effect on ELF MF induced increase in NOx levels in the regions of the brain. L-ARG significantly potentiated while L-NAME or 7-NI significantly antagonised increase in NOx levels. However, none of the drug treatments alone produced significant effect per se suggesting the involvement of NOS and subsequently the effect on NO production, specifically in cortex, hippocampus and hypothalamus.

Thus, the overwhelming production of NO in the cortical region could be one of the cause for ELF MF induced depression as the dysfunction of the prefrontal cortex has been proposed in depression (George et al., 2000). Also, it has been suggested that prefrontal cortex deep brain stimulation in rats shows antidepressant like effects (Hamani et al., 2010). Thus, implicating the role of cortex in ELF MF induced observed behavioral effect.

Substantial investigations document the role of the hippocampus in depression. In rodents, the hippocampus has been studied extensively as it is responsible for most of the behavioral effects (Seo et al., 2011; VanGuilder et al., 2011). In addition, significant influence of magnetic fields on hippocampal physiology has been documented (Ahmed and Wieraszko, 2008). Therefore, the observed behavioral effect on exposure to ELF MF could be linked with the elevated level of NO in hippocampus, as prolonged exposure to ELF MF is reported to produce the effects in frontal cortex, parietal cortex, and hippocampus in the rodents (Zecca et al., 1998). There is growing evidence supporting the role of hippocampus in depression, since depression has been reported to be associated with hippocampal atrophy (Sheline et al., 2002). Furthermore, recent evidences have revealed that reduction of NO levels within the hippocampus can induce antidepressant like effects, thus implicating the role of hippocampus and NO in the neurobiology of depression (Joca and Guimaraes, 2006; Joca et al., 2007). Moreover, hypothalamic function could be the cause of the psychological problems as well as neurochemical imbalance that affect health of the animals and humans. In the present investigation, experimental data also demonstrated increased NO level in hypothalamus showing the involvement of hypothalamus in EMF induced observed behavioral consequence. Previous investigations on patients of depression also reported effect on number of NOS containing neurons in the hypothalamus (Bernstein et al., 2005; Bernstein et al., 1998) and hippocampus (Oliveira et al., 2008). In addition, increased NO metabolites (NO_2 and NO_3) have been observed in the samples of suicide attempters (Lee et al., 2006). Collectively, it is reasonable to speak that ELF MF exposure induced depression and indicated the involvement of NO.

In conclusion, the exposure of ELF MF induced depression, resulting from the elevation in NO level in the cortex, hippocampus and hypothalamus. The consequential behavioral effect of ELF MF was shown to be associated with NOS, and subsequent surplus generation of NO. Perhaps it may be mediated by changes in conformation, of the activation site in NOS. However, the exact mechanism of the elevation of NO in our study is not elucidated. Further studies are warranted to critically evaluate the role of NOS, cortex, hippocampus and hypothalamus in ELF MF induced depression.

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