Chapter-3

Effects of ELF-PEMF Exposure on Spontaneous Alternation, Anxiety, Motor Co-ordination and Locomotor Activity of Adult Wistar Rats and Viability of C6 (Glial) Cells in Culture

3.1 Introduction

The EM fields in our environment are intensively increasing due to industrial expansion, gradually rising electrical distribution and broad usage of electrical and electronic appliances, and most recently, mobile phones (Azab and Ebrahim, 2017; Ibrahim et al., 2018; Swanson and Kaune, 1999). The measurements of background MFs have shown an increasing trend due to the usage of household appliances in several countries, including Australia, North America, and the U.K. (Coghill et al., 1996; Karipidis, 2015; Linet et al., 1997). Recently, several research articles supported the therapeutic applications of EM fields for cognitive disorders based on the field strength, frequency, and length of exposure (Arendash, 2012; Capelli et al., 2017; Demitrack and Thase, 2009; DiCarlo et al., 1999; Hong, 1995; Sandyk, 1994a, 1994b, 1994a; Sandyk and Iacono, 1994). The ELF-PEMF therapy is safe and non-invasive to reduce pain and neuroinflammation. Moreover, we can also use it to supplement and enhance currently existing healthcare modalities. The ELF-PEMF exposure is also able to reduce the amyloid-β peptide levels, modulate miRNAs in Alzheimer's disease, attenuate post-traumatic stress disorder (PTSD), improve adenosine phosphate (ATP) production, increase oxygenation and circulation, promote hydration, facilitate detoxification and support better absorption of nutrients (Capelli et al., 2017; Levin, 2002; Mohammad Alizadeh et al., 2019; Perez et al., 2021).

The biological effects of PEMF are mainly due to the induction of small electrical currents (Polk and Postow, 1995). The magnetism produces a voltage in the tissue that the following expression can represent:

$$V = N * A * \left(\frac{dB}{dt}\right) \qquad (3.1)$$

Here,

$V =$ Induced voltage in volts $(V)(M^1L^2T^{-3}A^{-1})$

$N = \text{Number of wire turns}$

$A = \text{Area of coil } (M^0L^2T^0)$

$\frac{dB}{dt} = \text{ rate of change in magnetic field}$

As shown in equation (3.1), a PEMF is more suitable than a static MF for bioelectromagnetic applications since the dB/dt component of the equation is zero for the static MF. The Maxwell equations explain the interactions of physical and biological mechanisms (Téglás et al., 2018). Numerous studies also reported that ELF-PEMF (< 20 mT) with duration (≥ 2 h/day to several days in some studies) affected cognitive functions (HE et al., 2011; Zecca et al., 1998). However, there is a paucity of post-exposure effects of 50 Hz ELF-PEMF of 1-3 mT with an exposure duration of < 1 h/day. How could they influence spontaneous alternation, anxiety, motor co-ordination, and locomotor activity in the rodent model? Therefore, evaluating the effects of 50 Hz ELF-PEMF exposure on the rats might shed further light on the relationship between MFs exposure and cognitive performance.

In the present work, we have studied the effects of ELF-PEMF exposure of 1-3 mT, 50 Hz with an exposure duration of 20 min (twice) with a 4 h gap on the cellular proliferation and morphologies of C6 (Glial) cells and spontaneous alternation, anxiety, motor co-ordination, and locomotor activity of Wistar rats under *in vitro* and *in vivo* conditions, respectively. We also performed histological analysis of the selected brain sections (hippocampus and cortex) and estimated the cortical cell counts, tissue structure, and morphology following the 50 Hz ELF-PEMF exposure.

3.2 Material and methods

3.2.1 Materials

We received a C6 (Glial) cell line from the National Center for Cell Sciences (NCCS) Pune. We also received Dulbecco's modified Eagle's medium (DMEM) high glucose, phosphate

buffer saline (PBS), ethylenediaminetetraacetic acid (EDTA), 4′,6-diamidino-2-phenylindole (DAPI), bovine serum albumin (BSA), antibiotics penicillin-streptomycin solution (100X), and fetal bovine serum (FBS) from HiMedia, India. We used absolute ethanol (99.9%) and distilled water for *in vitro* experiments.

3.2.2 50 Hz ELF-PEMF exposure system

The 50 Hz ELF-PEMF exposure was provided by a custom-made monoaxial Helmholtz coils system, as illustrated in figures 3.1 (A-D). The prototype of the Helmholtz coil system is fabricated using a 0.5-inch thick plywood circular frame of a circumference ($2\pi r = 1.664$ m), wire length ($L = 898.668$ m) for coil radius ($R = 0.265$ m) using the copper wires of diameter ($d = 0.914 \times 10^{-3}$m) for 20 Standard Wire Gauge (SWG) and cross-sectional area ($A = 6.558 \times 10^{-7}$m²) with wire turns ($N = 540$), coil winding was done manually but machine-aided, as depicted in figures 3.1 (A-B). The exposure system was powered by the sinusoidal current at a frequency (50 Hz), time (20 msec.), and duty cycle (50%), as depicted in figure 3.1 (C). The incubation chamber for *in vitro* studies was fabricated with a 0.5 cm thick acrylic sheet with dimensions $\text{length} = 30\ \text{cm}, \text{height} = 17\ \text{cm}, \text{and width} = 25\ \text{cm}$, as shown in figures 3.2 (A-D). The following expressions can explain the magnetic field intensity, current, and duty cycle

$$B = \left(\frac{4}{5}\right)^{3/2} \left(\frac{I*n*\mu}{R}\right) \qquad (3.2)$$

Here,

$B = \text{Magnetic flux density (Tesla)}\ (M^1L^2T^{-2}A^{-1})$

$N = \text{Number of turns}$

$R = \text{Coil radius (meters)}\ (M^0L^1T^0)$

$I = \text{Current flow through coils (Ampere)}\ (M^0T^0L^0A^1)$

$r = \text{Coil separation distance}$

and

$$\mu = \text{Permiability of free space} \left(4\pi \text{ x } 10^{-7} \frac{\text{H}}{\text{m}}\right) (M^1L^1T^{-2}A^{-2})$$

$$\text{Duty cycle} = \frac{T_{on}}{T_{on}+T_{off}} \qquad (3.3)$$

As shown in equation (3.2), the magnetic flux density (B) is directly proportional to the number of wires turn (N) and supplied current (I) but inversely proportional to the coil radius (R). T_{ON} and T_{OFF} are the time durations for A.C. current on cycle and off cycle, respectively, and I=1, 2, 3 A, as shown in figure 3.1 (C).

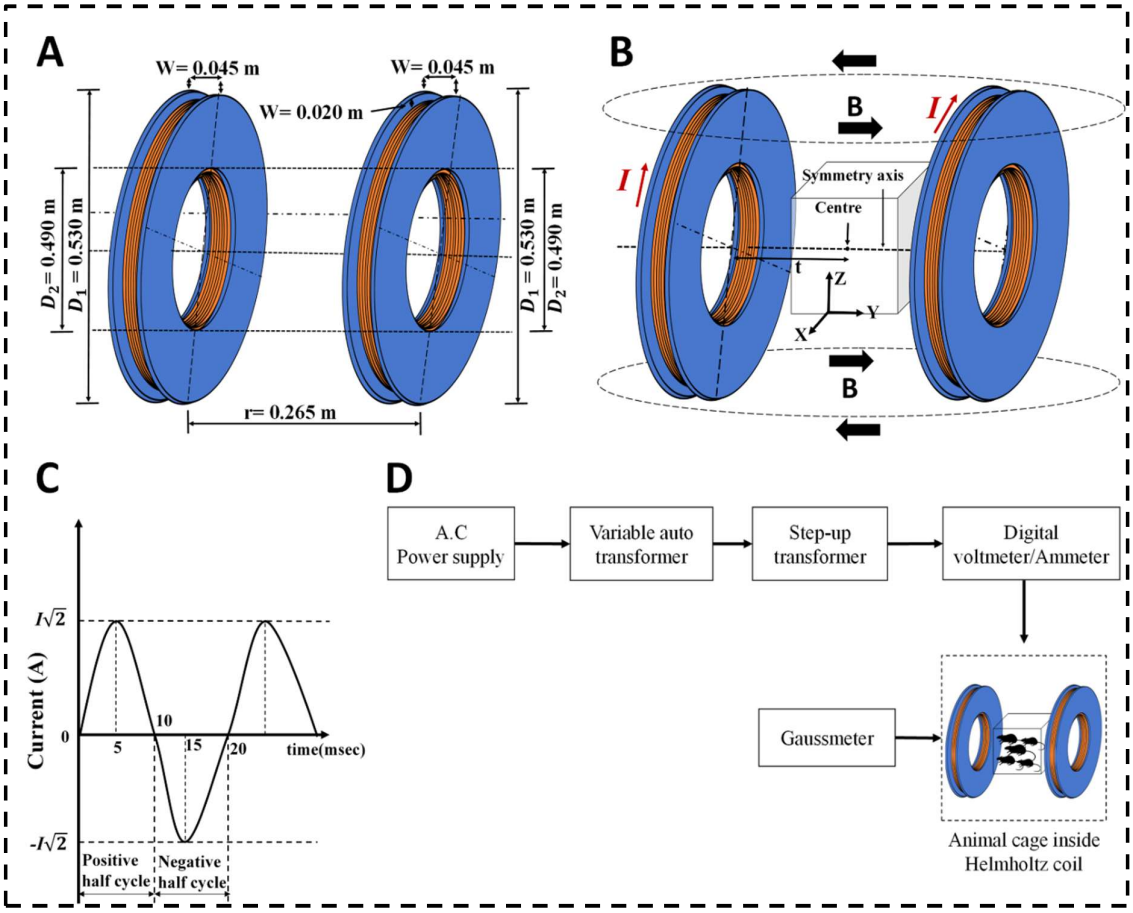

Figure 3.1 Schematic representation of Helmholtz coil system. (A) Monoaxial Helmholtz coil structural profile, (B) magnetic field direction inside exposure system, (C) Sinusoidal current characteristics, (D) Schematic arrangement of the Helmholtz coil setup for bioelectromagnetic studies.

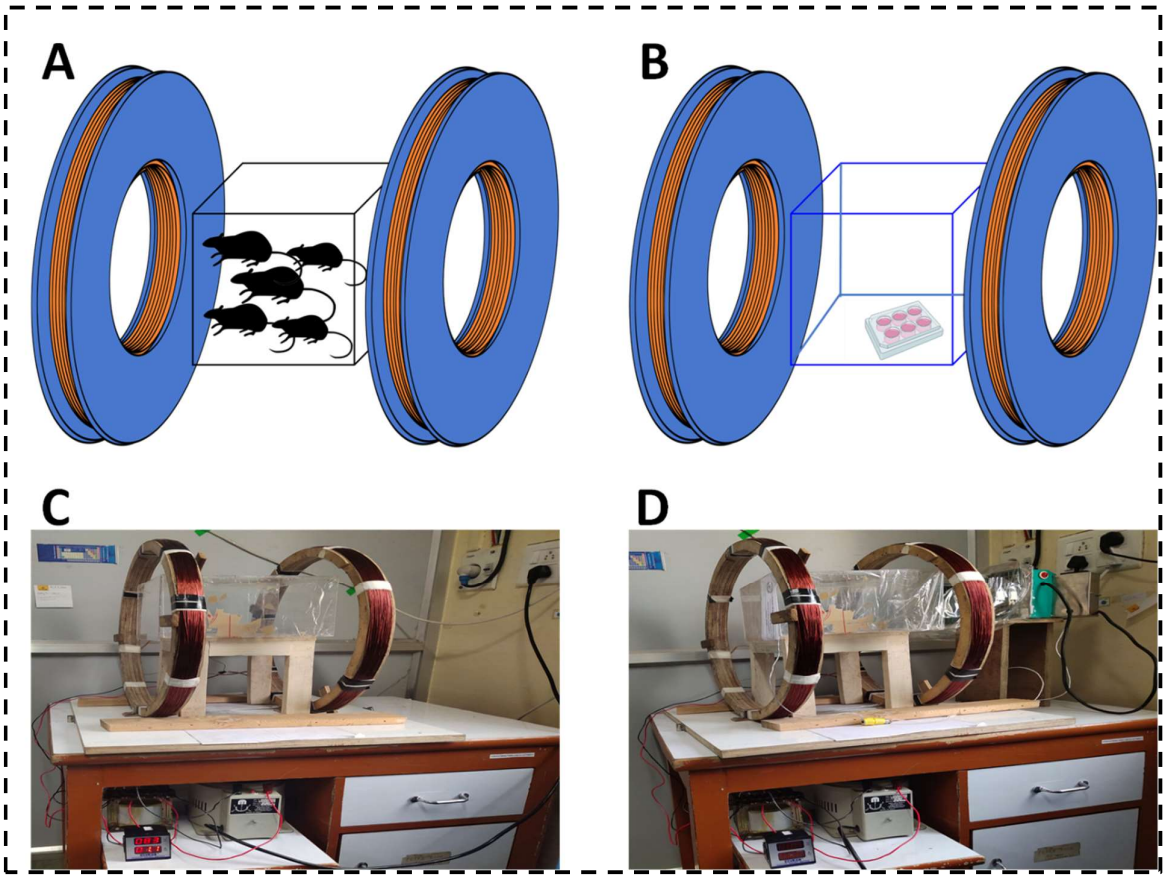

Figure 3.2 50 Hz ELF-PEMF exposure system for *in vitro* and *in vivo* experiments. (A) Schematic representation for *in vivo* analysis, (B) Schematic representation for *in vitro* study, (C) Monoaxial Helmholtz coil system for *in vivo* analysis, (D) Monoaxial Helmholtz coil system for *in vitro* analysis.

3.2.3 The experimental measurement of ELF-PEMF

The intensity of the 50 Hz ELF-PEMF between two parallel coils was measured in real-time utilizing an AC/DC gaussmeter (MG3002, Lutron Electronic) at various loading voltages as depicted in figures 3.3 (A-D). After placement of cell culture platforms or rats inside the ELF-PEMF chamber, we continuously monitored the magnetic flux density throughout the experimental duration. During 50 Hz ELF-PEMF exposure, the cell monolayer was kept at a temperature (37 ± 2˚C) inside an incubation chamber, and we performed ELF-PEMF exposure for 20 min (twice) with a 4 h gap. In contrast, we maintain identical conditions for control groups except for MF exposure.

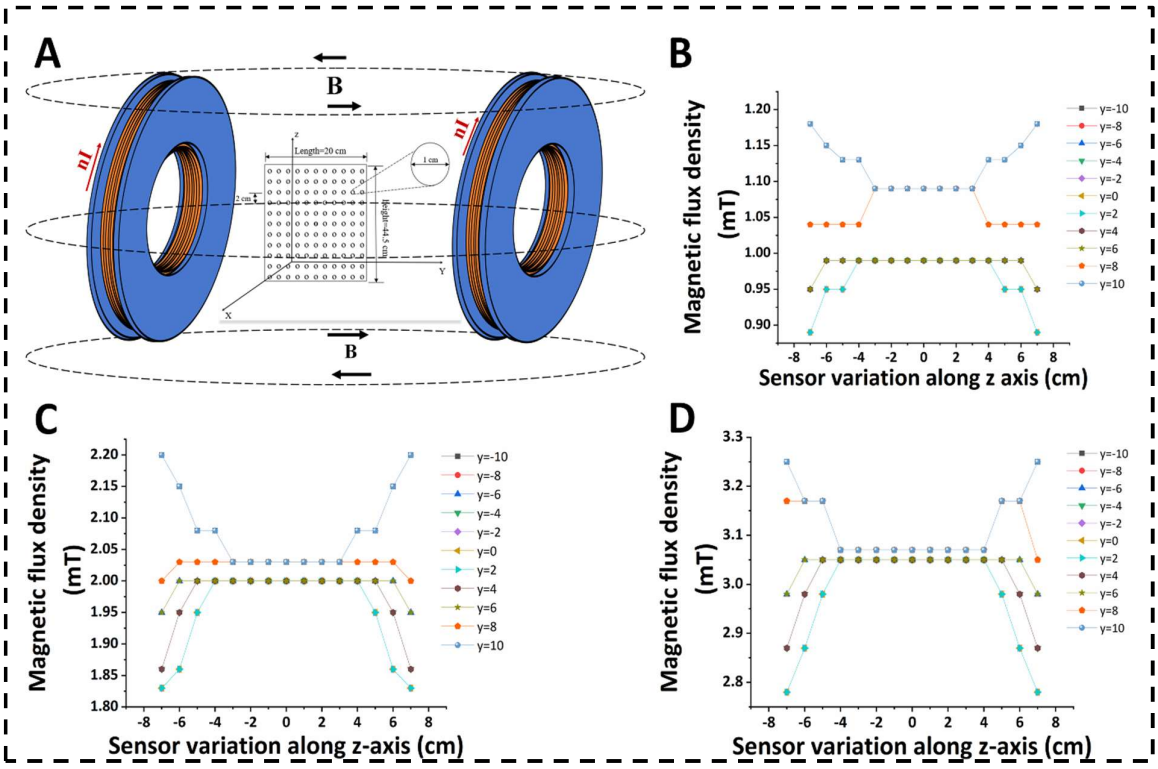

Figure 3.3 50 Hz ELF-PEMF distributions between Helmholtz coils at different loading voltages. (A) Schematic for acrylic sheet placement along x, y, and z-axis in 50 Hz ELF-PEMF exposure system (B) Magnetic flux density vs sensor movement along the z-axis for a voltage and current range (V = 75 volts, I = 1.0 A), (C) Magnetic flux density vs sensor movement along the z-axis for a voltage and current range (V = 120 volts, I = 2.0 A), (D) Magnetic flux density vs sensor movement along the z-axis for a voltage and current range (V = 190 volts, I = 3.0 A).

3.2.4 *In Vitro* analysis

3.2.4.1 Experimental protocol

The cell culture platform was divided into four groups: control, ELF-PEMF1 (B = 1 mT), ELF-PEMF2 (B = 2 mT), and ELF-PEMF3 (B = 3 mT) with an exposure duration of 20 min (twice) with a 4 h gap at a voltage range of $V = 75 - 190$ volts till 6th day or confluency (90%) whichever was earlier as depicted in figure 3.4. Simultaneously, temperature and humidity were identical for the control and exposed groups. Cells cultured in wells without exposure were taken as the negative control, i.e. (the control group). The 50 Hz ELF-PEMF exposure system for *in vitro* studies is depicted in figures 3.2 (B & D). During MF

measurement, the magnetic flux densities exhibited no significant difference; a slightly high magnetic flux density near the coil surface was observed due to the proximity of the magnetic sensor from the coil surface. The cell culture medium was changed every alternate day just before when a bright-field microscope captured images. All the experiments under the same condition were repeated thrice.

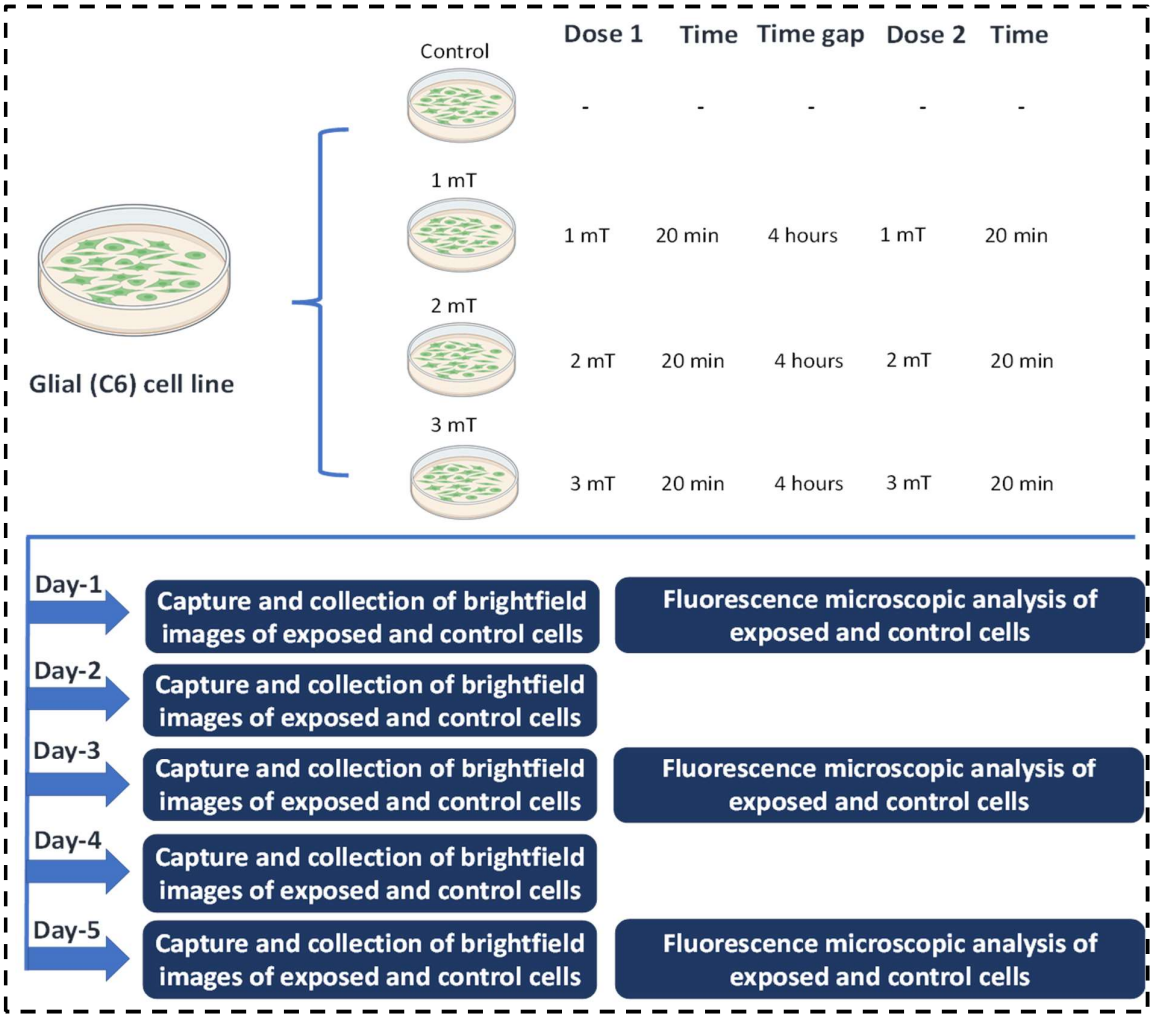

Figure 3.4 Schematic representation of repeated 5-day MF exposure study. In this study, C6 cell lines were exposed to 50 Hz ELF-PEMF (1-3 mT, 20 min (twice) with 4 h gap) under *in vitro* conditions and observed daily for changes in cell proliferation and morphology.

3.2.4.2 Glial cell culture and preparation

C6 is a glial cell line isolated from the brain of a rat with glioma, and it is spindle-like cells that simulate human glioblastoma multiforme (GBM) (Giakoumettis et al., 2018; Xu et al., 2019). It is widely used in toxicological studies to observe any harmful effects of new therapy methods. We cultured the cells in Dulbecco's Modified Eagle medium (DMEM, HiMedia) supplemented with 10% fetal bovine serum (FBS, HiMedia) and 1% antibiotics (Penicillin and streptomycin, HiMedia). The cell culture was maintained at temperature ($37 \pm 2^{\circ}C$), CO_2 (5%), and humidity (95%) inside the incubator (Galaxy 170S, Eppendorf, Germany). After the cells reached confluency (i.e., 90%), trypsin (0.5%) and EDTA (0.2%) were utilized to detach, and a hemocytometer counted the cell. The cells were seeded at 1×10^3 cells/well and kept inside an incubator to promote growth and wait for 12-15 h for adequate cell adhesion on the surface before exposure to the magnetic field intensities.

3.2.4.3 Cell proliferation

Cell viability indicates healthy cells for understanding the mechanism or pathways involved in cell survival or death. The cell culture platform was exposed to 50 Hz ELF-PEMF of 1-3 mT for an exposure duration of 20 min (twice) with a 4 h gap at a loading voltage of $V = 75 - 190$ volts, respectively, for 5 days. We captured bright-field images of C6 cells daily to analyze cell count and proliferation upon exposure.

3.2.4.4 Fluorescence microscopic analysis

Fluorescence analysis of cells is a simple and effective approach to provide qualitative data on the day-to-day progress of cell cultivation. F-actin is responsible for structural, mechanical, and enzymatic functions within eukaryotic cells (Dominguez and Holmes, 2011). Rhodamine Phalloidin is a series of phalloidin conjugates used to stain actin filaments in fluorescence microscopy (Lichtman and Conchello, 2005; Lu et al., 2014). DAPI staining was utilized to

determine the number of nuclei to understand changes in cell morphologies after therapy or drug treatment (Tarnowski et al., 1991). The cells were plated at a density of 1×10^3 on 22 mm coverslips to visualize cell structure and morphology. On days 1, 3, and 5, after 50 Hz ELF-PEMF exposure, the cells were rinsed with PBS three times, followed by fixing (15 min.) with paraformaldehyde (4%). The cells were permeabilized with 0.5% Triton X 100 for 5 min at temperature (37 ± 2 ˚C) followed by treatment with 1% BSA for 10 minutes after being rinsed three times with PBS. After PBS wash, 1X Rhodamine-phalloidin (1:150, Invitrogen, Carlsbad, CA, USA) for f-actin staining was added followed by 4,6-diamidino-2-phenylindole (DAPI, 1:800, Roche, Basel, Switzerland). In the end, glycerol was added to keep the cells from drying out while they were being examined under a fluorescent microscope (Leica SP8, Wetzlar, Germany) for any changes in cell morphology.

3.2.5 *In Vivo* analysis

3.2.5.1 Animal

Adult Wistar male rats (8 weeks, 150-180 g) were received from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Uttar Pradesh, India. The rats were housed in polypropylene cages under standard laboratory conditions of 12:12 h light-dark cycle, temperature (25 ± 2 ˚C), and relative humidity (45-55%) with free access to commercial rat feed (Doodhdhara Pashu Ahar, India) and water ad libitum during experiment. The experiments were carried out following the guidelines of the Central Animal Ethical Committee of Banaras Hindu University, Varanasi, India (Serial no. Dean/2017/CAEC/261).

3.2.5.2 Experimental protocol

In the G*power analysis performed, we selected α error probability as 0.05 with power (1-β err probability), effect size, number of groups, number of measurements, and the non-sphericity correction (€) as 0.80, 0.30, 4, 5, and 1, respectively. We calculated the total sample size as 24,

and the rats were divided into four groups, i.e., control, ELF-PEMF1 (B = 1 mT), ELF-PEMF2 (B = 2 mT), and ELF-PEMF3 (B = 3 mT) (n = 6 male rats in each group) accordingly. Further, we also used the resource equation method for sample size calculation, which is based on the law of diminishing returns (Festing, 2006; Festing and Altman, 2002). In this method, a value E (degree of freedom of analysis of variance (ANOVA)) was calculated. As suggested in previous reports, E's value must lie between 10 and 20. If E (< 10), then adding more animals will increase the probability of significant results, but if E (>20), then adding more animals will not increase the chance of getting substantial results (Charan and Kantharia, 2013).

$$\mathrm{E} = \text{Total number of animals} - \text{Total number of groups} \qquad (3.4)$$

In our study, we took 6 rats per group, and there were 4 groups. So, according to the formulae,

$$\mathrm{E} = (6 \times 4) - (4) \qquad (3.5)$$

and

$$\mathrm{E} = 20 \qquad (3.6)$$

Hence, 6 rats per group can be considered an appropriate sample size per the “resource equation method” in our present experiment.

An exposure duration of 20 min (twice) with a 4 h gap was given for 14 days (experimental period). The ELF-PEMF exposure system for *in vivo* studies is depicted in figures 3.2 (A & C). We conducted all behavioral analyses between 10.30 h and 16.30 h, and observations were recorded and quantified with ANY-MAZE ™ (version-3.72; USA) video tracking system. We maintained a time interval of 30 min between each behavioral test (Bhattacharjee et al., 2021; Krishnamurthy et al., 2013; Prajapati et al., 2020). Animals were subjected to an Elevated plus maze (EPM) (anxiety), Actophotometer (locomotor analysis), Rotarod (motor coordination),

and Y-maze (spontaneous alternation) on respective days, as depicted in figure 3.5. On day 14, after behavioral tests, rats were decapitated, and the brain was micro dissected into the cortex and hippocampus by following the standard procedure (Garabadu et al., 2015; Prajapati et al., 2019).

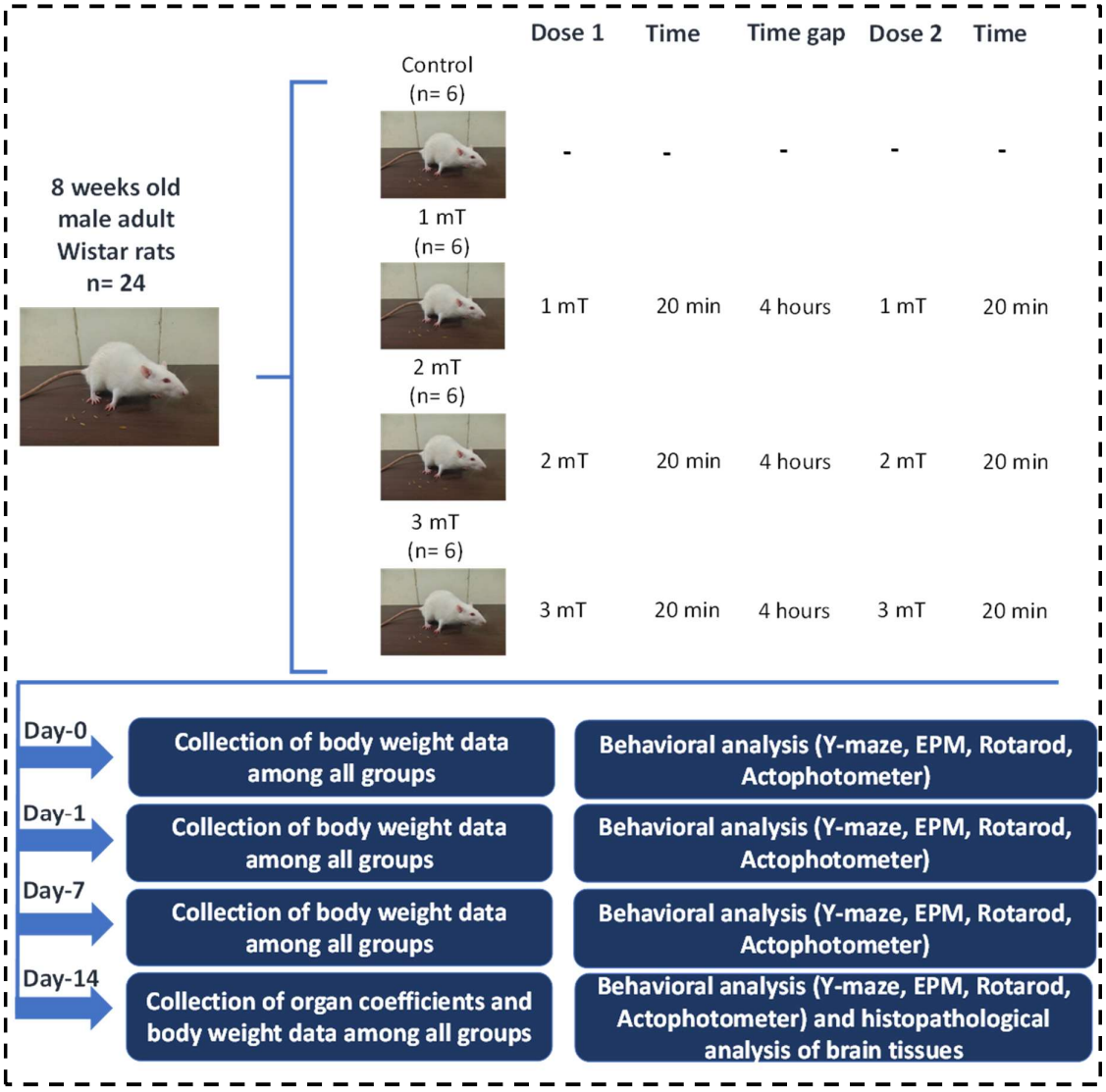

Figure 3.5 Schematic representation of repeated 14 days *in vivo* study. In this study, adult Wistar rats were exposed to 50 Hz ELF-PEMF exposure (1-3 mT, 20 min (twice) with a 4 h gap). We observed any significant changes in total body weight, organ coefficient, cognitive abilities, brain tissue structure, and morphology.

3.2.5.3 Organ coefficient analysis

Organ weight is a sensitive indicator of any aberrant changes induced by chemical or non-chemical means. In various toxicological studies, organ weight assessment has been utilized to determine the toxic effects of test therapy between treated and control animals (Majumdar and Krishnamurthy, 2022; Michael et al., 2007; Peters and Boyd, 1966; Pfeiffer, 1968). Since the organ coefficient is an integral part of the toxicity assessment of therapy and medical devices, we must perform evaluation and interpretation of data with scientific diligence. The organ coefficient of the brain was calculated using the following formula.

$$\text{Organ coefficient} = [\frac{\text{Weight of the organ}}{\text{Total body weight}}] \times 100 \qquad (3.7)$$

3.2.5.4 Total body weight analysis

The total body weight analysis is crucial for assessing biological impacts and abnormal behavioral changes during the study (Margonato et al., 1995). According to the experimental design, we matched all animals for baseline body weight and divided them into four groups, namely control, ELF-PEMF1, ELF-PEMF2, and ELF-PEMF3. Total body weight was measured on days 0, 1, 7, and 14 of the experiment to determine any aberrant changes due to ELF-PEMF exposure intensities.

3.2.5.5 Behavioral analysis

3.2.5.5.1 Y-maze test

Y-maze is an instrument utilized for the assessment of spontaneous alternation. It was performed on days 0, 1, 7, and 14 to negate the effects of testing history, as mentioned in the literature (Garabadu et al., 2015). It consists of three identical arms starting, known (open in trials 1 and 2) and novel arm (open in trial 2 only) with dimensions (50×16×32 cm) at 120˚ angle to each other. During tests, coloured papers were used for visual cues but not in repetition

to maintain the novelty to rats. The rats were introduced to the starting arm and allowed to visit the known arm (kept open in trials 1 and 2) adjacent to the novel arm (blocked in trials 1 but open in trials 2). During trial 1, rats were free to visit the starting and known arms (15 min), followed by an inter-trial interval (4 h) before starting trial 2 (Kraeuter et al., 2019). In trial 2, rats were free to visit all arms (5 min.). The total number of entries in all arms (during trials 1 and 2 in 5 min.) and the percentage of entries in known versus novel arms (during trial 2 for 5 min.) are a measure of spontaneous alternation (Prajapati et al., 2019).

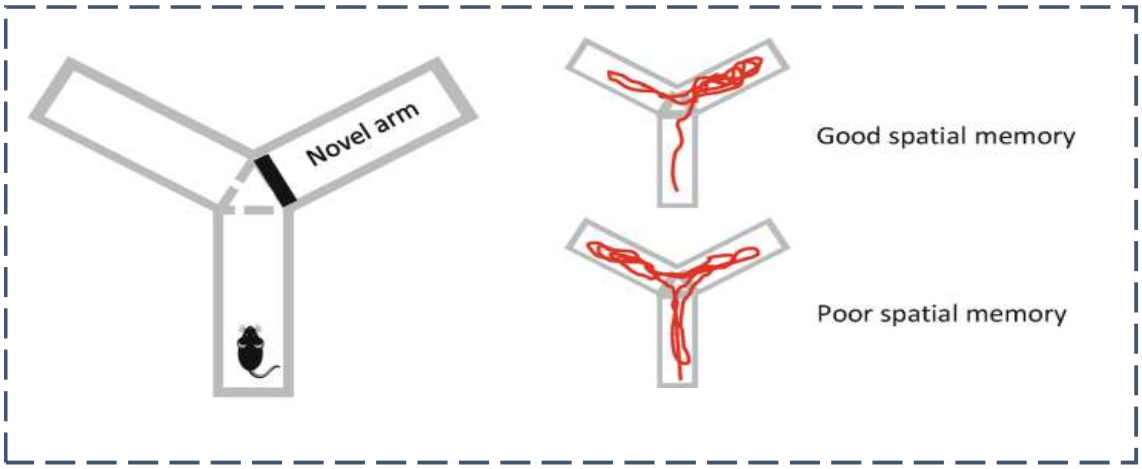

Figure 3.6 Schematic representation of Y-maze test for spontaneous alternation (Kraeuter et al., 2019).

3.2.5.5.2 Elevated plus maze (EPM)

EPM is used to assess anxiety-like symptoms in the exposed rats (Krishnamurthy et al., 2013). The appearance of an instrument is of a plus sign with two open arms (50 cm ×10 cm) and two closed (50 cm × 10 cm × 40 cm) with a central square (10 cm ×10 cm) having a height of 50 cm. The study was performed between 12.00 and 14.00 h in a moderately lit room (20 lx), and rats were relocated to the test room for habituation (30 min). The rats were positioned on open arms facing away from the center, and we collected the data for 5 min on day 1, day 7, and day 14 of the experimental protocol (Krishnamurthy et al., 2013).

3.2.5.5.3 Rotarod test

Rotarod (orchid scientific) was designed to evaluate impaired motor coordination ability in rodents (Lundblad et al., 2003). The rats were trained for five successive days at a speed of 4 to 10 rpm over 10 min for stable baseline performance. During the test, the cylinder (75 mm diameter) rotates at a constant or increasing speed, and animals try to make balance on it for the cut-off period (60 sec.) rather than falling onto a platform directly below. During the test, rats were positioned over the cylinder in the opposite orientation of the rod to acquire skilled behavior. The increase in retention time is considered a sign of the acquisition of competent behavior (Prajapati et al., 2017).

3.2.5.5.4 Actophotometer test

Actophotometer (ICON instrument) is a test used to evaluate the locomotor ability of rats, and during the assessment, we kept the instrument in a dark, sound-proof, and ventilated room. The rat movement cut off the light on the photocell, and the count was recorded. The animals were kept inside an activity cage for habituation (3 min), followed by measurement of locomotor activity for the next 5 min on days 0, 1, 7, and 14. The locomotor activity was expressed as counts/5 min per animal (Reddy and Kulkarni, 1998).

3.2.5.6 Histological analysis

Histopathological studies were performed on perfused brain tissue samples from control and 50 Hz ELF-PEMF exposed groups at day 14 of the experimental duration. The brain tissues were stored in formalin solution (10%) followed by dehydration in graded ethanol series, cleared in benzene/xylene, and embedded in paraffin. The tissues were sectioned at 6 μm, stained with periodic acid-schiff (PAS), and constrained with hematoxylin. The sections were observed and photographed using Leica DFC 290 (Leica Microsystems Ltd, Wetzler, Germany) at different magnifications (Gupta et al., 2019; Srivastava et al., 2017).

3.3 Statistical analysis

The G*power analysis was utilized for the calculation of the total sample size. All behavioral data are expressed as mean ± standard deviation (SD) and analyzed by two-way ANOVA followed by the Bonferroni post hoc test. All other statistical analyses were performed using one-way ANOVA followed by the Newman-Keuls post hoc test. All statistical analyses were performed on Prism 5.0 for Windows (Graph Pad Prism, RRID: SCR-002798 Software, San Diego, California). All the data are presented as mean ± S.D., and $p < 0.05$ was considered significant.

3.4 Results

3.4.1 Influence of ELF-PEMF *in vitro*

3.4.1.1 Cellular proliferation and morphology of Glial cells

Effects of 50 Hz ELF-PEMF exposure on the cellular proliferation and morphology of C6 (Glial) cells over 5 days are depicted in figure 3.7 (A). The cell numbers at day 0 were visualized 12 h after initial cell seeding to examine how many cells adhered to the plate. Cell numbers significantly increased successively from day 1 to day 5. In addition, we also observed no significant alterations in cell morphology between the exposed and control groups, as shown in figure 3.7 (B).

The relationship between cell proliferation and 50 Hz ELF-PEMF exposure is observed. 50 Hz ELF-PEMF exposure (1-3 mT) with the different MFs did not appear to change the average rate of cell proliferation and morphological features of cells in the above-mentioned experimental conditions. The function of the 50 Hz ELF-PEMF exposure and the duration of the experiment determined the cell viability. The cell count was performed with the help of software (NIS-Elements Viewer 4.20 and Image J). The C6 cell count was unaffected by 50

Hz ELF-PEMF exposure compared to the control group on respective days during the study, as depicted in figure 3.7 (C).

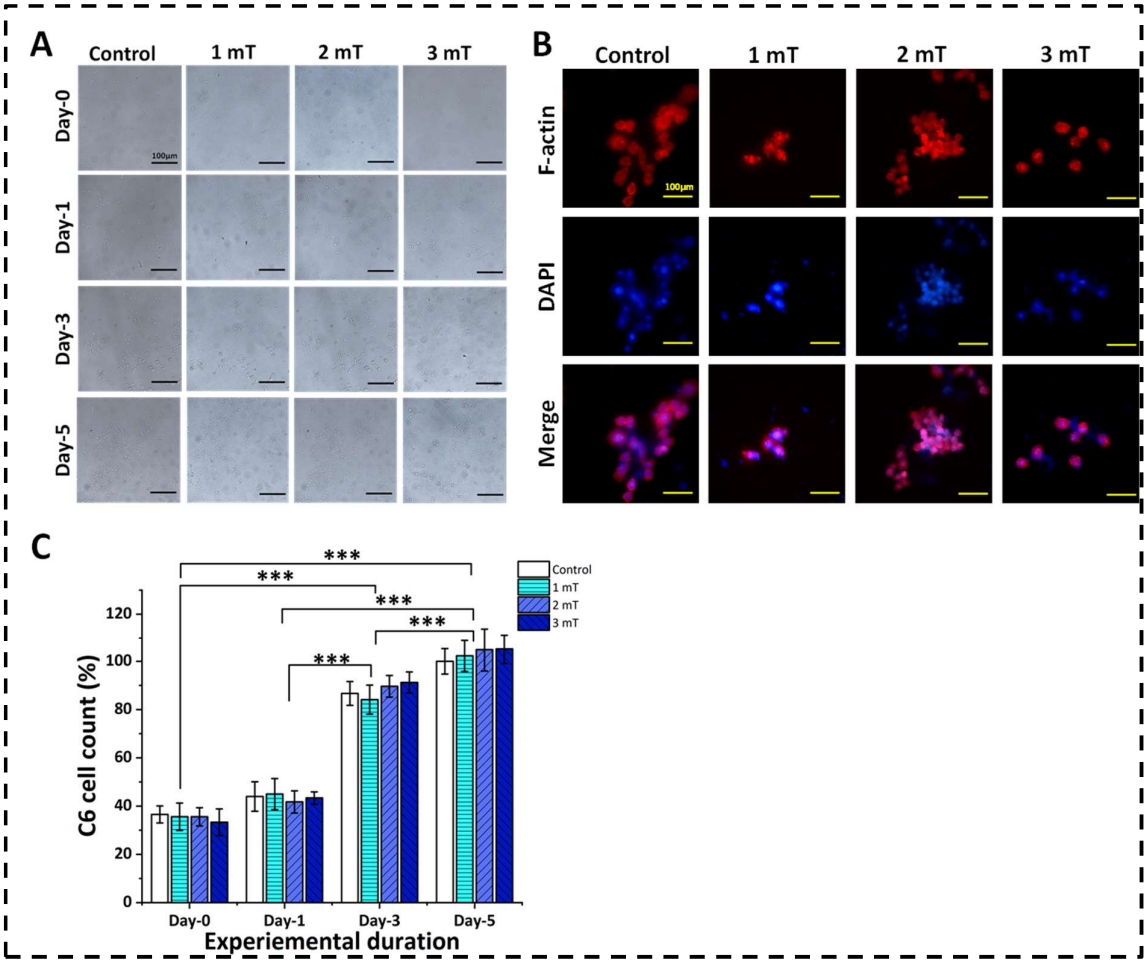

Figure 3.7 Influence of 50 Hz ELF-PEMF on the proliferation and morphology of C6 cell lines. (A) A representative panel of bright-field images of C6 cells stimulated by ELF-PEMF of different strengths, (B) a Representative panel of fluorescent images of C6 cells stimulated by ELF-PEMF of varying strength, (C) C6 cells count at different MF intensities over 5 days. The initial seeding concentration was 1×10^3 cells/well and ELF-PEMF with an exposure duration of 20 min (twice) with a 4 h gap till the 6th day or confluency (90%). Scale bar: 100 μm for bright-field, fluorescent, and merged images. The values are expressed as mean ± S.D.

3.4.2 Influence of ELF-PEMF *in vivo*

3.4.2.1 Organ coefficient

Effects of 50 Hz ELF-PEMF (1-3 mT) exposure on the organ coefficient of the brain rats are depicted in figure 3.8 (A). As shown in table 3.1, the organ coefficient of the brain changes

from 1.0976 ± 0.0983 gm (control value) to 1.133 ± 0.0459 gm, 1.159 ± 0.0124 gm, 1.076 ± 0.046 gm. There was no significant difference in the organ coefficient of the brain observed among 50 Hz ELF-PEMF exposed and control group as measured by One-way ANOVA ([F (3,16) =0.35; $p > 0.05$]) depicted in figure 3.8 (A).

Table 3.1. Effect of 50 Hz ELF-PEMF exposure (1-3 mT) on organ coefficient of the brain of rats.

Exposure (mT)	Control (gm)	ELF-PEMF1 (gm)	ELF-PEMF2 (gm)	ELF-PEMF3 (gm)
Organ coefficient of the brain (mean ± S.D.)	1.0976 ±0.0983	1.133 ± 0.0459	1.159 ± 0.0124	1.076 ± 0.046

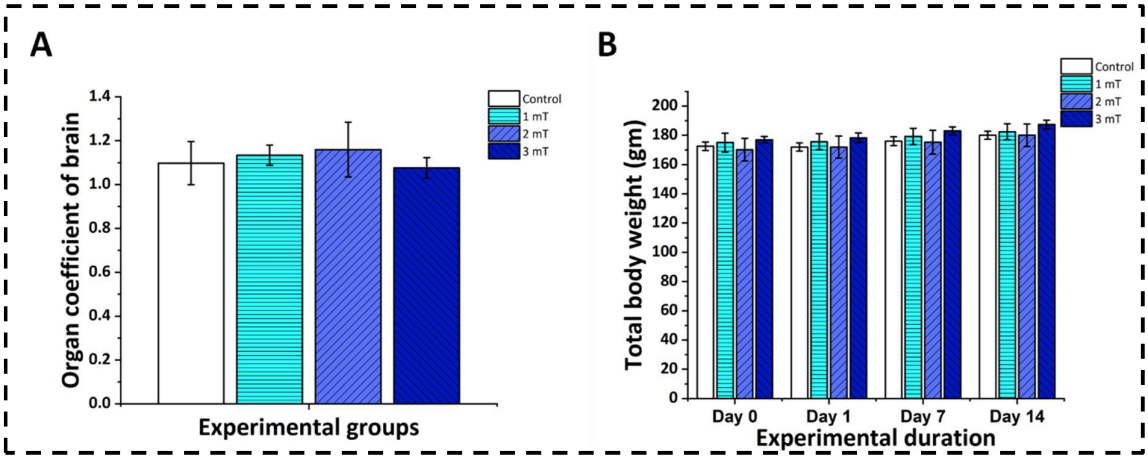

Figure 3.8 50 Hz ELF-PEMF influence on organ coefficient of the brain and total body weight. (A) Organ coefficient of brain, (B) Total body weight. The values are expressed as mean ± S.D. (n = 6).

3.4.2.2 Total body weight

Effects of 50 Hz ELF-PEMF (1-3 mT) exposure on the total body weight of rats are depicted in figure 3.8 (B). As shown in table 3.2, total body weight on day 14 was observed as 175.12 ± 3.705 (gm) in the control group, 178.05 ± 3.44 (gm) in ELF-PEMF1, 173.62 ± 5.05 (gm) in ELF-PEMF2, 181.42 ± 4.74 (gm) in ELF-PEMF3.

The statistical analysis performed by two-way ANOVA revealed group [F (3,80) = 8.96; $p > 0.05$], time [F (3,80) =14.18; $p > 0.05$], and interaction between group and time [F (9,80) =0.11; $p > 0.05$].

Table 3.2. Effect of 50 Hz ELF-PEMF exposure (1-3 mT) on total body weight of rats.

Exposure (mT)	Control (gm)	ELF-PEMF1 (gm)	ELF-PEMF2 (gm)	ELF-PEMF3 (gm)
Total Body weight (mean ± S.D.)	175.12 ± 3.705	178.05 ± 3.44	173.625 ± 5.05	181.42 ± 4.74

3.4.2.3 Behavioral analysis

3.4.2.3.1 ELF-PEMF induced behavioral changes in the Y-maze test

Y-maze is a verified method for assessing cognitive behavior in rats (Prajapati et al., 2020). The effects of 50 Hz ELF-PEMF exposure on spontaneous alternation behavior in trials-1 (acquisition memory) and trial-2 (retrieval memory) and spatial recognition memory are depicted in figures 3.9 (A, B, C & D). The two-way ANOVA revealed total arm entries in trial-1 [F (3,64) = 0.25; $p > 0.05$] among groups, time [F (3,64) = 0.40; $p > 0.05$] and interaction between groups and time [F (9,64) = 0.19; $p > 0.05$]. Similar results were found for total arm entries in trial-2 [F (3,64) = 1.36; $p > 0.05$], time [F (3,64) = 1.06; $p > 0.05$], and interaction between groups and time [F (9,64) = 0.23; $p > 0.05$].

There were no significant changes observed in arm discrimination behavior between known and novel arms among groups [F (3,64) = 0.38; $p > 0.05$], time [F (3,64) = 0.54; $p > 0.05$] and interaction between groups and time [F (9,64) = 0.48; $p > 0.05$], percentage of known novel arm entries [F (3,64) = 1.094; $p > 0.05$] among groups, time [F (3,64) = 1.303; $p > 0.05$] and interaction between groups and time [F (9,64) = 0.7199; $p > 0.05$]. ELF-PEMF did not attenuate spatial recognition memory in experimental rats.

3.4.2.3.2 ELF-PEMF-induced anxiety-like behavior in EPM

EPM is utilized to study anxiety-like behavior in experimental animals. It uses normal exploratory behavior in animals to determine anxiety levels (Krishnamurthy et al., 2013). In this study, 50 Hz ELF-PEMF-induced changes in percentage arm entries, total time spent in arms, and the total number of arm entries in the form of exploratory behavior are depicted in figures 3.9 (E, F & G). The two-way ANOVA demonstrated no significant changes in the percentages of open arm entries among groups [F (3,64) = 0.18; $p > 0.05$], time [F (3,64) = 0.24; $p > 0.05$] and interaction between groups and time [F (9,64) = 0.08; $p > 0.05$], percentage of time spent in open arms among groups [F (3,64) = 0.39; $p > 0.05$], time [F (3,64) = 0.25; $p > 0.05$] and interaction between groups and time [F (9,64) = 0.17; $p > 0.05$]. Moreover, no changes were observed in total arm entries among groups [F (3,64) = 0.18; $p > 0.05$], time [F (3,64) = 0.33; $p > 0.05$], and interaction between groups and time [F (9,64) = 0.13; $p > 0.05$]. Post hoc analysis revealed no significant changes in open-arm entries and time spent in the open-arm during the experimental duration.

3.4.2.3.3 ELF-PEMF-induced motor co-ordination ability in Rotarod

Motor co-ordination ability in terms of retention time on the rotating bar is shown in figure 3.9 (H). The two-way ANOVA revealed no significant differences among groups [F (3,64) = 2.93; $p > 0.05$], time [F (3,64) = 1.303; $p > 0.05$], and interaction of the groups and the time [F (9,64) = 0.41; $p > 0.05$], respectively. Post hoc analysis demonstrated that repeated ELF-PEMF exposure during the experimental duration did not affect the retention time.

3.4.2.3.4 ELF-PEMF-induced alterations in locomotor activity

The locomotor activity was expressed in total beam counts in 5 minutes per animal. In the present study, ELF-PEMF exposure caused no changes in locomotor activity, as depicted in figure 3.9 (I). The two-way ANOVA showed no significant differences among groups [F (3,64)

= 0.73; $p > 0.05$], time [F (3,64) = 0.34; $p > 0.05$], and interaction of the groups and the time [F (9,64) = 0.16; $p > 0.05$]. Post-hoc tests also showed no changes in the locomotor activity of experimental animals.

Table 3.3 Effects of 50 Hz ELF-PEMF exposure (1-3 mT) on behavioral changes in adult Wistar rats during Y-maze, EPM, Rotarod, and Actophotometer test.

Repeated exposure of ELF-PEMF (1-3 mT, 50 Hz)						
Time: 2 times, 20 min (twice) with 4 h gap						
Groups	**D0**	**D1**	**D7**	**D14**	**Observation**	**Conclusion**
Total arm entries (trial-1)						
Control	7 ± 0.707	7.2 ± 0.836	6.8 ± 0.836	7.2 ± 1.095	$p > 0.05$	No significant changes were observed among the groups.
ELF-PEMF1	6.8 ± 0.836	7 ± 0.707	7.2 ± 1.303	6.8 ± 0.836		
ELF-PEMF2	6.6 ± 0.894	6.8 ± 0.836	7 ± 1.414	6.8 ± 0.836		
ELF-PEMF3	6.6 ± 0.547	7 ± 0.707	7.2 ± 1.643	7.2 ± 0.447		
Total arm entries (trial-2)						
Control	13.6 ± 1.51	12.8 ± 0.83	13 ± 0.707	13.8 ± 0.8366	$p > 0.05$	No significant changes were observed among the groups.
ELF-PEMF1	12 ± 1.140	12.6 ± 1.51	13 ± 1.414	12.8 ± 1.788		
ELF-PEMF2	12 ± 1.414	12.2 ± 0.83	12.4 ± 1.14	13.2 ± 1.923		
ELF-PEMF3	12.8 ± 1.64	12.6 ± 2.07	13.2 ± 1.09	13.4 ± 1.673		
Percentage of known arm entries						
Control	34.6 ± 4.87	33.2 ± 4.711	33.8 ± 3.76	32.2 ± 4.43	$p > 0.05$	No significant changes were observed
ELF-PEMF1	32 ± 1.414	32.2 ± 1.92	32.6 ± 1.81	33.6 ± 2.408		
ELF-PEMF2	32.6 ± 2.40	31.8 ± 1.48	31.8 ± 1.92	34.6 ± 3.361		
ELF-PEMF3	32.8 ± 2.77	33.2 ± 4.08	32.8 ± 2.68	34.8 ± 4.147		

						among the groups.
Percentage of known novel entries						
Control	41.6 ± 3.36	42.8 ± 5.63	43 ± 3.162	42.8 ± 2.949	$p > 0.05$	No significant changes were observed among the groups.
ELF-PEMF1	40.2 ± 2.28	44 ± 2.121	39.4 ± 1.81	40.4 ± 1.673		
ELF-PEMF2	40.8 ± 4.96	42.8 ± 3.63	39.6 ± 1.14	39.6 ± 2.073		
ELF-PEMF3	40 ± 5.244	41.2 ± 4.71	42.2 ± 4.14	42.8 ± 2.387		
Percentage open-arm entries						
Control	33.8 ± 3.193	34.2 ± 2.588	34.6 ± 4.56	35 ± 5.52	$p > 0.05$	No significant changes were observed among the groups.
ELF-PEMF1	34 ± 3.60	35 ± 3.464	34.6 ± 4.39	36.6 ± 3.049		
ELF-PEMF2	35.4 ± 5.176	35 ± 3.535	35.4 ± 4.03	35.6 ± 4.159		
ELF-PEMF3	34.4 ± 4.722	35.2 ± 3.033	34.8 ± 4.43	34.8 ± 5.16		
Percentage time spent in open arms						
Control	8.4 ± 1.140	8 ± 1.870	8.2 ± 1.303	8.8 ± 1.095	$p > 0.05$	No significant changes were observed among the groups.
ELF-PEMF1	8.2 ± 0.836	8 ± 1.58	8.2 ± 1.30	7.8 ± 2.58		
ELF-PEMF2	8 ± 1	7.4 ± 1.816	7.8 ± 1.788	8.2 ± 0.44		
ELF-PEMF3	7.8 ± 1.64	8.2 ± 1.303	8 ± 0.707	8.4 ± 1.51		
Total arm entries (min)						
Control	11.2 ± 1.303	11.6 ± 2.07	11.4 ± 1.51	11.8 ± 1.095	$p > 0.05$	No significant changes were
ELF-PEMF1	12 ± 2	11.8 ± 1.303	11.6 ± 1.81	11.8 ± 2.28		
ELF-PEMF2	11.8 ± 1.923	11.2 ± 0.836	11.2 ± 2.04	12.4 ± 1.516		

ELF-PEMF3	11.6 ± 2.408	12 ± 1.581	11.8 ± 3.03	12.2 ± 0.836		observed among the groups.
Retention time on the rod (seconds)						
Control	154.2 ± 6.457	158.6 ± 8.26	157 ± 5.83	158.4 ± 6.84	$p > 0.05$	No significant changes were observed among the groups
ELF-PEMF1	148.8 ± 3.27	151 ± 11.42	149.2 ± 2.5	151 ± 6		
ELF-PEMF2	146.4 ± 4.92	157.4 ± 11.37	153.6 ± 4.1	149.8 ± 6.97		
ELF-PEMF3	151.2 ± 8.64	152.2 ± 10.59	155.2 ± 8.2	152.8 ± 11.16		
Locomotor activity (score/5 min)						
Control	34.2 ± 4.74	33.2 ± 4.86	35.2 ± 3.34	34 ± 4.74	$p > 0.05$	No significant changes were observed among the groups.
ELF-PEMF1	32.2 ± 4.65	34.4 ± 3.64	35 ± 2.91	34.4 ± 2.07		
ELF-PEMF2	32.4 ± 2.70	33.2 ± 3.11	32.6 ± 5.50	32 ± 4		
ELF-PEMF3	32.8 ± 3.27	33.6 ± 4.615	34 ± 5.52	33.2 ± 5.76		

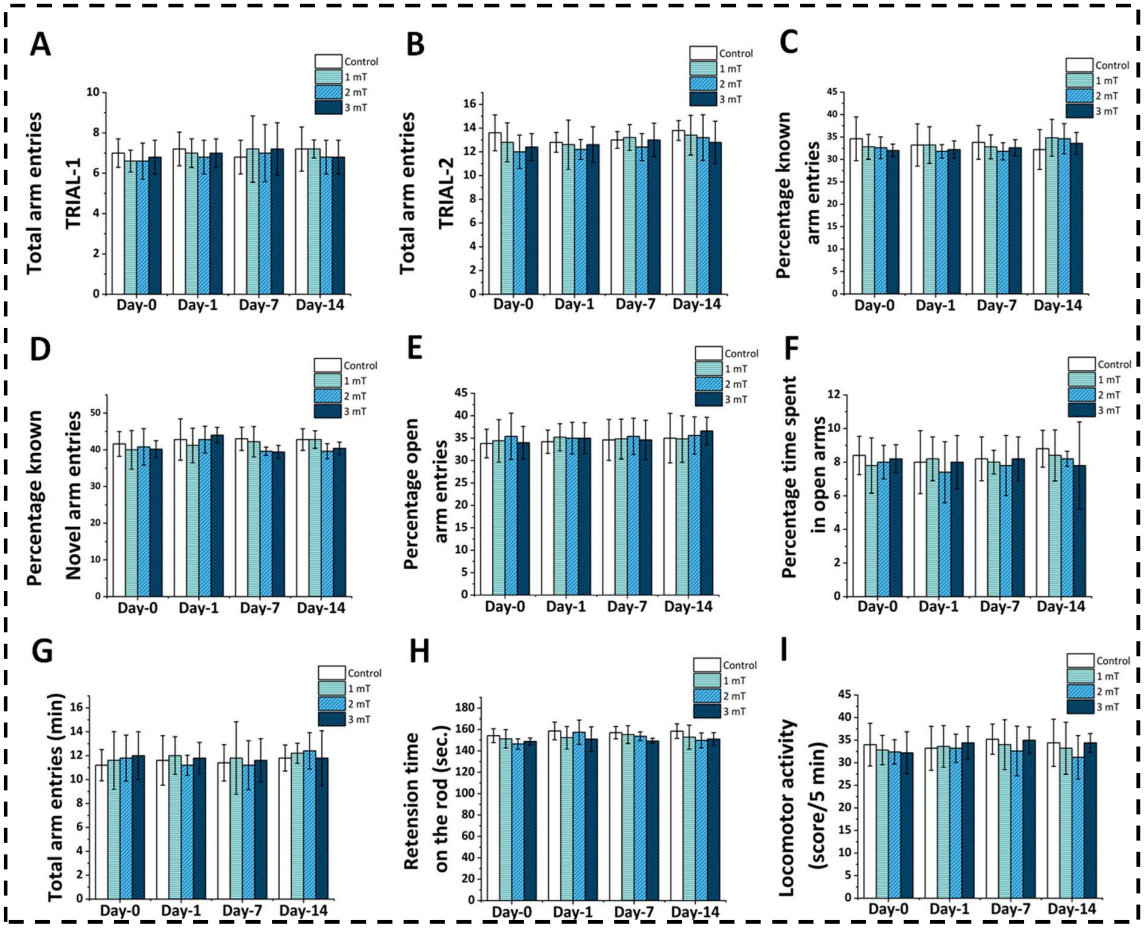

Figure 3.9 Influence of 50 Hz ELF-PEMF on spontaneous alternation, anxiety, motor coordination, and locomotor activity of adult Wistar rats. (A) Total arm entries Trial-1, (B) Total arm entries Trial-2, (C) Percentage known arm entries, (D) Percentage known novel arm entries, (E) Percentage open arm entries, (F) Percentage time spent in open arms, (G) Total arm entries, (H) Retention time on rod (sec.), (I) Locomotor activity (score/5 min.). Two-way ANOVA was utilized for statistical analysis, and values are expressed as mean ± S.D. (n = 6).

3.4.2.4 Histological analysis

Hematoxylin and eosin (H&E) stain is one of the principal tissue stains used in histology (Titford, 2005). The hematoxylin and eosin stain cell nuclei with purplish blue and extracellular matrix with cytoplasm pink, respectively, with other structures taking combinations of these colours (Bancroft and Gamble, 2008; Chan, 2014). The histopathological alterations in the Dentate gyrus (DG) region and Cornu ammonis (CA2) region of the hippocampus, respectively, and cortex regions of brain tissues are depicted in figures 3.10 (A, B & C). 50 Hz

ELF-PEMF caused no notable decrease in cortical cell counts and no noticeable structural changes in the hippocampus and cortex regions, indicating no signs of neurodegeneration. The cell count in the brain's cortex region was performed with the help of software (NIS-Elements Viewer 4.20 and Image J). We observed no significant change in the cell counts of the cortex region among 50 Hz ELF-PEMF exposed and control group as measured by one-way ANOVA ([F (3,8) =1.23; $p > 0.05$]) depicted in figure 3.10 (D).

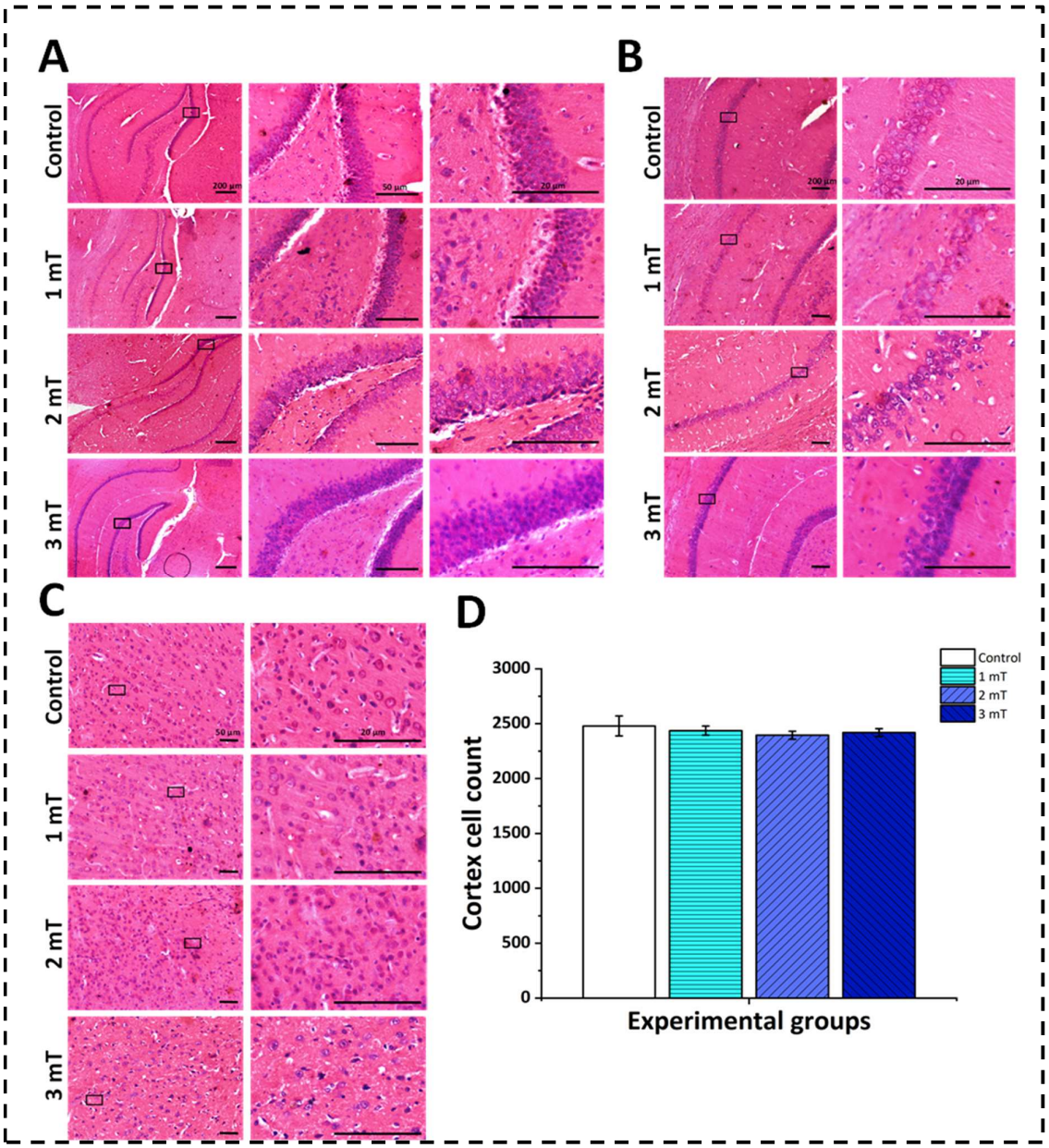

Figure 3.10 Influence of 50 Hz ELF-PEMF exposure on hippocampus and cortex brain region. Representative H&E-stained images of brain tissues in control and ELF-PEMF exposed groups on day 14; (A) Hippocampus DG region (control, 1 mT, 2 mT & 3 mT), (B) Hippocampus CA2 region (control, 1 mT, 2 mT & 3 mT), (C) cortex region (control, 1 mT, 2 mT & 3 mT), (D) cortex cell count in exposed and control groups. No aberrant changes existed between control, ELF-PEMF1, ELF-PEMF2, and ELF-PEMF3 exposed rats. The values are expressed as mean ± S.D. (n = 6).

3.5 Discussion:

EM exposure has increased following the widespread use of modern telecommunication systems and household appliances in daily life (Kaune et al., 2000; Kheifets and Oksuzyan, 2008; Swanson (INVITED), 1999). Brain tissues with different electrical characteristics can exhibit other effects under variable magnetic field intensities. Not much evidence is available for the impact of ELF-PEMF with an exposure duration of < 1 h/day on brain cells (Glial cells) and behavioral parameters. Thus, the present study was designed to explore the EM stimulation induced by 50 Hz ELF-PEMF in male Wistar rats and C6 cells. C6 (Glial) cells are non-neuronal cells mainly found in the central nervous system (CNS) and peripheral nervous system (PNS) and responsible for homeostasis, protection, and support for neurons. C6 cells are long and spindle-shaped, with a large nucleus at the center. It is helpful to analyze the effects of pro-inflammatory molecules on tau phosphorylation and amyloid precursor protein (APP) expression, temozolomide (TMZ) induced cognitive impairment, magnetic and R.F. effects on cell proliferation (Martínez-Díaz et al., 2020; Pathak et al., 2020; Stagg et al., 1997; Xu et al., 2019). We exposed C6 cells to ELF-PEMF with adjustable intensities, voltage, and current but with a fixed frequency (50 Hz) and exposure duration. The bright-field images showed no fragmentation of C6 cells after the exposure, and fluorescent microscopy also revealed that f-actin distribution and nucleus are unaffected by 50 Hz ELF-PEMF exposure. Based on the above findings, we anticipate that 50 Hz ELF-PEMF is non-destructive under current experimental conditions. Xu et al. studied the effects of a pulsed millisecond magnetic field on

C6 (Glial) cells and observed cumulative time effects, and it is non-destructive (Xu et al., 2019). Akbarnejad et al. studied the impact of ELF-PEMF in different amplitudes on the Glioblastoma Multiforme (GBM) cell line (U87) *in vitro*. They observed proliferative and inhibiting effects for selective PEMF frequencies and intensities (Akbarnejad et al., 2017). Based on the above findings, we can suggest that 50 Hz ELF-PEMF is non-destructive under current experimental conditions.

The influence of 50 Hz ELF-PEMF (1-3 mT) exposure on the organ coefficient of the rat brain is shown in figure 3.8 (A). The organ coefficient is the most preliminary indicator of drug toxicity, which often precedes morphological alterations in tissue structure and morphology (Michael et al., 2007). The results showed no aberrant changes in the organ coefficient of the brain and revealed no significant alteration in tissue morphology and structure under current experimental conditions. Chung et al. studied the ELF magnetic effects on female Sprague-Dawley (S.D.) rats. There were no changes in organ weights, mean maternal body weight, and hematological parameters (Chung et al., 2003). Persinger et al. studied the effect of a rotating magnetic field (RMF) on wet organ weight and thyroid morphology. They observed no significant changes in wet adrenal and pituitary weights and body weight in adult female rats (Persinger et al., 1978).

Figure 3.8 (B) illustrates the effects of 50 Hz ELF-PEMF (1-3 mT) with an exposure duration of 20 min (twice) with a 4 h gap on the total body weight of rats within the experimental period. The statistical analysis of total body weight shows a comparable pattern with no significant difference in body weight between exposed and control groups at the end of the experiment. Akhras et al. studied the influence of a 50 Hz sinusoidal magnetic field on male Sprague-Dawley (SD) rats and observed no significant changes in absolute body weight and weight of testes (Al-Akhras et al., 2006). Cakir et al. studied the ELF-EMF effects on body weight and

hematological parameters and observed no variation in body weight and blood serum parameters (Cakir et al., 2009).

Scientists often perform several tests on each animal to assess the behavioral phenotype of various rodents since a single behavioral test cannot provide all the necessary information. Moreover, repeated stress can influence learning, memory, and cognition, altering the results (Brown et al., 2000; Hånell and Marklund, 2014). In the present study, we performed tests for anxiety and exploratory behavior first since results can be significantly affected by testing history (repeated tests). Therefore, multiple days of resting time between tests were maintained (Lad et al., 2010; McIlwain et al., 2001; Peng et al., 2016; Wolf et al., 2016). Animals exposed to 50 Hz ELF-PEMF showed no significant changes in cognitive behavior. In the Y-maze test, dependent variables were general exploratory behavior and spatial recognition memory regarding the total number of arm entries in trial-1(1^{st} 5 min.), trial-2 (5 min.), and the percentage of known and novel arm entries, respectively. In the present study, no noted changes were detected in exploratory and coping behavior within the experimental duration, as depicted in figures 3.9 (A, B, C & D), suggesting that 50 Hz ELF-PEMF possibly caused no loss in spatial recognition memory. Earlier studies also supported that ELF-PEMF exposure does not affect spatial recognition memory (Cai et al., 2013; Conrad et al., 1997; Fu et al., 2008; McEwen et al., 1997; Trzeciak et al., 1993). Thus, results indicate that the repeated exposure of adult Wistar rats to ELF-PEMF with an exposure duration of 20 min (twice) with a 4 h gap may reflect no impairment in spatial recognition capability.

Anxiety-like behavior is generally observed in humans and rats with PTSD (Post-traumatic stress disorder). The reduction in open-arm entries and time spent in open arms are symptoms of anxiety-like behavior in the EPM (Patki et al., 2014). In the present study, repeated exposure to 50 Hz ELF-PEMF did not significantly affect the percentage of open-arm entries and time spent on open arms, as depicted in figures 3.9 (E, F & G). The literature also reports a similar

observation (HE et al., 2011). Thus, the current finding indicates that repeated exposure of adult Wistar rats to 50 Hz ELF-PEMF with an exposure duration of 20 min (twice) with a 4 h gap did not induce noticeable anxiety-like behavior. Further, no tolerance was observed for repeated trials on EPM in the control group animals, as mentioned in (Prajapati and Krishnamurthy, 2021). It may be due to a one-week interval gap between exposures (Bhattacharjee et al., 2021). Similarly, in another study, rats did not show tolerance to open arms following exposure to EPM for 18 consecutive days (Treit et al., 1993).

In the current study, the outcomes revealed that the repeated exposure to 50 Hz ELF-PEMF with an exposure duration of 20 min (twice) with a 4 h gap did not significantly change spontaneous motor behavior in adult Wistar rats, as depicted in figure 3.9 (H). We observed no significant change in retention time in the exposed groups compared to the control. Also, the results suggested that repeated exposure to ELF-PEMF with an exposure duration of 20 min (twice) with a 4 h gap did affect motor coordination abilities in rats. Similarly, the repeated exposure to 50 Hz ELF-PEMF, with an exposure duration of 20 min (twice) with a 4 h gap, did not affect spontaneous locomotion, as depicted in figure 3.9 (I). We observed no noticeable changes in the basal activity (score/5 min.) of exposed groups compared to the control.

The hippocampus is a curved seahorse-shaped organ responsible for memory and learning (Burgess et al., 2002). Hippocampus is divided into subfields CA1, CA2, and CA3. CA1 neurons primarily form and retrieve hippocampus-dependent memories (Bartsch et al., 2011). The CA2 region of the hippocampus is involved in creating social recognition memory and hippocampal information processing (Lehr et al., 2021; Tzakis and Holahan, 2019). CA3 region has a specific role in memory processes, seizure susceptibility, and neurodegeneration (Cherubini and Miles, 2015). DG region forms hippocampal memory (Hainmueller and Bartos, 2020).

Hippocampal neurogenesis is the formation of new granules for the maintenance of learning and memory, and damage to it can impair the formation and recall of new memories and the remembrance of past experiences. The cerebral cortex is the brain's outermost layer of nerve cell tissue. It is also responsible for memory, thinking, learning, reasoning, problem-solving, and functions related to your senses (Jawabri and Sharma, 2022). The 50 Hz ELF-PEMF exposure did not result in any significant structural or morphological changes in the hippocampus or cortex regions of the brain, as evaluated by H&E staining and shown in figures 3.10 (A, B & C). We also observed through the statistical analysis that the cortical cell counts remain unaffected in the exposed groups compared to the control, as shown in figure 3.10 (D).

3.6 Conclusion

In the present work, we have studied the effects of 50 Hz ELF-PEMF in both *in vitro* (C6 Glial cells) and *in vivo* (adult Wistar rats) models. The present study reveals that exposure to ELF-PEMF for 20 min (twice) with a 4 h gap could be non-destructive for C6 cells and result in unaltered effects on spontaneous alternation, anxiety, motor coordination, and locomotor activity of adult Wistar rats. The results demonstrated that 50 Hz ELF-PEMF exposure did not induce any significant levels of cellular fragmentation and changes in the morphology of glial cells. Also, the outcomes revealed no noticeable effects on spontaneous alternation, anxiety, motor coordination, and locomotor activity in PEMF-exposed groups compared with the control. Histological analysis of the selected brain sections (hippocampus and cortex) following ELF-PEMF exposure revealed no significant changes at the level of cortical cell counts and tissue structure and morphology. Our efforts provide the theoretical underpinnings and experimental support for the safe range of magnetic fields for their application in the biomedical field.

3.7 References

Akbarnejad, Z., Eskandary, H., Vergallo, C., Nematollahi-Mahani, S.N., Dini, L., Darvishzadeh-Mahani, F., Ahmadi, M., 2017. Effects of extremely low-frequency pulsed electromagnetic fields (ELF-PEMFs) on glioblastoma cells (U87). Electromagn. Biol. Med. 36, 238–247.

Al-Akhras, M.-A., Darmani, H., Elbetieha, A., 2006. Influence of 50 Hz magnetic field on sex hormones and other fertility parameters of adult male rats. Bioelectromagnetics 27, 127–131. https://doi.org/10.1002/bem.20186

Arendash, G.W., 2012. Transcranial electromagnetic treatment against Alzheimer's disease: why it has the potential to trump Alzheimer's disease drug development. J. Alzheimers Dis. 32, 243–266.

Azab, A.E., Ebrahim, S.A., 2017. Exposure to electromagnetic fields induces oxidative stress and pathophysiological changes in the cardiovascular system. J Appl Biotechnol Bioeng 4, 00096.

Bancroft, J.D., Gamble, M., 2008. Theory and practice of histological techniques. Elsevier health sciences.

Bartsch, T., Döhring, J., Rohr, A., Jansen, O., Deuschl, G., 2011. CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness. Proc. Natl. Acad. Sci. 108, 17562–17567. https://doi.org/10.1073/pnas.1110266108

Bhattacharjee, A., Prajapati, S.K., Krishnamurthy, S., 2021. Supplementation of taurine improves ionic homeostasis and mitochondrial function in the rats exhibiting post-traumatic stress disorder-like symptoms. Eur. J. Pharmacol. 908, 174361.

Brown, R.E., Stanford, L., Schellinck, H.M., 2000. Developing standardized behavioral tests for knockout and mutant mice. ILAR J. 41, 163–174.

Burgess, N., Maguire, E.A., O'Keefe, J., 2002. The Human Hippocampus and Spatial and Episodic Memory. Neuron 35, 625–641. https://doi.org/10.1016/S0896-6273(02)00830-9

Cai, Z.-L., Wang, C.-Y., Jiang, Z.-J., Li, H.-H., Liu, W.-X., Gong, L.-W., Xiao, P., Li, C.-H., 2013. Effects of cordycepin on Y-maze learning task in mice. Eur. J. Pharmacol. 714, 249–253.

Cakir, D.U., Yokus, B., Akdag, M.Z., Sert, C., Mete, N., 2009. Alterations of Hematological Variations in Rats Exposed to Extremely Low Frequency Magnetic Fields (50Hz). Arch. Med. Res. 40, 352–356. https://doi.org/10.1016/j.arcmed.2009.07.001

Capelli, E., Torrisi, F., Venturini, L., Granato, M., Fassina, L., Lupo, G.F.D., Ricevuti, G., 2017. Low-frequency pulsed electromagnetic field is able to modulate miRNAs in an experimental cell model of Alzheimer's disease. J. Healthc. Eng. 2017.

Chan, J.K., 2014. The wonderful colors of the hematoxylin–eosin stain in diagnostic surgical pathology. Int. J. Surg. Pathol. 22, 12–32.

Charan, J., Kantharia, N., 2013. How to calculate sample size in animal studies? J. Pharmacol. Pharmacother. 4, 303–306.

Cherubini, E., Miles, R., 2015. The CA3 region of the hippocampus: how is it? What is it for? How does it do it? Front. Cell. Neurosci. 9, 19. https://doi.org/10.3389/fncel.2015.00019

Chung, M.-K., Kim, J.-C., Myung, S.-H., Lee, D.-I., 2003. Developmental toxicity evaluation of ELF magnetic fields in Sprague–Dawley rats. Bioelectromagnetics 24, 231–240. https://doi.org/10.1002/bem.10114

Coghill, R.W., Steward, J., Philips, A., 1996. Extra low frequency electric and magnetic fields in the bedplace of children diagnosed with leukaemia: a case-control study. Eur. J. Cancer Prev. 153–158.

Conrad, C.D., Lupien, S.J., Thanasoulis, L.C., McEwen, B.S., 1997. The effects of Type I and Type II corticosteroid receptor agonists on exploratory behavior and spatial memory in the Y-maze. Brain Res. 759, 76–83. https://doi.org/10.1016/S0006-8993(97)00236-9

Demitrack, M.A., Thase, M.E., 2009. Clinical significance of transcranial magnetic stimulation (TMS) in the treatment of pharmacoresistant depression: synthesis of recent data. Psychopharmacol Bull 42, 5–38.

DiCarlo, A.L., Farrell, J.M., Litovitz, T.A., 1999. Myocardial protection conferred by electromagnetic fields. Circulation 99, 813–816.

Dominguez, R., Holmes, K.C., 2011. Actin Structure and Function. Annu. Rev. Biophys. 40, 169–186. https://doi.org/10.1146/annurev-biophys-042910-155359

Festing, M.F.W., 2006. Design and Statistical Methods in Studies Using Animal Models of Development. ILAR J. 47, 5–14. https://doi.org/10.1093/ilar.47.1.5

Festing, M.F.W., Altman, D.G., 2002. Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals. ILAR J. 43, 244–258. https://doi.org/10.1093/ilar.43.4.244

Fu, Y., Wang, C., Wang, J., Lei, Y., Ma, Y., 2008. LONG-TERM EXPOSURE TO EXTREMELY LOW-FREQUENCY MAGNETIC FIELDS IMPAIRS SPATIAL RECOGNITION MEMORY IN MICE. Clin. Exp. Pharmacol. Physiol. 35, 797–800. https://doi.org/10.1111/j.1440-1681.2008.04922.x

Garabadu, D., Ahmad, A., Krishnamurthy, S., 2015. Risperidone attenuates modified stress–re-stress paradigm-induced mitochondrial dysfunction and apoptosis in rats exhibiting post-traumatic stress disorder-like symptoms. J. Mol. Neurosci. 56, 299–312.

Giakoumettis, D., Kritis, A., Foroglou, N., 2018. C6 cell line: the gold standard in glioma research. Hippokratia 22, 105–112.

Gupta, S.K., Patel, S.K., Tomar, M.S., Singh, S.K., Mesharam, M.K., Krishnamurthy, S., 2019. Long-term exposure of 2450 MHz electromagnetic radiation induces stress and anxiety like behavior in rats. Neurochem. Int. 128, 1–13.

Hainmueller, T., Bartos, M., 2020. Dentate gyrus circuits for encoding, retrieval and discrimination of episodic memories. Nat. Rev. Neurosci. 21, 153–168. https://doi.org/10.1038/s41583-019-0260-z

Hånell, A., Marklund, N., 2014. Structured evaluation of rodent behavioral tests used in drug discovery research. Front. Behav. Neurosci. 8, 252.

HE, L., SHI, H., LIU, T., XUYing-chun, YE, K., WANG, S., 2011. Effects of extremely low frequency magnetic field on anxiety level and spatial memory of adult rats. Chin. Med. J. (Engl.) 124, 3362–3366. https://doi.org/10.3760/cma.j.issn.0366-6999.2011.20.028

Hong, F.T., 1995. Magnetic field effects on biomolecules, cells, and living organisms. Biosystems 36, 187–229.

Ibrahim, N., Hajalan, S., Wajih, A., Khudhair, N., Khalid, A., Thaker, A.A., 2018. Study the effect of electromagnetic field on some physiological and histological characteristics on the liver of mice. Asian Jr Microbiol Biotech Env Sc 20, S41–S46.

Jawabri, K.H., Sharma, S., 2022. Physiology, Cerebral Cortex Functions, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Karipidis, K.K., 2015. Survey of residential power-frequency magnetic fields in Melbourne, Australia. Radiat. Prot. Dosimetry 163, 81–91.

Kaune, W. t., Miller, M. c., Linet, M. s., Hatch, E. e., Kleinerman, R. a., Wacholder, S., Mohr, A. h., Tarone, R. e., Haines, C., 2000. Children's exposure to magnetic fields produced by U.S. television sets used for viewing programs and playing video games. Bioelectromagnetics 21, 214–227. https://doi.org/10.1002/(SICI)1521-186X(200004)21:3<214::AID-BEM8>3.0.CO;2-Y

Kheifets, L., Oksuzyan, S., 2008. Exposure assessment and other challenges in non-ionizing radiation studies of childhood leukaemia. Radiat. Prot. Dosimetry 132, 139–147.

Kraeuter, A.-K., Guest, P.C., Sarnyai, Z., 2019. The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice, in: Guest, P.C. (Ed.), Pre-Clinical Models, Methods in Molecular Biology. Springer New York, New York, NY, pp. 105–111. https://doi.org/10.1007/978-1-4939-8994-2_10

Krishnamurthy, S., Garabadu, D., Joy, K.P., 2013. Risperidone ameliorates post-traumatic stress disorder-like symptoms in modified stress re-stress model. Neuropharmacology 75, 62–77. https://doi.org/10.1016/j.neuropharm.2013.07.005

Lad, H.V., Liu, L., Paya-Cano, J.L., Parsons, M.J., Kember, R., Fernandes, C., Schalkwyk, L.C., 2010. Behavioural battery testing: evaluation and behavioural outcomes in 8 inbred mouse strains. Physiol. Behav. 99, 301–316.

Lehr, A.B., Kumar, A., Tetzlaff, C., Hafting, T., Fyhn, M., Stöber, T.M., 2021. CA2 beyond social memory: Evidence for a fundamental role in hippocampal information processing. Neurosci. Biobehav. Rev. 126, 398–412. https://doi.org/10.1016/j.neubiorev.2021.03.020

Levin, M.E., 2002. Management of the Diabetic Foot: Preventing Amputation. (Featured CME Topic: Diabetes Mellitus). South. Med. J. 95, 10–21.

Lichtman, J.W., Conchello, J.-A., 2005. Fluorescence microscopy. Nat. Methods 2, 910–919. https://doi.org/10.1038/nmeth817

Linet, M.S., Hatch, E.E., Kleinerman, R.A., Robison, L.L., Kaune, W.T., Friedman, D.R., Severson, R.K., Haines, C.M., Hartsock, C.T., Niwa, S., Wacholder, S., Tarone, R.E., 1997. Residential Exposure to Magnetic Fields and Acute Lymphoblastic Leukemia in Children. N. Engl. J. Med. 337, 1–8. https://doi.org/10.1056/NEJM199707033370101

Lu, H., Mack, J., Yang, Y., Shen, Z., 2014. Structural modification strategies for the rational design of red/NIR region BODIPYs. Chem. Soc. Rev. 43, 4778–4823. https://doi.org/10.1039/C4CS00030G

Lundblad, M., Vaudano, E., Cenci, M.A., 2003. Cellular and behavioural effects of the adenosine A2a receptor antagonist KW-6002 in a rat model of l-DOPA-induced dyskinesia. J. Neurochem. 84, 1398–1410. https://doi.org/10.1046/j.1471-4159.2003.01632.x

Majumdar, S., Krishnamurthy, S., 2022. In vivo toxicological evaluation of barium-doped bioactive glass in rats. Ceram. Int. 48, 33288–33305. https://doi.org/10.1016/j.ceramint.2022.07.272

Margonato, V., Nicolini, P., Conti, R., Zecca, L., Veicsteinas, A., Cerretelli, P., 1995. Biologic effects of prolonged exposure to ELF electromagnetic fields in rats: II. 50 Hz magnetic fields. Bioelectromagnetics 16, 343–355. https://doi.org/10.1002/bem.2250160602

Martínez-Díaz, J.A., Hernández-Aguilar, M.E., Rojas-Durán, F., Herrera-Covarrubias, D., García-Hernández, L.I., Mestizo-Gutiérrez, S.L., Aranda-Abreu, G.E., 2020. Expression of proteins linked to Alzheimer's disease in C6 rat glioma cells under the action of lipopolysaccharide (LPS), nimesulide, resveratrol and citalopram. Turk. J. Biochem. 45, 793–801. https://doi.org/10.1515/tjb-2020-0091

McEwen, B.S., Conrad, C.D., Kuroda, Y., Frankfurt, M., Magarinos, A.M., McKittrick, C., 1997. Prevention of stress-induced morphological and cognitive consequences. Eur. Neuropsychopharmacol. 7, S323–S328.

McIlwain, K.L., Merriweather, M.Y., Yuva-Paylor, L.A., Paylor, R., 2001. The use of behavioral test batteries: effects of training history. Physiol. Behav. 73, 705–717.

Michael, B., Yano, B., Sellers, R.S., Perry, R., Morton, D., Roome, N., Johnson, J.K., Schafer, K., 2007. Evaluation of organ weights for rodent and non-rodent toxicity studies: a

review of regulatory guidelines and a survey of current practices. Toxicol. Pathol. 35, 742–750.

Mohammad Alizadeh, M.A., Abrari, K., Lashkar Blouki, T., Ghorbanian, M. taghi, Jadidi, M., 2019. Pulsed electromagnetic field attenuated PTSD-induced failure of conditioned fear extinction. Iran. J. Basic Med. Sci. 22, 650–659. https://doi.org/10.22038/ijbms.2019.32576.7797

Pathak, N., Cheruku, S.P., Rao, V., Vibhavari, R.J.A., Sumalatha, S., Gourishetti, K., Rao, C.M., Kumar, N., 2020. Dehydrozingerone protects temozolomide-induced cognitive impairment in normal and C6 glioma rats besides enhancing its anticancer potential. 3 Biotech 10, 1–13.

Patki, G., Li, L., Allam, F., Solanki, N., Dao, A.T., Alkadhi, K., Salim, S., 2014. Moderate treadmill exercise rescues anxiety and depression-like behavior as well as memory impairment in a rat model of posttraumatic stress disorder. Physiol. Behav. 130, 47–53. https://doi.org/10.1016/j.physbeh.2014.03.016

Peng, M., Zhang, C., Dong, Y., Zhang, Y., Nakazawa, H., Kaneki, M., Zheng, H., Shen, Y., Marcantonio, E.R., Xie, Z., 2016. Battery of behavioral tests in mice to study postoperative delirium. Sci. Rep. 6, 1–13.

Perez, F.P., Maloney, B., Chopra, N., Morisaki, J.J., Lahiri, D.K., 2021. Repeated electromagnetic field stimulation lowers amyloid-β peptide levels in primary human mixed brain tissue cultures. Sci. Rep. 11, 621. https://doi.org/10.1038/s41598-020-77808-2

Persinger, M.A., Lafrenière, G.F., Carrey, N.J., 1978. Thyroid morphology and wet organ weight changes in rats exposed to different low intensity 0.5 Hz magnetic fields and pre-experimental caging conditions. Int. J. Biometeorol. 22, 67–73. https://doi.org/10.1007/BF01553142

Peters, J.M., Boyd, E.M., 1966. Organ Weights and Water Levels of the Rat following Reduced Food Intake. J. Nutr. 90, 354–360. https://doi.org/10.1093/jn/90.4.354

Pfeiffer, C.J., 1968. A mathematical evaluation of the thymic weight parameter. Toxicol. Appl. Pharmacol. 13, 220–227. https://doi.org/10.1016/0041-008X(68)90096-3

Polk, C., Postow, E., 1995. Handbook of Biological Effects of Electromagnetic Fields, -2 Volume Set. CRC press.

Prajapati, S.K., Dangi, D.S., Krishnamurthy, S., 2019. Repeated caffeine administration aggravates post-traumatic stress disorder-like symptoms in rats. Physiol. Behav. 211, 112666. https://doi.org/10.1016/j.physbeh.2019.112666

Prajapati, S.K., Garabadu, D., Krishnamurthy, S., 2017. Coenzyme Q10 prevents mitochondrial dysfunction and facilitates pharmacological activity of atorvastatin in 6-OHDA induced dopaminergic toxicity in rats. Neurotox. Res. 31, 478–492.

Prajapati, S.K., Krishnamurthy, S., 2021. Non-selective orexin-receptor antagonist attenuates stress-re-stress-induced core PTSD-like symptoms in rats: Behavioural and neurochemical analyses. Behav. Brain Res. 399, 113015.

Prajapati, S.K., Singh, N., Garabadu, D., Krishnamurthy, S., 2020. A novel stress re-stress model: modification of re-stressor cue induces long-lasting post-traumatic stress disorder-like symptoms in rats. Int. J. Neurosci. 130, 941–952.

Reddy, D.S., Kulkarni, S.K., 1998. Possible role of nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging- and dizocilpine-induced learning impairment. Brain Res. 799, 215–229. https://doi.org/10.1016/S0006-8993(98)00419-3

Sandyk, R., 1994a. Alzheimer's disease: improvement of visual memory and visuoconstructive performance by treatment with picotesla range magnetic fields. Int. J. Neurosci. 76, 185–225.

Sandyk, R., 1994b. A drug naive parkinsonian patient successfully treated with weak electromagnetic fields. Int. J. Neurosci. 79, 99–110.

Sandyk, R., Iacono, R.P., 1994. Reversal of Micrographia in Parkinson's Disease by Application of Picotesla Range Magnetic Fields. Int. J. Neurosci. 77, 77–84. https://doi.org/10.3109/00207459408986020

Srivastava, A., Verma, N., Mistri, A., Ranjan, B., Nigam, A.K., Kumari, U., Mittal, S., Mittal, A.K., 2017. Alterations in the skin of Labeo rohita exposed to an azo dye, Eriochrome black T: a histopathological and enzyme biochemical investigation. Environ. Sci. Pollut. Res. 24, 8671–8681. https://doi.org/10.1007/s11356-017-8517-4

Stagg, R.B., Thomas, W.J., Jones, R.A., Adey, W.R., 1997. DNA synthesis and cell proliferation in C6 glioma and primary glial cells exposed to a 836.55 MHz modulated radiofrequency field. Bioelectromagnetics 18, 230–236. https://doi.org/10.1002/(SICI)1521-186X(1997)18:3<230::AID-BEM5>3.0.CO;2-3

Swanson (INVITED), J., 1999. Residential Power-Frequency Electric and Magnetic Fields: Sources and Exposures. Radiat. Prot. Dosimetry 83, 9–14. https://doi.org/10.1093/oxfordjournals.rpd.a032670

Swanson, J., Kaune, W.T., 1999. Comparison of residential power-frequency magnetic fields away from appliances in different countries. Bioelectromagn. J. Bioelectromagn. Soc. Soc. Phys. Regul. Biol. Med. Eur. Bioelectromagn. Assoc. 20, 244–254.

Tarnowski, B.I., Spinale, F.G., Nicholson, J.H., 1991. DAPI as a useful stain for nuclear quantitation. Biotech. Histochem. Off. Publ. Biol. Stain Comm. 66, 297–302.

Téglás, T., Dörnyei, G., Bretz, K., Nyakas, C., 2018. Whole-body pulsed EMF stimulation improves cognitive and psychomotor activity in senescent rats. Behav. Brain Res. 349, 163–168. https://doi.org/10.1016/j.bbr.2018.04.036

Titford, M., 2005. The long history of hematoxylin. Biotech. Histochem. 80, 73–78.

Treit, D., Menard, J., Royan, C., 1993. Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44, 463–469.

Trzeciak, H.I., Grzesik, J., Bortel, M., Kuśka, R., Duda, D., Michnik, J., Ma\lecki, A., 1993. Behavioral effects of long-term exposure to magnetic fields in rats. Bioelectromagnetics 14, 287–297.

Tzakis, N., Holahan, M.R., 2019. Social Memory and the Role of the Hippocampal CA2 Region. Front. Behav. Neurosci. 13.

Wolf, A., Bauer, B., Abner, E.L., Ashkenazy-Frolinger, T., Hartz, A.M., 2016. A comprehensive behavioral test battery to assess learning and memory in 129S6/Tg2576 mice. PloS One 11, e0147733.

Xu, W., Sun, J., Le, Y., Chen, J., Lu, X., Yao, X., 2019. Effect of pulsed millisecond current magnetic field on the proliferation of C6 rat glioma cells. Electromagn. Biol. Med. 38, 185–197. https://doi.org/10.1080/15368378.2019.1608233

Zecca, L., Mantegazza, C., Margonato, V., Cerretelli, P., Caniatti, M., Piva, F., Dondi, D., Hagino, N., 1998. Biological effects of prolonged exposure to ELF electromagnetic fields in rats: III. 50 Hz electromagnetic fields. Bioelectromagn. J. Bioelectromagn. Soc. Soc. Phys. Regul. Biol. Med. Eur. Bioelectromagn. Assoc. 19, 57–66.