Research Article

Extremely low frequency electromagnetic fields promote cognitive function and hippocampal neurogenesis of rats with cerebral ischemia

https://doi.org/10.4103/1673-5374.301020

Date of submission: November 25, 2019

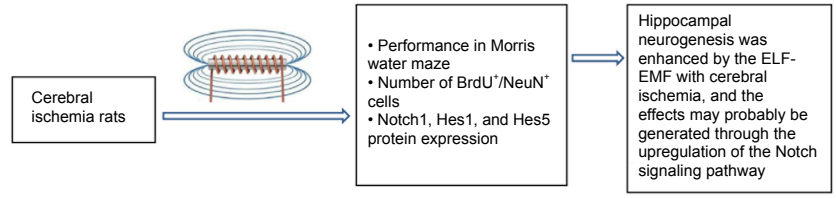
Date of decision: January 2, 2020

Date of acceptance: August 25, 2020

Date of web publication: December 12, 2020

Qiang Gao[1, 2, 3], Aaron Leung[2, 3, *], Yong-Hong Yang[1, 2, 4], Benson Wui-Man Lau[4], Qian Wang[1, 2], Ling-Yi Liao[1], Yun-Juan Xie[1], Cheng-Qi He[1, 2, *]

Graphical Abstract *Extremely low frequency electromagnetic fields (ELF-EMF) bear the potential to be a new therapeutic intervention in cerebral ischemia*



Abstract

Extremely low frequency electromagnetic fields (ELF-EMF) can improve the learning and memory impairment of rats with Alzheimer's disease, however, its effect on cerebral ischemia remains poorly understood. In this study, we established rat models of middle cerebral artery occlusion/reperfusion. One day after modeling, a group of rats were treated with ELF-EMF (50 Hz, 1 mT) for 2 hours daily on 28 successive days. Our results showed that rats treated with ELF-EMF required shorter swimming distances and latencies in the Morris water maze test than those of untreated rats. The number of times the platform was crossed and the time spent in the target quadrant were greater than those of untreated rats. The number of $BrdU^+/NeuN^+$ cells, representing newly born neurons, in the hippocampal subgranular zone increased more in the treated than in untreated rats. Up-regulation in the expressions of Notch1, Hes1, and Hes5 proteins, which are the key factors of the Notch signaling pathway, was greatest in the treated rats. These findings suggest that ELF-EMF can enhance hippocampal neurogenesis of rats with cerebral ischemia, possibly by affecting the Notch signaling pathway. The study was approved by the Institutional Ethics Committee of Sichuan University, China (approval No. 2019255A) on March 5, 2019.

Key Words: cerebral ischemia; cognitive function; electromagnetic fields; hippocampus; neurogenesis; plasticity; repair; signaling pathway; stroke; rat

Chinese Library Classification No. R454; R741; Q441.4

Introduction

Extremely low frequency electromagnetic fields (ELF-EMF) are those with the frequencies between 0 and 300 Hz (Feychting et al., 2005; Funk et al., 2009). ELF-EMF can affect cellular activities through ion channels, receptors or enzymes on the cell membrane or intracellularly. Clinical studies have shown that ELF-EMF had the effect of alleviating pain (Abdulla et al., 2019), reducing oxidative stress (Cichoń et al., 2017), accelerating the process of fracture healing (Ehnert et al., 2019), protecting chondrocytes in osteoarthritis (Ross et al., 2019) and increasing bone mineral density in osteoporosis (Wang et al., 2019). ELF-EMF could also be beneficial to cognitive and memory functions. Exposure to ELF-EMF for 2 hours each day over 9 days improved social recognition memory in normal adult rats (Vázquez-García et al., 2004). Moreover, ELF-EMF improved learning and memory impairments in rats with Alzheimer's disease (Akbarnejad et al., 2018). However, the effect of ELF-EMF on cognitive function is still controversial. It has been reported that low intensity ELF-EMF (50 Hz, 0.1 mT) did not improve the memory or change the β-amyloid deposition and the shape of neurons in rats with Alzheimer's disease (Zhang et al., 2015). Others found that a daily high dose of ELF-EMF (50 Hz, 8 mT) for 28 days could impair the memory function of rats by increasing neurotransmitters, including glutamate and gamma-aminobutyric acid, in the hippocampus (Duan

[1]Department of Rehabilitation Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China; [2]Institute of Disaster Management and Reconstruction, Sichuan University–The Hong Kong Polytechnic University, Chengdu, Sichuan Province, China; [3]Department of Biomedical Engineering, The Hong Kong Polytechnic University, Hong Kong Special Administrative Region, China; [4]Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hong Kong Special Administrative Region, China

*Correspondence to: Aaron Leung, PhD, aaron.leung@polyu.edu.hk; Cheng-Qi He, MD, hxkfhcq@126.com.

https://orcid.org/0000-0003-3603-749X (Qiang Gao)

Funding: *This work was supported by the National Natural Science Foundation of China, No. 81201513 (to QG).*

How to cite this article: *Gao Q, Leung A, Yang YH, Lau BWM, Wang Q, Liao LY, Xie YJ, He CQ (2021) Extremely low frequency electromagnetic fields promote cognitive function and hippocampal neurogenesis of rats with cerebral ischemia. Neural Regen Res 16(7):1252-1257.*

et al., 2014). Other research reported that ELF-EMF (50 Hz, 1 mT) significantly increased Hes1 and Mash1, which are the key factors in the Notch signaling pathway, and promoted neural stem cells (NSCs) differentiation into neurons in the dentate gyrus (Cuccurazzu et al., 2010). Furthermore, the new mature neurons integrated into the granule cell layer of the dentate gyrus and enhanced the synaptic plasticity of the hippocampus in normal adult rats. Recently, ELF-EMF was shown to increase the number of differentiated NSCs and new neurons in the dentate gyrus in mice with hippocampal injury (Sakhaie et al., 2017). The effect of ELF-EMF on cognitive function and neurogenesis in cerebral ischemia is still not clear. In this study, we aimed to test the hypothesis that ELF-EMF would promote the neurogenesis and improve cognitive functions after cerebral ischemia by its effect on the Notch signaling pathway.

Materials and Methods

Animals and ethical approval

Fifty-four male, specific-pathogen-free Sprague-Dawley rats aged 12 weeks and weighing 280–320 g were provided by Vitalriver Biotechnology, Chengdu, China [license No. SCXK (Chuan) 2015-030]. The rats were housed under controlled temperature 20 ± 2°C and 12/12 hours light/dark cycle (light on from 07:00 to 19:00), with food and water made available *ad libitum* throughout the experiments. All animal experimental protocols were conducted according to the Guide for the Care and Use of Laboratory Animals (National Institute of Health, Bethesda, MD, USA) and were approved by the Institutional Ethics Committee of Sichuan University, China (approval No. 2019255A) on March 5, 2019. All experiments were designed and reported according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.

Grouping

Rats were randomly assigned to the sham-surgery group (sham group, n = 12) and the middle cerebral artery occlusion (MCAO) group. All the rats in the MCAO group received the MCAO surgery. After surgery, the successfully induced model rats were randomly assigned into a control group (Con group, n = 12) and an experimental group (Exp group, n = 12) using a random number table. The sham group received the sham surgery (no MCAO) and no exposure of ELF-EMF; the Con group received MCAO surgery but no exposure of ELF-EMF; the Exp group received both MCAO surgery and ELF-EMF exposure. Six rats were randomly selected from each group for western blot assay on the 7th day after the surgery. The flow chart of the experiment is shown in **Figure 1**.

Focal ischemia model

MCAO was induced using the intraluminal filament technique to establish the focal cerebral ischemia (Longa et al., 1989). The full procedure was described in our previous study (Gao et al., 2011). Briefly, rats were intraperitoneally anesthetized with 40 mg/kg pentobarbital. The right common, internal and external carotid arteries were exposed. A filament was inserted 2 cm into the internal carotid artery via the external carotid artery. After 60 minutes of the occlusion, the filament was gently pulled out and the external carotid artery was tightly ligated. For the sham surgery, an incision was made in the neck skin with no vascular occlusion. Rectal temperature was maintained at 37.0–37.5°C during the procedures.

The model was considered successful if the rat presented one of following symptoms 24 hours after the surgery according to the Longa's neurological examination (Longa et al., 1989): (1) flexion and internal rotation of the left forelimb; (2) weakness of the left forelimb, or falling down to the left side; (3) circling towards the left side. Rats were excluded if they have: (1) subarachnoid hemorrhage observed in dissection; (2) died before sacrifice.

ELF-EMF interventions

An ELF-EMF device was designed and manufactured by the College of Manufacturing Science and Engineering in Sichuan University following devices detailed in previous studies (Vázquez-García et al., 2004; Varró et al., 2009; Capone et al., 2020). The device consists of a computer, power amplifier and Helmholtz coils (two parallel solenoids (diameter: 40 cm) 20 cm apart) (**Figure 2**). Both coils were surrounded by 300 turns of copper wire of 0.8 mm diameter. A 50 Hz audio signal was generated by a computer program, connected to the power amplifier that turned the audio signal into an electrical current to the coils to produce the ELF.

Finite element analysis software (ANSYS, Pittsburgh, PA, USA) was used to simulate the intensity and distribution of the EMF, the maximal variation between the simulation and real tested value of the EMF was ± 10%, as described in our previous study (Wang et al., 2014). The intensity was tested by a hand-held magnetometer during the intervention (Hengtong, Shanghai, China). The intensity was fixed at 1.0 ± 0.05 mT during the experiments. The room temperature was maintained at 20 ± 2°C, while the temperature in the space of the coils was between 21.5°C and 22°C when the device was used during the intervention.

For the intervention, the rats were placed in a plastic cage

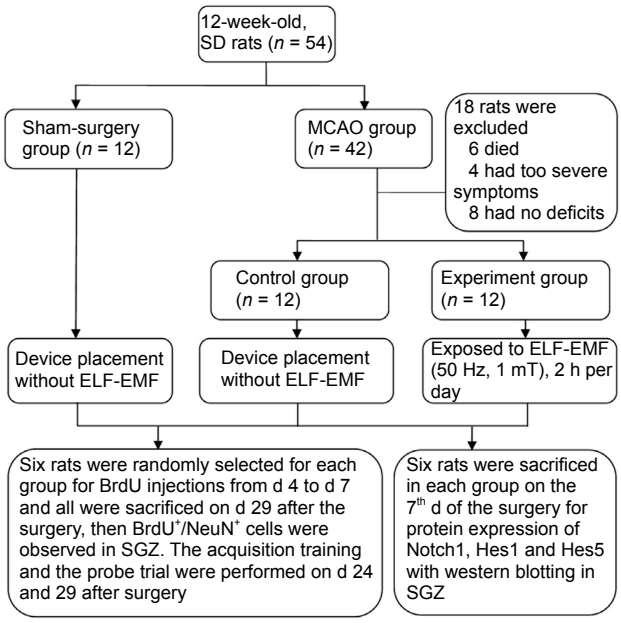


Figure 1 | **The flow chart of the study.**
BrdU: 5′-Bromo-2′-deoxyuridine; ELF-EMF: extremely low frequency electromagnetic fields; MCAO: middle cerebral artery occlusion; SD: Sprague-Dawley; SGZ: subgranular zone.

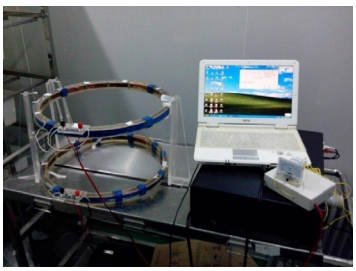


Figure 2 | **The extremely low frequency electromagnetic fields device.**
The device consists of a computer, power amplifier and Helmholtz coils. A 50 Hz audio signal is generated by a computer program connected to a power amplifier that turned the audio signal into an electrical current that drives the coils to generate extremely low frequency electromagnetic fields.

without restraint, 6 rats in a cage each time, and the cage was placed into the coils of the ELF-EMF device. Only the Exp group received the whole body ELF-EMF exposure (50 Hz, 1 mT) 24 hours after the surgery. The Sham group and the Con group were put into the plastic cage with the same procedure but with no ELF-EMF exposure. The intervention was given for 2 hours, once a day for 28 successive days.

Morris water maze

The Morris water maze was used to explore the cognitive function of the rats (Morris, 1984). A round pool with a diameter of 1.3 m and a height of 0.6 m was used. Rats learnt to use spatial cues to locate the fixed platform (diameter 10 cm) hidden under the water surface. One day before the first of the water maze tests, the cued version/visible platform trial was used to eliminate the influence of motor and sensory dysfunction. For the acquisition training, the rat was trained to identify the platform hidden under the water. The acquisition training, which mainly tests their learning ability, was started on the 23rd day of the intervention (day 24 after surgery), 4 times per day and was continued for 5 days. The video was then analyzed by the behavior analysis system (Panlab, Barcelona, Spain) to collect data on the latency (the duration from the time the rat was put into the water to when it detected the platform) and the swimming distance. The probe trial, which tests memory, was performed on the 2nd day after the acquisition training had finished (day 29 after the surgery). For the probe trial, the platform in the water was removed, then the rat was released from the opposite quadrant of the former platform for 60 seconds, the time that the rat spends in the former platform quadrant and the time it crossed the platform area was recorded. The tests were carried out at the same time every day to avoid circadian related effects. The temperature was maintained at 24–25°C in the laboratory and the water temperature was controlled at 23 ± 2°C during the experiment.

5′-Bromo-2′-deoxyuridine label

Intraperitoneal injection of the 5′-bromo-2′-deoxyuridine (BrdU) was given to rats at the dosage of 50 mg/kg to label proliferative cells (Sakhaie et al., 2017). Four successive intraperitoneal injections of BrdU were performed to all rats at intervals of 24 hours, started from day 4 to day 7 after the surgery (the 3rd day to the 6th day of the ELF-EMF intervention). All rats in the three groups were sacrificed and perfused transcardially by 4% paraformaldehyde on day 28 of the ELF-EMF intervention (day 29 after the surgery). Finally, brains were processed and fixed with 4% paraformaldehyde, then dehydrated and embedded in paraffin.

Immunofluorescence assays

Coronal slices (10 μm thickness) of the SGZ were obtained throughout the hippocampus. The slices were placed in an oven at 60°C for 0.5 hour, then transferred to xylene and graded ethanol for dewaxing and rehydration. Slices were incubated in antigen retrieval solution ethylenediaminetetraacetic acid (Amresco, Solon, OH, USA) at 97°C for 40 minutes, then incubated in 2 N HCl at 37°C for 30 minutes to denature the DNA. The slices were incubated overnight at 4°C with mouse anti-BrdU antibody (1:200; Cat# MS-1058-P0; NeoMarkers, Fremont, CA, USA), followed by re-incubating with Alexa Fluor 594 donkey anti-mouse IgG (1: 200; Cat# A21203; Thermo Fisher Scientific, Waltham, MA, USA). Double staining, to include neuron nuclei (NeuN), was achieved by antibody incubation with rabbit anti-NeuN polyclonal antibody (1: 300; Cat# ab104225; Abcam, Cambridge, MA, USA) overnight at 4°C. Markers were visualized with the second antibody of Alexa Fluor 488 conjugate anti-rabbit IgG (1: 300; Cat# 4412S; Cell Signaling Technology, Boston, MA, USA) for 1 hour. Staining of nuclei was performed with 4′,6-diamidino-2-phenylindole (Sigma, St. Louis, MO, USA) and slices were mounted with glycerol buffer.

Cell counting and image acquisition were performed using fluorescence microscopy (Leica Microsystems CMS, Leica, Solms, Germany) without any knowledge of the grouping. Slices were observed at 200× magnification, four fields were randomly selected in each slice for cell counting. The BrdU$^+$/NeuN$^+$ cells were counted in each field of each slice on the right (ischemic) side of the brain, then the average number of positive cells in each field for each rat was calculated (Arvidsson et al., 2002; Cuccurazzu et al., 2010).

Western blot analysis

Western blot analysis was used to measure the protein expression levels of Notch1, Hes1 and Hes5. Beta actin was used as control. Briefly, the fresh hippocampal tissue was collected and analyzed. Protein concentration was determined using a bicinchoninic acid protein assay kit (KeyGen Biotech, Nanjing, China). Equal amounts of proteins were electrophoresed on sodium dodecyl sulfate/10% polyacrylamide gel electrophoresis gels and transferred to nitrocellulose membranes and then incubated with the following primary antibodies: rat anti-Notch1 polyclonal antibody (Cat# 3447; Cell Signaling Technology), mouse anti-β-actin monoclonal antibody (Cat# TA-09; ZSGB-BIO, Beijing, China), rabbit anti-Hes1 polyclonal antibody (Cat# 11988; Cell Signaling Technology) and rabbit anti-Hes5 polyclonal antibody (Cat# ab25374; Abcam). The samples were then incubated with peroxidase conjugated secondary antibodies (goat anti-rat (Cat# ZB-2307), goat anti-mouse (Cat# ZB-2305) or goat anti-rabbit (Cat# ZB-2301); 1:5000; ZSGB-BIO) for 1 hour at 37°C. The optical density was quantified using the light density scanning analysis software (Image-Pro Plus 6.0, Media Cybernetics, Bethesda, MD, USA). β-Actin was used as an internal control and the results were normalized to β-actin.

Statistical analysis

Data were tested for normal distribution, descriptive data were expressed as mean ± standard error of the mean (SEM). Two-way repeated measure analysis of variance was performed for comparing differences among the three groups at each of the different time points for the data of the acquisition training in the Morris water maze test. One-way analysis of variance was employed for comparing the differences among the three groups for the results of the probe-trial in Morris water maze test, the number of BrdU$^+$/NeuN$^+$ cells in the subgranular zone (SGZ) and the expression of Notch1, Hes1 and Hes5. Significant analysis of variance results were further analyzed with least significant difference *post hoc* tests. Alpha errors were set at 0.05. SPSS 19.0 (IBM, Armonk, NY, USA) was used for all data processing.

Results

ELF-EMF promotes cognitive and memory function recovery in rats with cerebral ischemia

The focal cerebral ischemia impaired the memory functions of the rats in the water maze test. The Exp group performed better than the Con group in both the acquisition training and probe trial (**Figure 3**). In the acquisition training, shown in **Figure 3A** and **B**, two-way repeated measure analysis of variance revealed significant differences between groups on swimming distance (F = 0.684, P = 0.009) and latency (F = 6.166, P = 0.012). There were interaction effects between group and day on swimming distance (F = 0.715, P = 0.677) and latency (F = 0.721, P = 0.672). Results of the least

significant difference *post hoc* tests showed that both the swimming distance and the latency of the Con group were significantly longer than those in the sham group ($P < 0.05$ on each of the 5 days); the Exp group had significantly shorter swimming distances and latencies than in the Con group ($P < 0.05$ on day 3, 4 and 5).

Figure 3C and **D** show the results of the probe trial on day 29 after the surgery. One-way analysis of variance suggested that the difference of the number of crossings was significant among the three groups ($F = 5.232$, $P = 0.020$), with the least significant difference *post hoc* test showing that the Con group had a significantly reduced number of crossings comparing with Sham group ($P = 0.006$). The Exp group trended to perform better than the Con group, however, no significant difference was found ($P = 0.083$). The time spent in the target quadrant was significantly different between the three groups ($F = 5.372$, $P = 0.019$). The Con group spent less time than Sham group ($P < 0.01$), whereas, ELF-EMF treatment increased the time spent in the target quadrant compared with that of the Con group ($P < 0.05$).

ELF-EMF increases the numbers of newly born neurons in the SGZ of rats with cerebral ischemia

After the 28-day intervention, the results of one-way analysis of variance showed that the number of $BrdU^+/NeuN^+$ cells was significantly different between the three groups. The least-significant difference *post hoc* analysis showed that, compared with the sham group, the numbers of $BrdU^+/NeuN^+$ cells were significantly higher in the Con ($P < 0.05$) and Exp groups ($P < 0.01$). Comparing with the Con group, the number of $BrdU^+/NeuN^+$ cells was significantly increased in the Exp group ($P < 0.05$; **Figure 4**).

ELF-EMF improves the protein expression of the Notch signaling pathway in the SGZ of rats with cerebral ischemia

The expression of key components of the Notch signaling pathway was assessed in the SGZ of the hippocampus with western blot assay (**Figure 5**). The results showed that the protein expression of Notch1, Hes1 and Hes5 proteins in the SGZ was significantly higher in rats with cerebral ischemia than in the Sham group ($P < 0.01$). ELF-EMF enhanced the expression of Notch1, Hes1 and Hes5 proteins compared with the Con group ($P < 0.05$). These results indicated that there was increased upregulation of the Notch signaling pathway by ELF-EMF in rats after cerebral ischemia.

Discussion

Cerebral ischemia impairs the memory function in rats (Farokhi-Sisakht et al., 2020; Yuan et al., 2020). The acquisition training and probe trial we used were designed to test spatial learning and memory (Vorhees and Williams, 2006; He et al., 2019; Ryu et al., 2020). Our results indicate that ELF-EMF significantly improved the learning ability and memory of cerebral ischemic rats. In rats with hippocampal injury, ELF-EMF (50 Hz, 1 mT) significantly decreased the mean latency and distance traveled to find the hidden platform and increased the percentage of the entrance to the target quadrant in the probe trial. Consistent with our results, in a study on ischemic stroke patients, ELF-EMF (40 Hz, 7 mT) induced a significant improvement in cognition as measured by Mini-Mental State Examination (Cichoń et al., 2017). However, some studies reported that ELF-EMF (50 Hz, 0.1 mT) did not improve the memory in rats with Alzheimer's disease (Zhang et al., 2015) and that ELF-EMF (50 Hz, 8 mT) even impaired memory function in rats (Duan et al., 2014). We speculated that the effect might depend strongly on the exposure parameters, such as frequency and intensity (Mahdavi et al., 2014; Masoudian et al., 2015).

Studies *in vitro* showed that ELF-EMF induced sodium channel currents as well as the excitatory postsynaptic potentials of the rat hippocampal CA1 neurons (Zheng et al., 2017), promoted the differentiation of bone marrow mesenchymal stem cells into neurons (Bai et al., 2013; Kim et al., 2013) and promoted the neuronal differentiation and neurite outgrowth of embryonic NSCs (Ma et al., 2016). Some *in vivo* studies also showed beneficial results. ELF-EMF (60 Hz, 0.7 mT) considerably increased NSCs proliferation and migration and strengthen myelin repair in rats with white matter demyelination (Sherafat et al., 2012). Others reported that ELF-EMF increased the number of $BrdU^+$ and $NeuN^+$ cells in the dentate gyrus of adult mice with the hippocampal injury (Sakhaie et al., 2017). The $BrdU^+$ and $NeuN^+$ cells are the new proliferated NSCs that finally differentiate and develop into mature neurons. Our results showed that the number of $BrdU^+/NeuN^+$ cells was significantly higher in the Con group, demonstrating that cerebral ischemia could significantly promote endogenous NSCs to differentiate into neurons in the SGZ and repair damaged brain tissue, and the findings that are consistent with the literature (Arvidsson et al., 2002; Tobin et al., 2014). We found ELF-EMF further promoted the endogenous NSCs differentiation into neurons, thereby boosting the repair of brain tissue and possibly towards the recovery of brain function.

There has been increased interest in the effect of ELF-EMF on signaling pathways. Park et al. (2013) found that ELF-EMF (50 Hz, 1 mT) could activate the epidermal growth factor receptor pathway to promote the differentiation of human bone marrow mesenchymal stem cells into neurons. Others reported these effects could be generated by ELF-EMF (15 Hz, 1 mT) through the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway (Song et al., 2014). Treatment with ELF-EMF (60 Hz, 3 mT) has been reported to affect the collagen synthesis through the p38 mitogen activated protein kinase pathway and phosphatidylinositol 3-kinase pathway (Soda et al., 2008). Another study reported that ELF-EMF (50 Hz, 1 mT) inhibited the nuclear factor-kappaB signaling pathway to regulate the production of chemokine and the growth of glial cells and to inhibit the inflammatory process (Kim et al., 2017). Our study demonstrated that the ELF-EMF could upgrade the Notch signaling pathway. The proliferation and differentiation of endogenous NSCs were shown to be closely related to the Notch signaling pathway in cerebral ischemia (Wang et al., 2009; Qiao et al., 2020; Zhang et al., 2020). This has been linked to increased synaptic plasticity in the dentate gyrus (Cuccurazzu et al., 2010). These reports support our conclusion that the fundamental basis of the ELF-EMF intervention in cerebral ischemia is by regulating the Notch signaling pathway and that the associated increase in new born neurons in SGZ results in improved cognitive function.

There are still some limitations to our study that need to be considered. First, the ELF-EMF parameter setting was mainly based on the existing literature reports, it might not be the best setting for cerebral ischemia. Second, because of the restrictions of funding and time, no further experiments such as observing the proliferation and differentiation of NSCs in the condition of blocking the Notch signal pathway *in vivo* or *in vitro* were conducted. Medical practitioners seldom provide just a single treatment for the rehabilitation of patients. Therefore, before any clinical application is considered, the combination of the ELF-EMF with drugs, exercises or other treatments could play a part in an expansion of this project.

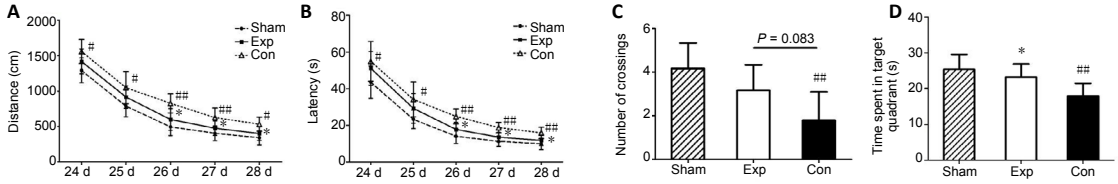


Figure 3 | **Effects of extremely low frequency electromagnetic fields on the cognitive and memory functions in rats with cerebral ischemia.**
(A, B) Swimming distance to escape water (A) and latency to reach the hidden platform (B) in the acquisition trials started on the 24th day after the surgery. (C, D) The times that rat crossed the former platform area in 60 seconds (C) and the time that rats stayed in the former platform quadrant (D) in the probe trial in Morris Water Maze on the 29th day after the surgery. Data are expressed as the mean ± SEM (n = 6). #P < 0.05, ##P < 0.01, *vs*. Sham group; *P < 0.05, *vs*. Con group (two-way repeated measure analysis of variance followed by the least significant difference *post hoc* tests). Con: Control; Exp: experiment; sham: sham-surgery

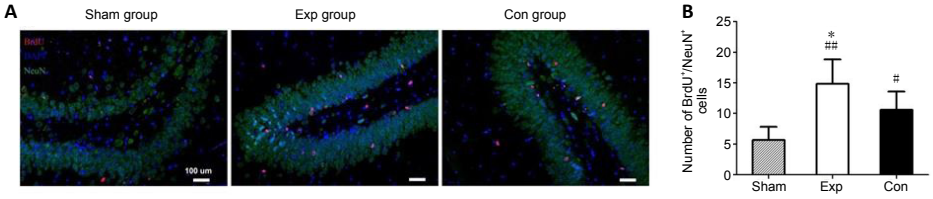


Figure 4 | **Extremely low frequency electromagnetic fields intervention increases the number of BrdU/NeuN double staining-positive cells in subgranular zone.**
(A) Representative images of BrdU/NeuN immunofluorescence. Red: BrdU, stained by Alexa Fluor 594; Green: NeuN, stained by Alexa Fluor 488. Original magnification: 400×. Scale bars: 100 μm. (B) Number of BrdU+/NeuN+ cells. The number of BrdU+/NeuN+ cells which indicates that the number of newborn neurons was significantly increased in the Exp group compared with the Con group. Data are expressed as the mean ± SEM (n = 6). #P < 0.05, ##P < 0.01, *vs*. Sham group; *P < 0.05, *vs*. Con group (one-way analysis of variance followed by the least significant difference *post hoc* tests). BrdU: 5′-Bromo-2′-deoxyuridine; Con: control; Exp: experiment; Sham: sham-surgery; NeuN: neuronal nuclei.

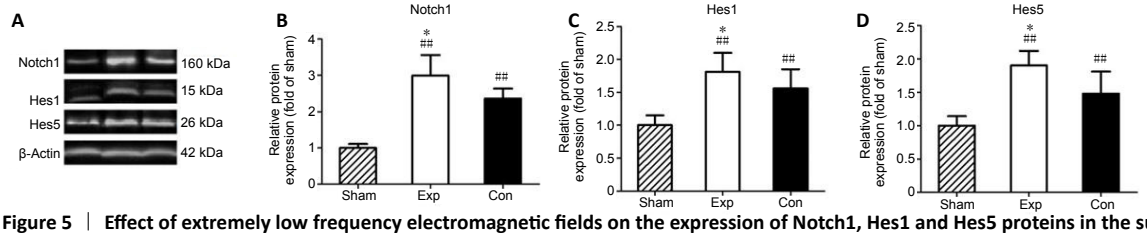


Figure 5 | **Effect of extremely low frequency electromagnetic fields on the expression of Notch1, Hes1 and Hes5 proteins in the subgranular zone.**
(A) Bands of western blot analysis from the subgranular zone. (B–D) Relative protein expression of Notch1, Hes1 and Hes5. Data are expressed as the mean ± SEM (n = 6). ##P < 0.01, *vs*. Sham group; *P < 0.05, *vs*. Con group (one-way analysis of variance followed by the least significant difference *post hoc* tests). Con: Control; Exp: experiment; Sham: sham-surgery.

Our findings have primarily provided a theoretical basis for the application of ELF-EMF in the treatment of cerebral ischemia but also suggest that it could treat other diseases in the central nervous system. Most importantly, this study has demonstrated that ELF-EMF has the potential to be a new therapeutic intervention in rehabilitation medicine.

Author contributions: *Data analysis and manuscript writing: QG; experiment performance and data analysis: GQ, YHY, QW, BWML, LYL and YJX; study design and manuscript writing: AL and CQH. All authors approved the final version of this paper.*
Conflicts of interest: *None declared.*
Financial support: *This work was supported by the National Natural Science Foundation of China, No. 81201513 (to QG). The funding source had no role in study conception and design, data analysis or interpretation, paper writing or deciding to submit this paper for publication.*
Institutional review board statement: *All experimental procedures described here were in accordance with the National Institute of Health (NIH) for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and approved by the Institutional Ethics Committee of Sichuan University (approval No. 2019255A) on March 5, 2019.*

Data sharing statement: *Datasets analyzed during the current study are available from the corresponding author on reasonable request.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open peer reviewers: *Hailong Song, University of Pennsylvania, USA; Syoichi Tashiro, Keio University School of Medicine, Japan.*
Additional files:
Additional file 1: *Open peer review reports 1 and 2.*
Additional file 2: *Original data of the experiment.*

References

Abdulla FA, Alsaadi S, Sadat-Ali M, Alkhamis F, Alkawaja H, Lo S (2019) Effects of pulsed low-frequency magnetic field therapy on pain intensity in patients with musculoskeletal chronic low back pain: study protocol for a randomised double-blind placebo-controlled trial. BMJ Open 9:e024650.

Akbarnejad Z, Esmaeilpour K, Shabani M, Asadi-Shekaari M, Saeedi Goraghani M, Ahmadi-Zeidabadi M (2018) Spatial memory recovery in Alzheimer's rat model by electromagnetic field exposure. Int J Neurosci 128:691-696.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 8:963-970.

Bai WF, Xu WC, Feng Y, Huang H, Li XP, Deng CY, Zhang MS (2013) Fifty-Hertz electromagnetic fields facilitate the induction of rat bone mesenchymal stromal cells to differentiate into functional neurons. Cytotherapy 15:961-970.

Capone F, Pellegrino G, Motolese F, Rossi M, Musumeci G, Di Lazzaro V (2020) Extremely low frequency magnetic fields do not affect LTP-like plasticity in healthy humans. Front Hum Neurosci 14:14.

Cichoń N, Bijak M, Miller E, Saluk J (2017) Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients. Bioelectromagnetics 38:386-396.

Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C (2010) Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol 226:173-182.

Duan Y, Wang Z, Zhang H, He Y, Fan R, Cheng Y, Sun G, Sun X (2014) Extremely low frequency electromagnetic field exposure causes cognitive impairment associated with alteration of the glutamate level, MAPK pathway activation and decreased CREB phosphorylation in mice hippocampus: reversal by procyanidins extracted from the lotus seedpod. Food Funct 5:2289-2297.

Ehnert S, Schröter S, Aspera-Werz RH, Eisler W, Falldorf K, Ronniger M, Nussler AK (2019) Translational insights into extremely low frequency pulsed electromagnetic fields (ELF-PEMFs) for bone regeneration after trauma and orthopedic surgery. J Clin Med 8:2028.

Farokhi-Sisakht F, Sadigh-Eteghad S, Mohaddes G, Ebrahimi-Kalan A, Karimi P, Farhoudi M (2020) Physical and cognitive training attenuate hippocampal ischemia-induced memory impairments in rat. Brain Res Bull 155:202-210.

Feychting M, Ahlbom A, Kheifets L (2005) EMF and health. Annu Rev Public Health 26:165-189.

Funk RH, Monsees T, Ozkucur N (2009) Electromagnetic effects- From cell biology to medicine. Prog Histochem Cytochem 43:177-264.

Gao Q, Yang YH, Li SS, He J, He CQ (2011) Changes in plasma calcitonin gene-related peptide and serum neuron specific enolase in rats with acute cerebral ischemia after low-frequency electrical stimulation with different waveforms and intensities. Neural Regen Res 6:2217-2221.

He HW, Zhang YL, Yu BQ, Ye G, You W, So KF (2019) Li X. Soluble Nogo receptor 1 fusion protein protects neural progenitor cells in rats with ischemic stroke. Neural Regen Res 14:1755-1764.

Kim HJ, Jung J, Park JH, Kim JH, Ko KN, Kim CW (2013) Extremely low-frequency electromagnetic fields induce neural differentiation in bone marrow derived mesenchymal stem cells. Exp Biol Med (Maywood) 238:923-931.

Kim SJ, Jang YW, Hyung KE, Lee DK, Hyun KH, Jeong SH, Min KH, Kang W, Jeong JH, Park SY, Hwang KW (2017) Extremely low-frequency electromagnetic field exposure enhances inflammatory response and inhibits effect of antioxidant in RAW 264.7 cells. Bioelectromagnetics 38:374-385.

Longa EZ, Weinstein PR, Carlson S, Cummins R (1989) Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20:84-91.

Ma Q, Chen C, Deng P, Zhu G, Lin M, Zhang L, Xu S, He M, Lu Y, Duan W, Pi H, Cao Z, Pei L, Li M, Liu C, Zhang Y, Zhong M, Zhou Z, Yu Z (2016) Extremely low-frequency electromagnetic fields promote in vitro neuronal differentiation and neurite outgrowth of embryonic neural stem cells via up-regulating TRPC1. PLoS One 11:e0150923.

Mahdavi SM, Sahraei H, Yaghmaei P, Tavakoli H (2014) Effects of electromagnetic radiation exposure on stress-related behaviors and stress hormones in male wistar rats. Biomol Ther (Seoul) 22:570-576.

Masoudian N, Riazi GH, Afrasiabi A, Modaresi SM, Dadras A, Rafiei S, Yazdankhah M, Lyaghi A, Jarah M, Ahmadian S, Seidkhani H (2015) Variations of glutamate concentration within synaptic cleft in the presence of electromagnetic fields: an artificial neural networks study. Neurochem Res 40:629-642.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11:47-60.

Park JE, Seo YK, Yoon HH, Kim CW, Park JK, Jeon S (2013) Electromagnetic fields induce neural differentiation of human bone marrow derived mesenchymal stem cells via ROS mediated EGFR activation. Neurochem Int 62:418-424.

Qiao J, Zhao J, Chang S, Sun Q, Liu N, Dong J, Chen Y, Yang D, Ye D, Liu X, Yu Y, Chen W, Zhu S, Wang G, Jia W, Xi J, Kang J (2020) MicroRNA-153 improves the neurogenesis of neural stem cells and enhances the cognitive ability of aged mice through the notch signaling pathway. Cell Death Differ 27:808-825.

Ross CL, Ang DC, Almeida-Porada G (2019) Targeting mesenchymal stromal cells/pericytes (MSCs) with pulsed electromagnetic field (PEMF) has the potential to treat rheumatoid arthritis. Front Immunol 10:266.

Ryu S, Jeon H, Kim HY, Koo S, Kim S (2020) Korean red ginseng promotes hippocampal neurogenesis in mice. Neural Regen Res 15:887-893.

Sakhaie MH, Soleimani M, Pourheydar B, Majd Z, Atefimanesh P, Asl SS, Mehdizadeh M (2017) Effects of extremely low-frequency electromagnetic fields on neurogenesis and cognitive behavior in an experimental model of hippocampal injury. Behav Neurol 2017:9194261.

Sherafat MA, Heibatollahi M, Mongabadi S, Moradi F, Javan M, Ahmadiani A (2012) Electromagnetic field stimulation potentiates endogenous myelin repair by recruiting subventricular neural stem cells in an experimental model of white matter demyelination. J Mol Neurosci 48:144-153.

Soda A, Ikehara T, Kinouchi Y, Yoshizaki K (2008) Effect of exposure to an extremely low frequency-electromagnetic field on the cellular collagen with respect to signaling pathways in osteoblast-like cells. J Med Invest 55:267-278.

Song M, Zhao D, Wei S, Liu C, Liu Y, Wang B, Zhao W, Yang K, Yang Y, Wu H (2014) The effect of electromagnetic fields on the proliferation and the osteogenic or adipogenic differentiation of mesenchymal stem cells modulated by dexamethasone. Bioelectromagnetics 35:479-490.

Tobin MK, Bonds JA, Minshall RD, Pelligrino DA, Testai FD, Lazarov O (2014) Neurogenesis and inflammation after ischemic stroke: what is known and where we go from here. J Cereb Blood Flow Metab 34:1573-1584.

Varró P, Szemerszky R, Bárdos G, Világi I (2009) Changes in synaptic efficacy and seizure susceptibility in rat brain slices following extremely low-frequency electromagnetic field exposure. Bioelectromagnetics 30:631-640.

Vázquez-García M, Elías-Viñas D, Reyes-Guerrero G, Domínguez-González A, Verdugo-Díaz L, Guevara-Guzmán R (2004) Exposure to extremely low-frequency electromagnetic fields improves social recognition in male rats. Physiol Behav 82:685-690.

Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1:848-858.

Wang L, Chopp M, Zhang RL, Zhang L, Letourneau Y, Feng YF, Jiang A, Morris DC, Zhang ZG (2009) The Notch pathway mediates expansion of a progenitor pool and neuronal differentiation in adult neural progenitor cells after stroke. Neuroscience 158:1356-1363.

Wang T, Yang L, Jiang J, Liu Y, Fan Z, Zhong C, He C (2019) Pulsed electromagnetic fields: promising treatment for osteoporosis. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 30:267-276.

Wang ZK, Fan QW, Wang DH, Zhong LH, He CQ, Gao Q (2014) Design and application of uniform magnetic field generator based on Helmholtz coils. Yiliao Weisheng Zhuangbei 35:1-3,10.

Yuan Y, Shan X, Men W, Zhai H, Qiao X, Geng L, Li C (2020) The effect of crocin on memory, hippocampal acetylcholine level, and apoptosis in a rat model of cerebral ischemia. Biomed Pharmacother 130:110543.

Zhang K, Guo L, Zhang J, Rui G, An G, Zhou Y, Lin J, Xing J, Zhao T, Ding G (2020) tDCS accelerates the rehabilitation of MCAO-induced motor function deficits via neurogenesis modulated by the Notch1 signaling pathway. Neurorehabil Neural Repair 34:640-651.

Zhang Y, Liu X, Zhang J, Li N (2015) Short-term effects of extremely low frequency electromagnetic fields exposure on Alzheimer's disease in rats. Int J Radiat Biol 91:28-34.

Zheng Y, Ma W, Dong L, Dou JR, Gao Y, Xue J (2017) Influence of the on-line ELF-EMF stimulation on the electrophysiological properties of the rat hippocampal CA1 neurons in vitro. Rev Sci Instrum 88:105106.

P-Reviewers: Song H, Tashiro S; C-Editor: Zhao M; S-Editors: Yu J, Li CH; L-Editors: Yu J, Song CP; T-Editor: Jia Y