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Extreme Low Frequency Electromagnetic Field Stimulation Induces Metaplastic-Like Effects on LTP/ LTD

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ABSTRACT In modern societies, people are more likely to be exposed to technological devices that emit extreme low frequency (ELF, < 300 Hz)-electromagnetic fields (EMFs). Although ELF-EMFs are successfully used as therapeutic agents in psychiatry treatment and rehabilitation practices, they are also considered to be environmental pollutants that pose a risk to human health. However, several studies have suggested that ELF-EMFs stimulation has the potential to ameliorate learning and memory processes in humans. Given that the underlying mechanisms of magnetic stimulation on the brain are not fully understood, this study aimed to investigate the effects of ELF-EMFs in learning and memory formation. Sprague-Dawley rats were used as a model system to evaluate learning and memory mechanisms based on the synaptic plasticity of the Schaffer-CA1 pathway in hippocampal slices using ELF-EMFs stimulation. Parameters were selected based on previous experiments (i.e., 15 hertz [Hz], 2 militesla [mT]), during, and after plasticity induction, basic frequencies of 1, 5, 20 and 100 Hz were applied and an on-line ELF-EMFs stimulation drive was used together, which previously defined as preceding, middle and post stimulation. Our results showed that the greatest effect on synaptic plasticity was observed when ELF-EMFs were paired with a plasticity induction protocol. Importantly, ELF-EMFs did not affect synapses that were weakly active or in synapses containing N-methyl-D-aspartate (NMDA) receptors that were blocked. This study highlights the metaplastic-like role of ELF-EMFs, acting as modulators of synaptic activity processes,as well as their regulation by NMDA receptor-dependent synaptic plasticity.

INDEX TERMS Preceding, middle and post ELF-EMFs stimulation, hippocampal slices, LTP and LTD, metaplasticity, NMDA receptors.

I. INTRODUCTION

In modern society, greater use of technologies leads to increased exposure to extremely low-frequency (ELF, <300 Hz) - electromagnetic fields (EMFs) generated by structures and appliances [1]. In addition, there is growing evidence that suggests that exposure to ELF-EMFs affects biological behavior [2]–[4]. Therefore, there exists a growing concern regarding the possibility of ELF-EMFs inducing biological phenomena, which might be harmful to human health and this is now currently under investigation in living systems, including their effects on brain activity, nervous system function and cognitive behaviors [5]. Recently, ELF-EMFs have been proposed to be involved in the modulation of hippocampal functions, including cell proliferation, neurogenesis and the regulation of behavioral activities [6]–[8]. Some studies have also shown that ELF-EMF exposure could cause notable long-term deficits in learning abilities [9] and memory formation in developing mice [10]. More recent studies have revealed that ELF-EMF of 60 hertz (Hz) and 0.7 militesla (mT) could also provide cognitive advantages in mice [11] and exert positive effects on the acquisition and maintenance of spatial memory [12]. Based on these data, we can conclude that several factors

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might indeed contribute to the promotional effect of electric and magnetic field exposure on learning and long-term memory. Nevertheless, the underlying mechanisms mediating these effects have not been fully elucidated.

Furthermore, it is well established that the hippocampus is involved in regulating cognitive functioning, including short-term memory and the long-term memory [13]. After the discovery of long-term potentiation (LTP) mechanisms by Dr. Professor Terje Lømo from the University of Oslo, Norway in 1966, LTP and long term depression (LTD) have been key cellular process of learning and memory in experiment models *in vitro* [14], [15]. Primarily, in neurosciences research, learning and memory are thought to be mediated by synaptic plasticity involving several processes, including LTP / LTD. Thus, synaptic activity is mediated by changes in synaptic strength via LTP / LTD mechanisms, which are further divided into three duration phases, before, during and after plasticity induction [16]. Marquez-Ruiz et al found that transcranial direct-current stimulation can modulate the cortical synaptic mechanisms which were involved in classical eyeblink conditioning in behaving rabbits (Marquez-Ruiz et al., PNAS USA, 2012). They also found that transcranial alternating-current stimulation can evoke tactile perception in behaving rabbits (Marquez-Ruiz et al., Scientific Rep, 2016) [17], [18]. Many *in vivo* experiments investigating EMF exposure have shown the difficulties involved in intervening exactly during these three duration phases [19], [20]. However, *in vitro* brain slices preparation allow for precise control over the EMFs being evaluated with respect to the different stages of synaptic activity. In a previous study, Park *et al*. reported that priming micro magnetic stimulation reduced LTP of Schaeffer collaterals from the CA3 region synapsing onto neurons in the CA1 region in C57BL/6 mice [21]. According to the scientific literature, adding a direct current stimulation (DCS) during the plasticity induction phase also has an impact on synaptic plasticity [22], and EMF exposure has the ability to induce a time-varying electric field (due to Maxwell-Faraday's law) [23]. Therefore, in this paper we put forward a hypothesis that ELF-EMF stimulation regulates synaptic plasticity at different stages, in other words, the phase during plasticity induction corresponds to the process of memory formation and the phase after plasticity induction corresponds to the process after memory formation and consolidation, which were equally important in this stimulation.

As a model of endogenous synaptic plasticity, we induced LTP / LTD using canonical protocols (e.g., pulse trains of stimuli delivered to Schaffer-CA1 synapses of rat hippocampal slices). Moreover, ELF-EMF stimulation was added before plasticity induction to determine the best magnetic stimulation parameters. We then confirmed that an ELF-EMF value of 15 Hz and 2 mT, was the best value among our test parameters and established it as a unified parameter for later studies. Subsequently, we further confirmed the influence of the magnetic stimulation added before, during and after plasticity induction that was generated using a base frequency of 1, 5, 20 and 100 Hz, which showed that the middle ELF-EMF stimulation had the greatest impact on LTP / LTD. Notably, the ELF-EMF stimulation did not affect synapses that were weakly active or synapses containing NMDA receptors that were blocked and inactive. Based on these results, we captured a new frequency response function (FRF), which has been widely used to study the predictions of the Bienenstock, Cooper and Munro (BCM) theory of synaptic plasticity [24]. To this end, we show that ELF-EMFs can shift the FRF and diminish LTP / LTD synaptic activity, similar to BCM-induced metaplasticity. Finally, we show that ELF-EMFs may not directly induce plasticity, but rather act as modulators of endogenous synaptic plasticity, which is crucial for our understanding of the effects of ELF-EMF stimulation on learning and memory formation.

II. METHODS

A. ETHICS STATEMENT

The experimental procedures used here were approved by the Animal Care and Use Committee of School of Electronics and Information Engineering, Tianjin Polytechnic University, Tianjin, China, and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised in 1996).

B. ANIMALS

A total of 85 Sprague–Dawley male rats, 14 to18 days old at the time of surgery, were purchased from the Institute of Academy of Military Medical Sciences (Tianjin, China), with the certification number SCXK (Jing) 2016-0006. Animals were housed in individual cages in a clean room maintained under a 12-hour light / dark cycle at a constant temperature ($25 \pm 2°C$) and had access to food and water *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee. The number of animals used and the experimental protocol were designed to minimize animal suffering. All brain slices were randomly assigned to either the control or the experimental group to minimize subjective bias.

C. HIPPOCAMPAL SLICE PREPARATION

Animals were deeply anesthetized with ether, and their brains were rapidly removed and submerged in a 4°C ice-cold cutting solution containing 90 mM sucrose, 87.2 mM NaCl, 2.5 mM KCl, 7 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 16.7 mM glucose. The solution was continuously bubbled with 95% O_2 and 5% CO_2. Next, 400 μm-thick slices were cut using a Vibratome 3000 tissue slicer (Technical Products International, St. Louis, MO) in ice-cold cutting solution. Slices were then incubated at 33°C in artificial cerebrospinal fluid (ACSF) consisting of 120 mM NaCl, 2.5 mM KCl, 2 mM $MgSO_4 \cdot 7H_2O$, 2 mM $CaCl_2$, 1.25 mM $NaH_2PO_4 \cdot 2H_2O$, 26 mM $NaHCO_3$, and 10 mM glucose continuously bubbled

with 95% O_2 and 5% CO_2 (pH 7.4). All reagents were of analytical grade and made in China.

D. ELECTROPHYSIOLOGICAL RECORDINGS

After incubation for 60 minutes, a slice was transferred onto the patch-clamp perfusion chamber and continuously superfused with oxygenated ACSF, the temperature was maintained at 33°C. We used standard procedures to record field excitatory postsynaptic potentials (fEPSPs) in the Schaffer-CA1 pathway of the hippocampus. The bipolar stimulating electrode, CBARC75, FHC^{TM}, (FHC, Inc., Bowdoin, ME) was placed in the stratum radiatum of the CA1 to deliver test and conditioning stimuli. Recording electrodes made of glass micropipettes pulled by a Sutter Instruments P-97 (Sutter Instrument, Novato, CA) filled with ACSF (resistance 1-8 MΩ) were placed in stratum radiatum (250 μm) from the stimulating electrode. fEPSPs were induced by test stimuli at 0.05 Hz with an intensity elicited 40-50% of the maximum response. Stable baseline fEPSPs were recorded every minute for at least 20 minutes before any plasticity induction was applied. Then, fEPSPs were recorded again every minute for 60 minutes after plasticity induction. Induction frequencies were chosen as 1, 5, 20, and 100 Hz. To induce LTD, 900 pulses were delivered at 1 and 5 Hz. To induce LTP, 900 pulses were delivered at 20 Hz and 400 pulses delivered at 100 Hz (duration: 1 second, repeated four times with 20-second breaks) [25], [26]. For NMDAR antagonist experiments, 100 μM MK-801 (Sigma-Aldrich, St. Louis, MO) was included in the ACSF perfused in the recording chamber throughout the experiment. Because MK-801 is an open channel blocker, slices were in the recording chamber 20 minutes before baseline fEPSPs recording to ensure the complete blockade of NMDAR channels [27].

E. ELECTROPHYSIOLOGICAL RECORDINGS

In order to allow for more accurate electrophysiological recordings after exposure to the magnetic field, an on-line magnetic stimulation device based on a patch clamp system was used. Our previous study had successfully demonstrated that the system may allow real-time observation of the effects of magnetic stimulation on neurons [28], [29]. In this study, preceding, middle, and post ELF-EMF stimulus protocols were used as shown in Fig. 1A1, A2 and A3, where the duration of preceding and post ELF-EMF stimulation was 20 minutes, and the duration of the middle ELF-EMF stimulation was the same as the one used for electric induction. In brief, the drive controller was composed of the function generator, SDG1020 (SLGLENT Technologies, Solon, OH) and the power amplifier, TDA7294 (STMicroelectronics, TX, USA) to amplify the current, which passed through the coil to produce a continuous sinusoidal magnetic field with intensities of 0.5, 1, and 2 mT and frequencies of 15, 50, and 100 Hz. The experimental magnetic field intensity of the position of the slices was measured by the militesla device, HT108 (Ningbo Haitian Magnets, Ningbo, China) to ensure the accuracy of the magnetic field.

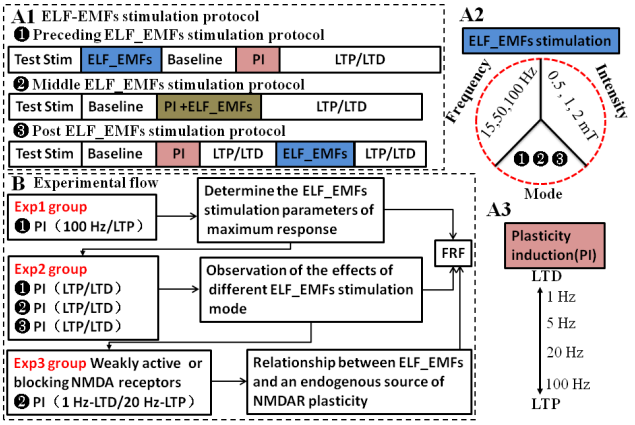

FIGURE 1. ELF-EMFs stimulation protocol and experimental flow. (A1) ELF-EMFs stimulation protocol. ❶, ❷, ❸ representing preceding, middle and post ELF-EMFs stimulation protocol, respectively. ❶ showed the time schedule of the test stimulus, 20 minutes of ELF-EMFs stimulation, baseline recording, Plasticity induction (PI) and LTP /LTD recording. ❷ showed the time schedule of test stimulus, baseline recording, PI + ELF-EMFs stimulation and LTP / LTD recordings. The brown rectangle box indicates that PI and ELF-EMFs stimulation are performed at the same time. ❸ showed the time schedule of test stimulus, baseline recording, PI, LTP / LTD recording, 20 minutes of ELF-EMFs stimulation and LTP / LTD recordings. The blue rectangle box, as shown in (A2), indicates the ELF-EMFs stimulation parameters with different magnetic frequencies (15, 50 and 100 Hz, respectively), magnetic intensities (0.5, 1 and 2 mT, respectively) and stimulation modes (❶, ❷ and ❸ respectively). The pink rectangle box, as shown in (A3), indicates electrical stimulation for induction of LTP / LTD with frequencies of 1, 5, 20 and 100 Hz, respectively. (B) Experimental flow. First, the ELF-EMFs stimulation parameters of the maximum response were determined by the Exp1 group using protocol ❶, plasticity induction was established by using 100 Hz to induce LTP. Moreover, by using the stimulation parameters from Exp1 group, the effects of different stimulation modes (❶, ❷, ❸) on LTP / LTD were investigated in the Exp2 group, all protocols were applied, plasticity induction using 1 Hz and 5 Hz to induce LTD, 20 Hz and 100 Hz to induce LTP. In the Exp3 group, by using protocol ❷, plasticity induction was established by using 1 Hz to induce LTD and 20 Hz to induce LTP. Finally, a new frequency response function (FRF) is obtained from the Exp1, 2, 3 groups.

The experimental process is shown in Fig. 1B, these slices were randomly divided into a control group and an experimental group, which were then further divided into 3 groups (Exp1, Exp2 and Exp3 groups). No ELF-EMF stimulation was applied to the slices of the control group. However, for the slices from the experimental group, 20 minutes preceding ELF-EMFs were introduced on the Exp1 Group to reach the maximum response on LTP under magnetic stimulation. Then, all the preceding, middle and post ELF-EMFs parameters were applied to the hippocampal slice, and the LTD / LTP induced by electrical stimulation frequencies of 1, 5, 20 and 100 Hz were recorded on Exp2 Group, in order to observe the effects of the different magnetic modes on synaptic plasticity. Finally, the Exp3 group was established to observe whether ELF-EMFs affected the synapses that were weakly active or synapses containing NMDA receptors that had been pharmacologically blocked.

F. STATISTICAL ANALYSIS

The raw data were processed with the Origin 8.0 data analysis software (OriginLab, Northampton, MA) and the statistical

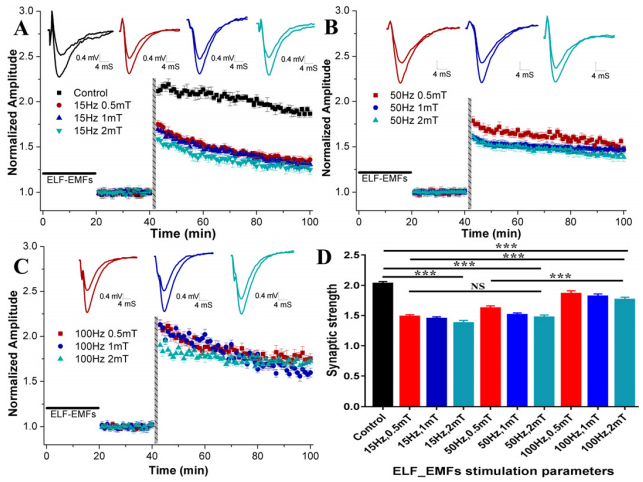

FIGURE 2. Determination of ELF-EMFs stimulation parameters for the Exp1 group. (A-C) Time course of 100 Hz HFS-induced LTP without (control group, black) and with 15, 50, and 100 Hz preceding ELF-EMFs (0.5 mT group, red; 1 mT group, blue; 1 mT group, green). Grey bars indicate the duration of induction. (D) Histogram showing the effect of preceding ELF-EMFs stimulation on LTP induction, synaptic strength is the average of the all normalized fEPSP amplitude in each condition (60 minutes post-induction). Compared with the control group, the synaptic strength increase with the decreased of magnetic field frequency and increase of magnetic field intensity, preceding ELF-EMFs stimulation shifts synaptic plasticity in SC-CA1 towards reduction. 15 Hz, 2 mT group showed the greatest difference. Data are represented as the mean ± SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; NS: not significant, one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test.

analysis was finalized with Graphpad Prism7 (GraphPad Software Incorporation, San Diego, CA). In the Exp1 group, all the data were analyzed with a one-way analysis of variance (ANOVA) using the Tukey's multiple comparisons test. In the Exp2 group, all data were analyzed with a two-way analysis of variance (ANOVA) on ranks with Tukey's post hoc test to evaluate the main effects of treatment and induction, and their interaction. Results were expressed as the mean ± SD. Differences were considered to be significant at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

III. RESULTS

A. PRECEDING ELF-EMFs STIMULATION MODULATES LTP FREQUENCY AND INTENSITY DEPENDENCE

For the Exp1 group (Fig. 1B), we first determined whether the preceding ELF-EMFs stimulation used was able to regulate the synaptic LTP, and identified the frequency and intensity dependence on LTP, using the stimulus protocol number ❶ as depicted in Fig. 1A1. We then applied 20 minutes of priming on-line sinusoidal ELF-EMFs using frequencies of 15, 50 and 100 Hz on Schaffer collateral pathways of the CA1 hippocampal region, before taking the baseline recording. Each frequency consisted of four magnetic field intensities: a control (without EMFs), 0.5 mT, 1 mT and 2 mT (Fig. 2A-C). A 100 Hz high frequency stimulation (HFS) generated

LTP in the slide recordings (Control: 202.5 ± 3.9%, n = 5, slices / 3 rats; 15 Hz, 0.5 mT: 147.6 ± 3.5%, p < 0.0001, n = 6, slices / 3 rats; 15 Hz, 1 mT : 144.2 ± 3.4%, p < 0.001, n = 6, slices / 3 rats; 15 Hz, 2 mT: 136.9 ± 4.7%, p < 0.0001, n = 5, slices / 3 rats; 50 Hz, 0.5 mT: 161.6 ± 3.9%, p < 0.001, n = 6, slices / 3 rats; 50 Hz, 1 mT: 150.4 ± 3.7%, p < 0.001, n = 5, slices / 3 rats; 50 Hz, 2 mT: 146.5 ± 4.2%, p < 0.001, n = 5, slices / 3 rats; 100 Hz, 0.5 mT: 185.3 ± 5.2%, p < 0.001, n = 4, slices / 2 rats; 100 Hz, 1 mT: 180.9 ± 4.5%, p < 0.001, n = 5, slices / 3 rats; 100 Hz, 2 mT: 175.7 ± 4.2%, p < 0.001, n = 5 slices / 3 rats). Moreover, ANOVA methods were used to analyze the data and the resulting statistical values are shown in Fig. 2D. These results showed that the preceding ELF-EMFs stimulation shifted LTP, and synaptic strength increased with decreasing values of magnetic field frequencies and increasing values of magnetic field intensities, compared with those of the control group. Notably, the group assigned to the 15 Hz, 2 mT ELF-EMFs parameters showed the greatest difference.

B. PRECEDING, MIDDLE, AND POST ELF-EMFs STIMULATION AS MODULATORS OF SYNAPTIC PLASTICITY

Subsequently, we applied to prime sinusoidal on-line to preceding, middle, and post ELF-EMFs stimulation using the parameters, 15 Hz and 2mT, in order to study the effects of synaptic plasticity events on the Exp2 group (Fig. 1B). Of note, all the stimulus protocols designed in this study were on the Exp2 group (i.e., protocols ❶, ❷, and ❸), as seen in Fig. 1A1. In addition, there were three types of ELF-EMFs stimulations used here. Each type consisted of four electrical induction frequencies, chosen as 1, 5, 20 and 100 Hz.

First, 20 minutes of ELF-EMFs stimulation was applied before the baseline recording was taken, we called this protocol "preceding ELF-EMFs stimulation", with plasticity induction frequencies of 1, 5, 20 and 100 Hz, lasting 15, 3, 0.75 minutes and 64 seconds, respectively. The time course of the four plasticities induced LTP / LTD recordings at 60 minutes is shown in Fig. 3A1-A4 (red = control group; black = preceding ELF-EMFs stimulation group). The experimental results showed that the preceding ELF-EMFs stimulation phase significantly attenuated LTD / LTP plasticity (1 Hz, low frequency stimulation (LFS): 89.6 ± 3.4%, p = 0.004, n = 4 slices / 2 rats; 20 Hz (HFS): 110.5 ± 4.5%, p = 0.04, n = 5 slices / 2 rats; 100 Hz (HFS): 136.9 ± 4.7%, p < 0.001, n = 5 slices / 3 rats). Moreover, the ELF-EMFs had a reduced effect at a frequency of 5 Hz, that was not statistically significant (Fig. 3A2: 96.9 ± 4%, p = 0.13, n = 5 slices / 2 rats), this was consistent with the effects we had observed in a previous study at the threshold between LTP and LTD [30].

Other studies investigating hippocampal CA1 plasticity have suggested that focusing on the induction of synaptic plasticity is crucial in order to fully understand the inner molecular mechanisms underlying neural plasticity events and memory formation [22]. Here, we were interested in the effects of ELF-EMFs stimulation on rat hippocampal slices during synaptic plasticity induction. We called this protocol "middle ELF-EMFs stimulation". Moreover, the duration of the magnetic field stimulation was equal to the duration of

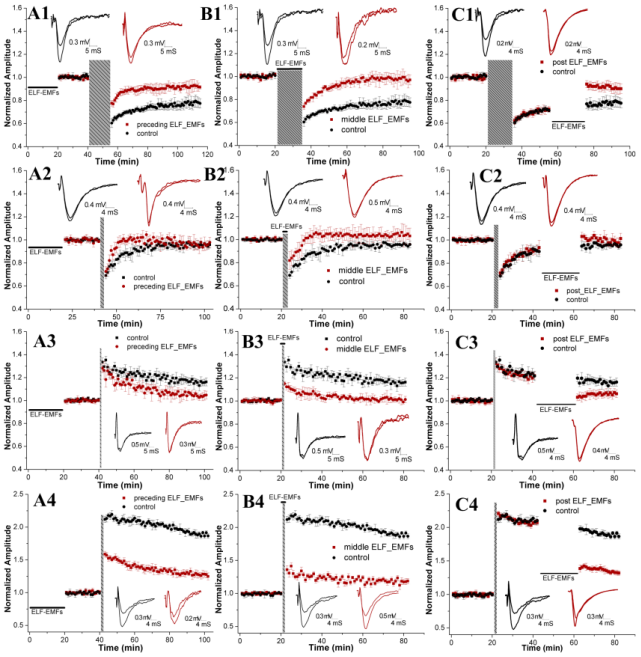

FIGURE 3. ELF-EMFs modulate LTP / LTD in the Exp2 group. The 2 mT magnetic field of 15 Hz was produced and applied to slices to study to effect of preceding, middle and post ELF-EMFs stimulation on synaptic plasticity. (A1-A4) Application protocol ❶, added 20 minutes of ELF-EMFs stimulation before the baseline recording, plasticity induction was 1 Hz (A1), 5 Hz (A2), 20 Hz (A3) and 100 Hz (A4), there was an obvious weakening effect on LTP / LTD. Grey bars indicate the duration of induction. (B1-B4) Application protocol ❷, added 900 s (B1), 180 s (B2), 45 s (B3) and 64 s (B4) ELF-EMFs stimulation during plasticity induction. The Middle phase of ELF-EMFs stimulation applied during plasticity-inducing attenuated LTP / LTD stronger than preceding ELF-EMFs. Grey bars indicate the duration of induction and concurrent ELF-EMFs stimulation. (C1-C4) Application protocol ❸ added 20 minutes ELF-EMFs stimulation in the middle of the entire recording on LTP / LTD after plasticity induction, and we found that post and preceding ELF-EMFs have similar effects. Grey bars indicate the duration of induction. The time course of fEPSP traces is provided for each condition (red: control group; black: 15 Hz, 2 mT ELF-EMFs stimulation group). Data are represented as the mean ± SEM across slices.

the electric induction (1 Hz: 15 minutes, 5 Hz: 3 minutes, 20 Hz: 0.75 minutes and 100Hz: 64 seconds). In addition, we showed that the middle ELF-EMFs stimulation resulted in a larger synaptic plasticity change together with the preceding ELF-EMFs stimulation as seen in Fig. 3B1-B4 (1 Hz (LFS): 93.4 ± 3.6%, $p < 0.001$, $n = 5$ slices / 3 rats; 5 Hz: 102 ± 5.5%, $p = 0.04$, $n = 5$, slices / 3 rats; 20 Hz (HFS): 103.9 ± 2.9%, $p = 0.004$, $n = 5$ slices / 3 rats; 100 Hz (HFS): 122.3 ± 3.9%, $p < 0.001$, $n = 5$, slices / rats). After this, we added 20 minutes of ELF-EMFs stimulation in the middle of the entire recording for LTD / LTP after plasticity induction. We called this protocol "post ELF-EMFs stimulation", and found that the "preceding" and "post ELF-EMFs stimulation" protocols, had similar effects as seen in Fig. 3C1-C4 (1 Hz: 91.7 ± 3.9%, $p < 0.001$, $n = 5$ slices / 3 rats; 5 Hz: 100.4 ± 3.6%, $p = 0.06$, $n = 4$ slices / 2 rats; 20 Hz: 105.5 ± 3.5%, $p = 0.002$, $n = 6$ slices / 3 rats; 100 Hz: 137.1 ± 3.8%, $p < 0.001$, $n = 4$ slices / 2 rats). Markedly, these experimental results showed that preceding, middle, and post ELF-EMFs stimulation events can inhibit

TABLE 1. Variance analysis of the mean normalized fEPSP amplitudes of 20 points (1 point / 1 minute) within 41-60 minutes of LTP / LTD recordings in the Exp2 group.

	Induction: Electrical induced frequency of synaptic plasticity				
	1 Hz	5 Hz	20 Hz	100 Hz	Induction (F, p)
Control	0.7343 ± 0.0304 (n=6, slices/3rats)	0.9129 ± 0.0314 (n=4, slices/2rats)	1.2105 ± 0.0400 (n=6, slices/3rats)	2.0247 ± 0.0394 (n=5, slices/2rats)	F (3, 64) = 67.38, p <0.001
Preceding ELF-EMFs	0.9182 ± 0.0368 (n=4, slices/3rats)	0.9631 ± 0.0431 (n=5, slices/3rats)	1.0507 ± 0.0342 (n=5, slices/3rats)	1.3697 ± 0.0470 (n=5, slices/3rats)	
Middle ELF-EMFs	1.0205 ± 0.0495 (n=5, slices/3rats)	1.0591 ± 0.0523 (n=5, slices/3rats)	0.9840±0.0 315 (n=5, slices/3rats)	1.2228 ± 0.0393 (n=5, slices/3rats)	
Post ELF-EMFs	0.9168 ± 0.0389 (n=5, slices/3rats)	0.9841 ± 0.0357 (n=4, slices/2rats)	1.0547 ± 0.0351 (n=6, slices/3rats)	1.3699 ± 0.0385 (n=4, slices/2rats)	
Treatment (F, p)	F (3, 64) = 913.4, p < 0.001				
Treatment * Induction (F, p)	F (9, 64) = 144.6, p < 0.001				

LTP / LTD plasticity, with the middle magnetic phase having the most obvious effect on plasticity.

C. ELF-EMFs CAN SHIFT THE FREQUENCY RESPONSE FUNCTION, SIMILAR TO METAPLASTICITY

Three different modes of magnetic stimulation and four different electrical induced frequencies were applied to Schaffer collateral pathways synapsing on CA1 stratum radiatum of hippocampal slices from the Exp2 group. LFS generated LTD, while HFS generated LTP, all the resulting statistical data are shown in Table 1. In addition, we tested the interaction between the treatment groups (control, preceding, middle, and post ELF-EMFs modulation phase groups) and induction groups (1, 5, 20, and 100 Hz electrical induced frequency groups) (Table 1, two-way ANOVA: $F_{Treatment(3,64)} = 913.4$, $p < 0.001$; $F_{Induction(3,64)} = 67.38$, $p < 0.001$; $F_{Treatment*Induction(9,64)} = 144.6$, $p < 0.001$). Compared to the control group, both the treatment and induction groups presented significantly altered patterns of activity resulting from the experimental procedures. For instance, when the electrical induced frequency was constant, the effects of the magnetic stimulation mode are shown in Fig. 4A, except for a comparison between preceding ELF-EMFs stimulation and post ELF-EMFs stimulation groups, other groups presented significant differences ($*p < 0.05$, $**$ $p < 0.01$, $***p < 0.001$). Based on the above statistical analysis we established a new FRF as shown in Fig. 4B. The resulting preceding, middle, and post ELF-EMFs stimulation FRF were significantly shifted compared to the control FRF, which mapped the degree of synaptic activity during induction to the degree of the resulting synaptic plasticity, which is consistent with the existing literature reports [31].

D. THE EFFECTS OF ELF-EMFs STIMULATION REQUIRE A CONCURRENT ENDOGENOUS SOURCE OF NMDAR PLASTICITY

As seen in the scientific literature, HFMS (High-frequency magnetic stimulation) can induce long-term potentiation in

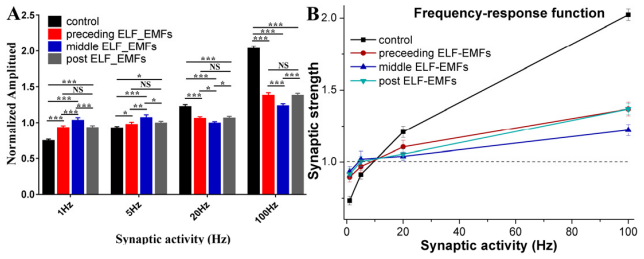

FIGURE 4. ELF-EMFs stimulation induces metaplastic-like effects on LTP/LTD. (A) Histogram showing the effect of preceding, middle and post ELF-EMFs stimulation phases on LTP / LTD induction, mean normalized fEPSP amplitudes of 20 points (1 point/ 1min) within 41-60 minutes LTP / LTD recording. The LTP / LTD in the middle phase ELF-EMFs stimulation group was most significantly reduced compared with the control group. (B) Preceding ELF-EMFs (red), middle ELF-EMFs (blue) and post ELF-EMFs (green) shifts the BCM-like frequency-response function towards potentiation, similar to metaplasticity, synaptic strength was the average of the last twenty normalized fEPSP amplitude in each condition (41-60 minutes post plasticity induction). Data are represented as the mean ± SEM across slices. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; NS: not significant, two-way analysis of variance (ANOVA) and Tukey's multiple comparisons test.

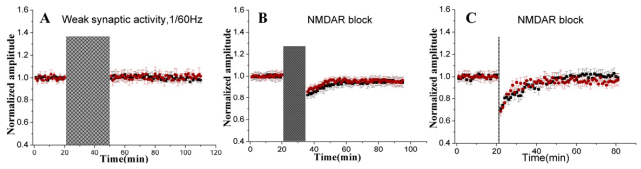

FIGURE 5. ELF-EMFs effects on neural activity require an endogenous source of NMDAR plasticity for the Exp3 group. (A) ELF-EMFs stimulation (red) applied during the synaptic activity that is too weak to induce plasticity (30 pulses at 1 / 60 Hz) has no effect on synaptic strength. (B) ELF-EMFs with 1 Hz LFS has no effect on synaptic strength when NMDARs are blocked with the antagonist MK-801. (C) ELF-EMFs stimulation of 20 Hz HFS has no effect on synaptic strength when NMDARs are blocked with the antagonist MK-801. Grey bars indicate the duration of induction and concurrent ELF-EMFs stimulation. Data are represented as the mean ± SEM across slices.

rat hippocampal slices [32]. Here, we propose that the ELF-EMFs stimulation can only modulate the synaptic plasticity that is regulated by the activity of NMDA pathways (i.e., NMDA receptors). ELF-EMFs stimulation, would therefore, require a concurrent endogenous source of plasticity to modulate synapse formation. To test this requirement, we applied an ELF-EMFs stimulation as in the middle phase induction, but this time removed endogenous NMDAR-dependent plasticity in two ways: first by weakening synaptic activity to well below the plasticity threshold, and second by directly blocking NMDAR currents during strong synaptic activity. ELF-EMFs stimulation applied during weak synaptic activity (30 pulses, 1 / 60 Hz), had no discernable effects on neural plasticity (Fig. 5A; control: 100.2 ± 2.7%, n = 6 slices / 3 rats; ELF-EMFs [15Hz, 2 mT]: 100.6 ± 3.8%, n = 6 slices / 3 rats; p = 0.88). When paired with synaptic activity (1 Hz LFS and 20 Hz HFS), while at the same time blocking NMDAR activity with the antagonist, MK-801, ELF-EMFs stimulation also presented no effect in synaptic changes (Fig. 5B, control: 93.8 ± 3.7%, n = 7, slices / 4 rats; ELF-EMFs: 94.7 ± 3%, n = 8, slices / 4 rats; p = 0.55; Fig. 5C, control: 93.7 ± 3.7%, n = 7 slices / 3 rats; ELF-EMFs: 94.7 ± 4.2%, n = 8 slices / 4 rats; p = 0.63). These results suggest that stimulation with ELF-EMFs can modulate LTP / LTD plasticity in an NMDAR dependent manner, which strongly suggest the involvement of NMDA receptor signaling pathways.

IV. DISCUSSIONS

In conclusion, the present study shows the influence of preceding ELF-EMFs stimulation on LTP and in addition identifies the optimal frequency and strength of ELF-EMFs generating the greatest impact on synaptic plasticity. From an experimental point of view, we further illustrated the possible effects of middle and post ELF-EMFs stimulation phases on synaptic plasticity, which can help understand directly the role of ELF-EMFs in the processes associated with learning and memory. Furthermore, blocking NMDA receptor activity using the MK-801 antagonist, confirmed an important actionable target of ELF-EMFs through NMDA receptor pathways. In addition, using the on-line ELF-EMFs system on rat hippocampal slices provided a basis for neural pathway research, as plasticity induction processes strongly appear to be impacted by ELF-EMFs, which may aid future studies investigating mechanisms associated with learning and memory formation.

A. THE POTENTIAL EFFECT ELF-EMFs HAVE ON SYNAPTIC PLASTICITY

There is now compelling evidence demonstrating the role of both LTP and LTD-like processes in learning and memory neuroplastic events [33]. Both of these processes not only provide strong evidence for activity-dependent synaptic plasticity in higher animals but also provide an ideal model for studying the neural mechanisms underpinning learning and memory processes at the synaptic level. Some scholars believe that a decline in gene expression is a possible mechanism affecting plasticity [34]. Another possible explanation for the inhibitory effects of ELF-EMF's on neural plasticity may be an increase in intracellular Ca^{2+} levels. A rise in intracellular Ca^{2+} concentration is a direct result of excessive and/or persistent activation of glutamate-gated ion channels, which may also cause neuronal degeneration [35]. Interestingly, it was reported that ELF-EMFs stimulation increased the concentrations of inhibitory amino acids, such as glycine, the inhibitory neurotransmitter, GABA, and the microtubule protein, Tau [36]. In addition, other studies have shown that extended EMF exposure could cause notable long-term deficits in learning ability and memory formation in rats [10], [37]. Another key feature of synaptic plasticity is that the process itself is, indeed, plastic. This characteristic of synaptic plasticity has been termed metaplasticity (i.e., higher-order synaptic plasticity) [38] and it involves a myriad of innate neural processes, For instance, vesicles of neurotransmitters have the ability to fuse with the synaptic membrane, as glutamate molecules bind on post-synaptic

2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propanoic acid (AMPA) and NMDA receptors, that are both of major significance for LTP and LTD events.

B. ELF-EMFs STIMULATION-INDUCED METAPLASTICITY

It has been proposed that the horizontal axis of the FRF can be equated with the degree of postsynaptic calcium influx during induction. In addition, it believed that HFS leads to a strong calcium influx and triggers LTP, while LFS leads to a moderate calcium influx and LTD [39]. Based on this calcium control hypothesis, we expected the ELF-EMFs stimulation-induced plasticity to modulate calcium influx through NMDAR activity and produce similar horizontal shifts in the FRF. Instead, our results showed that no matter what kind of stimulation protocol we used, the largest shift in LTP was the 100 Hz frequency group. We were convinced that the reason for this phenomenon was, perhaps, that the 100 Hz frequency control group had the largest shift compared with the LFS group and the 20 Hz frequency group. According to previous studies, ELF-EMFs stimulation has the potential to change cell membrane permeability, and thereby calcium activity, triggering the signal transduction cascade, which affects neural activity [40]. We also believe, that ELF-EMFs stimulation can regulate other important molecules, given that it has been suggested that HFS can elevate brain-derived neurotrophic factor (BDNF) levels in the hippocampus, which can, in turn, induce neuroplasticity in the pre-limbic cortex and stimulates neuropeptide release [41].

In addition, Tokay and others recorded that HFMS significantly reduced the propensity of subsequent electrical LTP, and found that NMDA receptor activation was involved in this form of HFMS-induced metaplasticity [42]. Markedly, our results support this hypothesis, as ELF-EMFs stimulation had no discernable effects on plasticity changes, when the synaptic input was weakened (Fig. 5A) or when NMDAR signals were blocked during strong synaptic inputs (Fig. 5B and C). Together, these results strongly indicate that synaptic efficacy can be modulated by ELF-EMFs only in the presence of NMDAR-dependent plasticity.

V. LIMITATIONS OF THE STUDY

Evoked LTP can interfere with synaptic changes in strength evoked by actual associative learning in behaving mice [43]. However, in this study, we performed ELF-EMFs stimulation induces metaplastic-like effects on *in vitro* brain slices not in behaving rats. Therefore, the correlation between the effect of *in vitro* ELF-EMFs stimulation on LTP / LTD and the synaptic changes evoked by actual associative learning in behaving mice needs to be further verified.

VI. CONCLUSION

Previous studies have shown that ELF-EMFs can be widely used as neural regulators [44], [45]. In addition, data from animals and humans demonstrate a correlation of ELF-EMFs with synaptic plasticity [46], [47]. More recently, other studies showed that repetitive transcranial magnetic stimulation (rTMS) can be applied as a therapeutic modality to directly or indirectly modulate neuronal excitability and synaptic plasticity in a specific neural region or in the entire brain. For example, high-frequency rTMS (HF-rTMS, 5 to 20 Hz) induces LTP, whereas low-frequency rTMS (LF-rTMS, $\leq$ 1 Hz) induces LTD [48]. Standard TMS has been demonstrated to be able to modulate cortical excitability up to a maximum depth of 1.5 to 2.5 cm from the scalp [49]. Nonetheless, it is still unknown exactly how EMF-EMFs can regulate deep brain regions, such as the hippocampus. Organotypic brain slice cultures can allow for precise control over the EMF stimulus with respect to different stages of synaptic activity. The results of this study show that ELF-EMFs can inhibit synaptic plasticity in the CA1 region of hippocampal slices in vitro. This highlights the importance of middle ELF-EMFs stimulation effects, which have received little attention in the EMFs literature. An earlier study has also shown that a 2 T pulsed magnetic field can induce LTP without electrical HFS in the CA1 region [32], in a similar manner to rTMS. However, sinusoidal ELF-EMFs with an mT order of magnitude cannot induce plasticity, instead it can only act as a modulator, similar to the BCM-proposed metaplasticity. Whether wider frequency ranges, stronger doses, or more complex EMF stimulation protocols, are necessary to induce endogenous synaptic activity will require further research. Despite this complexity, we highlight that a middle phase of ELF-EMFs stimulation, may be an effective mean of regulating synaptic activity when paired with a learning process.

ABBREVIATIONS

ELF-EMFs, extreme low frequency electromagnetic fields; cAMP, cyclic adenosine monophosphate; LTP, long-term potentiation; LTD, long-term depression; FRF, frequency-response function; BCM theory, theoretical model proposed by Bienenstock, Cooper, and Munro; ACSF, artificial cerebrospinal fluid; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; LFS, low-frequency stimulation; NMDA, N-methyl-D-aspartate; AMPA, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propanoic acid; rTMS, repetitive transcranial magnetic stimulation.

AUTHOR CONTRIBUTIONS

Y.Z. designed research. C.X.T., and K.H.Z. performed research. Y. G. and C.S.L. analyzed data. L.D., X. X.M., C.X., and C.X.T. wrote the paper.

REFERENCES

[1] H. Huuskonen, M. L. Lindbohm, and J. Juutilainen, "Teratogenic and reproductive effects of low-frequency magnetic fields," *Mutation Res./Rev. Mutation Res.*, vol. 410, no. 2, pp. 167–183, Apr. 1998.

[2] M. B. Bracken, K. Belanger, K. Hellenbrand, L. Dlugosz, T. R. Holford, J.-E. McSharry, K. Addesso, and B. Leaderer, "Exposure to electromagnetic fields during pregnancy with emphasis on electrically heated beds: Association with birthweight and intrauterine growth retardation," *Epidemiology*, vol. 6, no. 3, pp. 263–270, May 1995.

[3] J. Juutilainen, P. Matilainen, S. Saarikoski, E. Läärä, and S. Suonio, "Early pregnancy loss and exposure to 50-Hz magnetic fields," *Bioelectromagnetics*, vol. 14, no. 3, pp. 229–236, 1993.
[4] D. A. Savitz and C. V. Ananth, "Residential magnetic fields, wire codes, and pregnancy outcome," *Bioelectromagnetics*, vol. 15, no. 3, pp. 271–273, 1994.
[5] Z. Sienkiewicz, N. Jones, and A. Bottomley, "Neurobehavioural effects of electromagnetic fields," *Bioelectromagnetics*, vol. 26, no. S7, pp. S116–S126, 2005.
[6] T. Oda and T. Koike, "Magnetic field exposure saves rat cerebellar granule neurons from apoptosis *in vitro*," *Neurosci. Lett.*, vol. 365, no. 2, pp. 83–86, Jul. 2004.
[7] M. V. Podda, L. Leone, S. A. Barbati, A. Mastrodonato, D. D. L. Puma, R. Piacentini, and C. Grassi, "Extremely low-frequency electromagnetic fields enhance the survival of newborn neurons in the mouse hippocampus," *Eur. J. Neurosci.*, vol. 39, no. 6, pp. 893–903, Mar. 2014.
[8] B. Cuccurazzu, L. Leone, M. V. Podda, R. Piacentini, E. Riccardi, C. Ripoli, G. B. Azzena, and C. Grassi, "Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice," *Exp. Neurology*, vol. 226, no. 1, pp. 173–182, Nov. 2010.
[9] L. Sakhnini, S. Al-Ghareeb, S. Khalil, R. Ahmed, A. A. Ameer, and A. Kamal, "Effects of exposure to 50 Hz electromagnetic fields on Morris water-maze performance of prenatal and neonatal mice," *J. Assoc. Arab Universities Basic Appl. Sci.*, vol. 15, no. 1, pp. 1–5, 2014.
[10] M. Jadidi, S. M. Firoozabadi, A. Rashidy-Pour, A. A. Sajadi, H. Sadeghi, A. A. Taherian, "Acute exposure to a 50 Hz magnetic field impairs consolidation of spatial memory in rats," *Neurobiol. Learn. Memory*, vol. 88, no. 4, pp. 387–392, Nov. 2007.
[11] O. Arias-Carrión, L. Verdugo-Díaz, A. Feria-Velasco, D. Millán-Aldaco, A. A. Gutiérrez, A. Hernández-Cruz, and R. Drucker-Colín, "Neurogenesis in the subventricular zone following transcranial magnetic field stimulation and nigrostriatal lesions," *J. Neurosci. Res.*, vol. 78, no. 1, pp. 16–28, 2004.
[12] T. Liu, S. Wang, L. He, and K. Ye, "Chronic exposure to low-intensity magnetic field improves acquisition and maintenance of memory," *Neurorep. Rapid Commun. Neurosci. Res.*, vol. 19, no. 5, pp. 549–552, Mar. 2008.
[13] T. V. P. Bliss and G. L. Collingridge, "A synaptic model of memory: Long-term potentiation in the hippocampus," *Nature*, vol. 361, pp. 31–39, Jan. 1993. doi: 10.1038/361031a0.
[14] T. Lømo, "The discovery of long-term potentiation," *Phil. Trans. Roy. Soc. B, Biol. Sci.*, vol. 358, no. 1432, pp. 617–620, Apr. 2003.
[15] S. F. Cooke and T. V. P. Bliss, "Plasticity in the human central nervous system," *Brain*, vol. 129, no. 7, pp. 1659–1673, Jul. 2006.
[16] M. Migliore, F. Alicata, and G. F. Ayala, "A model for long-term potentiation and depression," *J. Comput. Neurosci.*, vol. 2, no. 4, pp. 335–343, Dec. 1995.
[17] J. Marquez-Ruiz, R. Leal-Campanario, R. Sánchez-Campusano, B. Molaee-Ardekani, F. Wendling, P. C. Miranda, G. Ruffini, A. Gruart, and J. M. Delgado-García, "Transcranial direct-current stimulation modulates synaptic mechanisms involved in associative learning in behaving rabbits," *Proc. Nat. Acad. Sci. USA*, vol. 109, no. 17, pp. 6710–6715, Apr. 2012.
[18] J. Márquez-Ruiz, C. Ammann, R. Leal-Campanario, G. Ruffini, A. Gruart, and J. M. Delgado-García, "Synthetic tactile perception induced by transcranial alternating-current stimulation can substitute for natural sensory stimulus in behaving rabbits," *Sci. Rep.*, vol. 6, p. 19753, Jan. 2016.
[19] Z. Ahmed and A. Wieraszko, "The mechanism of magnetic field-induced increase of excitability in hippocampal neurons," *Brain Res.*, vol. 1221, pp. 30–40, Jul. 2008.
[20] A. Komaki, A. Khalili, I. Salehi, S. Shahidi, and A. Sarihi, "Effects of exposure to an extremely low frequency electromagnetic field on hippocampal long-term potentiation in rat," *Brain Res.*, vol. 1564, pp. 1–8, May 2014.
[21] H. J. Park, H. K. Kang, M. Wang, J. Jo, E. Chung, and S. Kim, "A pilot study of planar coil based magnetic stimulation using acute hippocampal slice in mice," in *Proc. 39th Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. (EMBC)*, Jul. 2017, pp. 1118–1121.
[22] G. Kronberg, M. Bridi, T. Abel, M. Bikson, and L. C. Parra, "Direct current stimulation modulates LTP and LTD: Activity dependence and dendritic effects," *Brain Stimulation*, vol. 10, no. 1, pp. 51–58, 2016.
[23] D. M. Long, "Transcranial magnetic stimulation: A neurochronometrics of mind," *Neurosurgery Quart.*, vol. 14, no. 2, pp. 116–117, Jun. 2004.
[24] L. N. Cooper and M. F. Bear, "The BCM theory of synapse modification at 30: Interaction of theory with experiment," *Nature Rev. Neurosci.*, vol. 13, no. 11, pp. 798–810, Oct. 2012.
[25] S. Nabavi, H. W. Kessels, S. Alfonso, J. Aow, R. Fox, and R. Malinow, "Metabotropic NMDA receptor function is required for NMDA receptor-dependent long-term depression," *Proc. Nat. Acad. Sci. USA*, vol. 110, no. 10, pp. 4027–4032, 2013.
[26] M. Mayford, J. Wang, E. R. Kandel, and T. J. O'Dell, "CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP," *Cell*, vol. 81, no. 6, pp. 891–904, 1995.
[27] H. W. Kessels,, S. Nabavi, and R. Malinow, "Metabotropic NMDA receptor function is required for ß-amyloid–induced synaptic depression," *Proc. Nat. Acad. Sci. USA*, vol. 110, no. 10, pp. 4033–4038, 2013.
[28] Y. Zheng, W. Ma, L. Dong, J.-R. Dou, Y. Gao, and J. Xue, "Influence of the on-line ELF-EMF stimulation on the electrophysiological properties of the rat hippocampal CA1 neurons *in vitro*," *Rev. Sci. Instrum.*, vol. 88, no. 10, Oct. 2017, Art. no. 105106.
[29] Y. Zheng, L. Dong, Y. Gao, J.-R. Dou, and Z.-Y. Li, "A comparison of 15 Hz sine on-line and off-line magnetic stimulation affecting the voltage-gated sodium channel currents of prefrontal cortex pyramidal neurons," *EPL (Europhys. Lett.)*, vol. 116, no. 1, 2016, Art. no. 018002.
[30] S. R. Hulme, O. D. Jones, D. R. Ireland, and W. C. Abraham, "Calcium-dependent but action potential-independent BCM-like metaplasticity in the hippocampus," *J. Neurosci.*, vol. 32, no. 20, pp. 6785–6794, May 2012.
[31] M. F. Bear, "Bidirectional synaptic plasticity: From theory to reality," *Phil. Trans. Biol. Sci.*, vol. 358, no. 1432, pp. 649–655, Apr. 2003.
[32] T. Tokay, N. Holl, T. Kirschstein, V. Zschorlich, R. Köhling, "High-frequency magnetic stimulation induces long-term potentiation in rat hippocampal slices," *Neurosci. Lett.*, vol. 461, no. 2, pp. 150–154, Sep. 2009.
[33] W. Loscher, "Animal and cellular studies on carcinogenesis effects of low frequency (50/60-Hz) magnetic fields," *Mutation Res.*, vol. 410, no. 2, pp. 185–220, Apr. 1998.
[34] F. Mignini, C. Nasuti, and M. Artico,, "Effects and trimethyltin on hippocampal dopaminergic markers and cognitive behaviour," *Int. J. Immunopathol. Pharmacol.*, vol. 25, no. 4, pp. 1107–1119, 2012.
[35] A. Lisi, M. T. Ciotti, M. Ledda, M. Pieri, C. Zona, D. Mercanti, S. Rieti, L. Giuliani, and S. Grimaldi, "Exposure to 50 Hz electromagnetic radiation promote early maturation and differentiation in newborn rat cerebellar granule neurons," *J. Cellular Physiol.*, vol. 204, no. 2, pp. 532–538, Aug. 2005.
[36] Y. H. Li, D. W. Wang, R. Y. Peng, Z. J. Li, B. Dong, F. T. Dong, Y. Q. Liang, and W. H. Hu, "Effects of electromagnetic pulse on contents of amino acids in hippocampus of rats," *Chin. J. Ind. Hygiene Occupational Diseases*, vol. 21, no. 5, pp. 323–325, Oct. 2003.
[37] L. Sakhnini, S. Al-Ghareeb, S. Khalil, R. Ahmed, A. A. Ameer, and A. Kamal, "Effects of exposure to 50 Hz electromagnetic fields on morris water-maze performance of prenatal and neonatal mice," *J. Assoc. Arab Universities Basic Appl. Sci.*, vol. 15, no. 1, pp. 1–5, 2014.
[38] Z. A. Bortolotto, M. Amici, W. W. Anderson, J. T. R. Isaac, and G. L. Collingridge, "Synaptic plasticity in the hippocampal slice preparation," *Current Protocols Neurosci.*, vol. 54, no. 6, pp. 6–13, Jan. 2011.
[39] S. Nabavi, R. Fox, C. D. Proulx, J. Y. Lin, R. Y. Tsien, and R. Malinow, "Engineering a memory with LTD and LTP," *Nature*, vol. 511, no. 7509, pp. 348–352, Jul. 2014.
[40] R. Karabakhtsian, N. Broude, N. Shalts, S. Kochlatyia, R. Goodman, and A. S. Henderson, "Calcium is necessary in the cell response to EM fields," *FEBS Lett.*, vol. 349, no. 1, pp. 1–6, Jul. 1994.
[41] R. Gersner, E. Kravetz, J. Feil, G. Pell, and A. Zangen, "Long-term effects of repetitive transcranial magnetic stimulation on markers for neuroplasticity: Differential outcomes in anesthetized and awake animals," *J. Neurosci.*, vol. 31, no. 20, pp. 7521–7526, May 2011.
[42] T. Tokay, T. Kirschstein, M. Rohde, V. Zschorlich, and R. Köhling, "NMDA receptor-dependent metaplasticity by high-frequency magnetic stimulation," *Neural Plasticity*, vol. 2014, no. 2014, pp. 1–8, Oct. 2014.
[43] A. Gruart, M. D. Muñoz, and J. M. Delgado-García, "Involvement of the CA3–CA1 synapse in the acquisition of associative learning in behaving mice," *J. Neurosci.*, vol. 26, no. 4, pp. 1077–1087, 2006.
[44] N. Dragicevic, P. C. Bradshaw, M. Mamcarz, X. Lin, L. Wang, C. Cao, and G. W. Arendash, "Long-term electromagnetic field treatment enhances brain mitochondrial function of both Alzheimer's transgenic mice and normal mice: A mechanism for electromagnetic field-induced cognitive benefit," *Neuroscience*, vol. 185, pp. 135–149, Jun. 2011.

[45] I. Tasset, F. J. Medina, I. Jimena, E. Agüera, F. Gascón, M. Feijóo, F. Sánchez-López, E. Luque, J. Peña, R. Drucker-Colín, and I. Túnez, "Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: Effects on neurotrophic factors and neuronal density," *Neuroscience*, vol. 209, pp. 54–63, May 2012.
[46] J. M. Hoogendam, G. M. J. Ramakers, and V. Di Lazzaro, "Physiology of repetitive transcranial magnetic stimulation of the human brain," *Brain Stimulation*, vol. 3, no. 2, pp. 95–118, Apr. 2010.
[47] H. Lu, T. Kobilo, C. Robertson, S. Tong, P. Celnik, and G. Pelled, "Transcranial magnetic stimulation facilitates neurorehabilitation after pediatric traumatic brain injury," *Sci. Rep.*, vol. 5, Oct. 2015, Art. no. 014769.
[48] C.-C. Huang, T.-P. Su, and I. H. Wei, "Repetitive transcranial magnetic stimulation for treating medication-resistant depression in taiwan: A preliminary study," *J. Chin. Med. Assoc.*, vol. 68, pp. 210–215, May 2005.
[49] F. S. Bersani, A. Minichino, P. G. Enticott, L. Mazzarini, N. Khan, G. Antonacci, R. N. Raccah, M. Salviati, R. D. Chiaie, G. Bersani, P. B. Fitzgerald, and M. Biondi, "Deep transcranial magnetic stimulation as a treatment for psychiatric disorders: A comprehensive review," *Eur. Psychiatry*, vol. 28, pp. 30–39, Jan. 2013.

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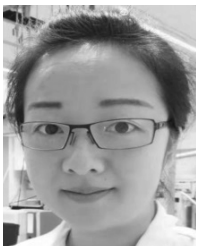

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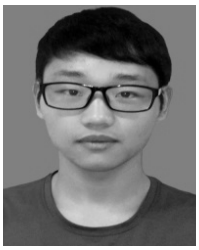

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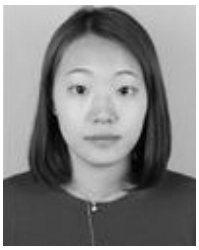

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