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Alleviation of Ferric Chloride-induced Seizures and Retarded Behaviour in Epileptic Rats by Cortical Electrical Stimulation Treatment

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OBJECTIVE: To study the effects of low-frequency cortical electrical stimulation (CES) on seizures and behaviour in a rat model of epilepsy induced by ferric chloride (FeCl3). METHODS: Rats were randomly assigned into four groups (n = 8 per group): normal healthy rats; saline-treated control rats; FeCl3-induced epileptic rats; CES-treated FeCl3-induced epileptic rats. Behavioural tests, analysis of the levels of brain-derived neurotrophic factor (BDNF) protein in brain tissue, and ultrastructural studies using transmission electron microscopy (TEM) were undertaken.

RESULTS: CES significantly decreased the number and grade of seizures, and improved rat behaviour, compared with untreated epileptic rats. CES reduced levels of BDNF protein in the forebrain and increased levels of BDNF protein in the hippocampus compared with untreated epileptic rats. TEM showed less damage and ultrastructural changes in the neurons of CES-treated epileptic rats. CONCLUSIONS: CES inhibited seizures in FeCl3-induced epileptic rats and improved their behaviour. These effects might be mediated by altering BDNF protein levels in the brain.

KEY WORDS: Cortical electrical stimulation; Epilepsy; Rat model; Behaviour; Brain-derived neurotrophic factor

Introduction

Epilepsy is a chronic neurological disorder invoked by the excessive abnormal discharge of brain neurons and is characterized by recurrent seizures. Its global prevalence is around 0.5 – 1%.1 A variety of factors such as brain trauma, cerebral haemorrhage and brain injury can lead to the occurrence of seizures. Although the outcome of pharmacological treatment is relatively good, seizures are unsatisfactorily controlled with antiepileptic drugs in around 30% of patients.2–4 For these patients, surgery can be a good option; however, identifiable epileptogenic foci are hard to find in many patients with refractory epilepsy, therefore alternative therapies are needed.5–7 The development of electrical stimulation therapy...
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offers hope of an effective treatment for patients with refractory epilepsy. Previous experiments have shown that electrical stimulation therapy is effective for controlling epilepsy and other functional brain diseases. Other studies have also confirmed that low-frequency electrical stimulation can inhibit epileptic seizures in animals subjected to electrical amygdala kindling (i.e. subthreshold electrical stimulation of the amygdala). Studies that have investigated brain slices have demonstrated that electrical stimulation – in particular low-frequency electrical stimulation – can inhibit epileptic discharges.

Although cortical electrical stimulation (CES) can effectively control seizures, its mechanism of action (and whether it has a protective effect on nerves damaged by epileptic seizures) remains unclear. Brain-derived neurotrophic factor (BDNF) is a small dimer protein. As a member of the neurotrophic factor family, BDNF, together with its receptor (the BDNF/NT-3 growth factors receptor [TrkB]), is widely distributed in the mammalian brain, where it plays a vital role in the functional stability and development of the central nervous system. BDNF regulates neural regeneration, synaptic transmission, synaptic plasticity, neurogenesis and mossy fibre sprouting. Several studies have found that appropriate electrical stimulations such as transient direct current stimulation (3V-DC, 100 µs, 20 Hz for 1 h), 22 epileptiform discharges and chronic electroconvulsive therapy can alter BDNF levels in the brain.

In addition to its unpredictable nature, the foremost complication of epilepsy is the recurrence-induced damage that adversely affects cognitive function and results in behavioural changes that present as very different clinical manifestations. Many patients present with no obvious differences compared with healthy people but, compared with other people of the same age and educational level, their cognitive abilities are impaired. Patients with different neuropathological abnormalities often have different cognitive impairments. For example, patients with temporal lobe epilepsy appear to have a decline in memory function, whereas patients with focal epilepsy on the right hemisphere are more likely to have linguistic dysfunction. Moreover, some special epileptic syndromes can cause specific cognitive and behavioural abnormalities. The damage caused by epileptic seizures can result in social and psychological abnormalities in the patient. Some experts believe that certain antiepileptic drugs, such as neurotrophic factors, can promote the recovery of this neurological damage. Most studies have shown, however, that traditional antiepileptic drugs often cause such damage. Most of the previous studies on electrical stimulation treatment of cognitive impairment have focused on depression; few have examined epilepsy-induced impairment of cognitive function. The effectiveness of CES on the inhibition of epileptic seizures has been demonstrated previously. Thus, the present study was designed to evaluate the effectiveness of CES in reducing epileptic seizures, and the potential mechanism underlying this approach, in a rat model of epilepsy.

Materials and methods

MATERIALS

All stainless-steel electrodes and wires used in the electrophysiological surgery were tissue compatible and were obtained from Plastics One (Roanoke, VA, USA). Chemical reagents used in the Western blot analysis were analytical grade and were obtained...
from Abcam® (Cambridge, MA, USA). Narishige ST–7 stereotactic instruments from Tritech Research Inc. (Los Angeles, CA, USA) and a Nihon Kohden JE-921A electroencephalographic (EEG) system (Nihon Kohden Corporation, Tokyo, Japan) were also used.

EXPERIMENTAL ANIMALS
Thirty-two male Sprague–Dawley rats weighing 250 – 350 g were obtained from the Experimental Animal Centre of the Fourth Military Medical University (FMMU; ShanXi, Xi’an, China). The rats were housed in pairs, in standard 8 × 12 × 5 cm laboratory cages, at a mean ± SD temperature of 26 ± 4 °C, under a 12-h light/12-h dark cycle, with free access to water and food. The use of the animals was approved by the FMMU Animal Care and Use Committee. Rats were randomly assigned to four groups using a computer-generated randomization schedule. The eight rats in the normal healthy group received no treatment; the remaining 24 rats underwent open-skull surgery. Of these, the eight rats in the control group were injected with saline into the right sensorimotor cortex, the eight rats in the epilepsy model group were injected with 200 mM ferric chloride (FeCl₃) into the right sensorimotor cortex, and the eight rats in the electrical stimulation (CES) treatment group were injected with 200 mM FeCl₃ (as above) and also treated with stereotactic electrical (1 Hz, 0.1 mA) stimulation every 0.3 ms for 2 h/day, for 7 days, using electrodes implanted during surgery; CES treatment started 1 h after FeCl₃ injection.

SURGICAL PROCEDURES AND EEG RECORDING
Surgical procedures were carried out in all rats excluding the group of normal healthy rats. Surgery was performed in all rats under mild anaesthesia induced by an intraperitoneal injection of 50 mg/kg pentobarbital via a stereotactic device. The rat scalp was incised along the midline and the skull was exposed after stripping off the soft tissue. The skull surface was sterilized with 3% hydrogen peroxide. After the hydrogen peroxide had dried, seven burr holes (each with a diameter of 0.5 mm) were drilled into the skull: two, named F1 and F2, were located 2.0 mm before the anterior fontanelle and 2.0 mm next to the midline, respectively; two, named P1 and P2, were located 2.0 mm behind the anterior fontanelle and 2.0 mm next to the midline, respectively; two, named O1 and O2, were located 6.0 mm behind the anterior fontanelle and 2.0 mm next to the midline, respectively; one, named R, was located on the nasal bone for the reference electrode. Odd numbers indicated the holes on the left of the midline; even numbers indicated the holes on the right of the midline. A 5-µl dose of saline or 200 mM FeCl₃ was microinjected slowly via hole P2 to the spot 2.5 mm inside the skull over a 5-min time period. All the holes were fitted with screw electrodes of length equal to the thickness of the skull (about 2 mm) so that they rested on the surface of the brain, and they were insulated with dental cement and fixed in the skull surface. After the scalps were sutured, rats were housed in a single cage.

At 3 h after surgery, rats had completely regained consciousness and continuous video–EEG was recorded using Nihon Kohden 9200 Studio software (QP-219BK; Nihon Kohden). Signals < 1 Hz or > 45 Hz were filtered by the software. In the EEG observation period, nonictal discharges were distinguished from ictal discharges based on waveform morphology, frequency and the associated behavioural alterations as described previously. Each animal was subjected to 5 h of video–EEG monitoring.
from 3 h after surgery. The grade of epileptogenesis was classified using the modified Racine scale. Spontaneous seizure rates were recorded and, based on the grades of seizure observed (Table 1), a chart was plotted to demonstrate the development of epileptiform activity.

**BEHAVIOURAL TESTS**

**Open-field behaviour tests**

Open-field tests were performed in all rats 1 week after surgery in a 106 cm × 70 cm × 70 cm unroofed rectangular wooden box. Each rat was placed in the centre of the box. Its exploratory behaviour was recorded for 15 min and quantified as the distance it moved horizontally in cm, and the number of times it stood on its hind limbs (rearing). The number of faecal boli expelled during the 15-min period was counted as the defaecation index. Before each test, the field was cleaned thoroughly with 0.1% acetic acid solution.

**Morris water maze test**

Morris water maze tests were conducted 8 days after surgery in all rats, in a circular tank (1.68 m in diameter and 0.5 m in depth) that was filled with water and divided into four quadrants with extra-maze cues of different shapes, sizes and colours in the above-water part. Water temperature was controlled at 22 – 25 °C. A black circular platform with a 15-cm diameter was positioned 2.0 cm under the surface of the water at the centre of one quadrant, so that the rat could stand and escape by swimming to the platform. After surgery, and 1 day before the test, each rat was placed once in the water maze for 120 s to adapt it to the environment. During the test, each rat was placed inside the water tank facing the tank wall at one of four randomly selected entry points, and its swimming ability was evaluated by the latency of the rat to reach the visible platform. If the animal
failed to reach the platform within 120 s, it was guided to the platform and allowed to remain on the platform for 20 s. Each rat was tested once a day for 4 consecutive days, starting from the day after surgery. On day 5, the platform was removed and the length of time that each rat spent in the original platform quadrant was recorded. The Morris water maze test was videoed by a web camera, mounted on the ceiling above the tank. The test was performed from 11:00 h to 14:00 h, to exclude variations resulting from the circadian rhythm.

**WESTERN BLOT ANALYSIS**

Seven days after injection of FeCl₃, four rats from each group were decapitated and their brain tissue was rapidly removed and placed on ice. The forebrain and hippocampus on the injection side were dissected and snap frozen in liquid nitrogen. Tissues were homogenized with 1 ml of precooled cell lysis buffer (0.5% Triton X-100, 1% sodium dodecyl sulphate [SDS], 300 mmol/l sucrose, 5 mmol/l Tris–HCl, 2 mmol/l ethylenediaminetetra-acetic acid, 1% protease inhibitor cocktail, 1 mmol/l sodium vanadate, 0.5 mmol/l phenylmethanesulphonylfluoride; pH 7.4) in a grinding tube for 20 min and then put on ice for 30 min. The homogenates were then centrifuged at 17,530 g for 10 min at 4°C and the supernatants were transferred into precooled 1.5 ml tubes. The protein concentration was measured using a bicinochoninic acid assay kit (Pierce Protein Research Products, Thermo Scientific, Rockford, IL, USA) according to the manufacturer’s instructions, and bovine serum albumin was used as the standard. Protein samples (20 µg) were subjected to 15% SDS–polyacrylamide gel electrophoresis and transferred electrophoretically onto nitrocellulose membranes. The membrane was blocked for 1 h in 5% skimmed milk in Tris-buffered saline Tween-20 (TBST; 30 mM Tris, 150 mM saline, 0.5% [vol/vol] Tween-20 [pH 7.4]). The blots were incubated with rabbit polyclonal anti-BDNF antibody (ab6201; 1 : 2000 dilution; Abcam®, Cambridge, MA, USA) or rabbit polyclonal anti-β-actin antibody (1 : 5000 dilution; Sigma-Aldrich, St Louis, MO, USA) overnight at 4°C. The membranes were washed three times for 10 min in TBST and incubated with the appropriate horseradish peroxidase-labelled goat antirabbit secondary antibodies (1 : 2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. Membranes were washed a final three times for 10 min in TBST and the signal was visualized using the ECL Plus™ kit (Amersham Biosciences, Little Chalfont, UK).

**TRANSMISSION ELECTRON MICROSCOPY**

Four rats from each group were anaesthetized by an intraperitoneal injection of 1% pentobarbital at 50 mg/kg. After perfusing with 4% paraformaldehyde, samples of the right forebrain and hippocampus (each measuring 1 × 1 × 1 mm) were rapidly removed by reference to their EEG findings, and immediately fixed in 30 ml/l glutaraldehyde for 120 min. After washing with 0.01 mM phosphate-buffered saline, pH 7.4, at 4°C for 120 min or overnight, the specimen was further fixed in 10 g/l osmium tetroxide for 60 min, rinsed with double-distilled or deionized water, and dehydrated in a graded ethanol series. The tissue samples were embedded in Epon 812 epoxy resin, sectioned with an LKB microtome JEM-2000EX (Nihon Kohden Corporation) to a thickness of approximately 50 nm, stained with uranyl acetate and lead citrate for 20 min, and examined with a JEOL JEM2 2000EX transmission electron microscope (JEOL Ltd, Beijing, China).
STATISTICAL ANALYSES
Data were analysed using the SPSS® software package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Data were expressed as mean ± SE. A one-way analysis of variance with Dunnett’s t-test was used to compare differences between the study groups as appropriate. A P-value of < 0.05 was considered to be statistically significant.

Results
SEIZURE ELECTROPHYSIOLOGY
No animals died during the surgery or EEG recording periods. Examples of EEG readings from the rat epilepsy model and the CES treatment model are shown in Fig. 1. No seizures were recorded in the normal healthy group, but spontaneous seizures were recorded in all three groups that underwent surgery and were graded according to the criteria shown in Table 1. Only grade I or II seizures were observed in the FeCl₃-induced epileptic rats undergoing CES, whereas grade III and IV seizures were observed in the FeCl₃-induced epileptic rats (Fig. 2). The mean ± SE number of seizures observed in a 5-h EEG recording period in the CES-treated FeCl₃-induced epileptic rats (12.13 ± 1.25) was higher than in the saline-treated control group (0.37 ± 8.25; P < 0.05 for all comparisons; Table 2). CES treatment of FeCl₃-induced epileptic rats significantly reduced the defaecation index, decreased rearing activity and increased the ambulatory activity compared with rats in the FeCl₃-induced epileptic group (P < 0.05 for all comparisons).

Morris water maze test
In the Morris water maze tests, at day 4, CES-treated FeCl₃-induced epileptic rats exhibited similar latency to rats in the normal healthy group and saline-treated control groups. Rats in all three of these groups had significantly shorter latency in finding the platform compared with FeCl₃-induced epileptic rats (P < 0.05; Fig. 5). On day 5, the latency of the CES-treated FeCl₃-induced epileptic rats in the quadrant where the platform had previously been placed was significantly longer (mean ± SE, 33.47 ± 5.20 s; P < 0.05) compared with the FeCl₃-induced epileptic rats (mean ± SE, 21.57 ± 4.83 s), and slightly longer than in the saline-treated control group (mean ± SE, 26.10 ± 7.51 s) (Fig. 6).

WESTERN BLOT ANALYSIS
As shown in Fig. 7, BDNF protein levels, examined by Western blot analysis, were decreased in the rat forebrain and increased in the hippocampus in CES-treated FeCl₃-induced epileptic rats, compared with FeCl₃-induced epileptic rats.

TRANSMISSION ELECTRON MICROSCOPY OBSERVATIONS
The results of the transmission electron microscopy analysis of rats from the four groups showed clear differences in the ultrastructural features (Fig. 8). In the forebrain cortical cells of the normal and saline-injected control rats, subcellular structures were normal and there were no
large extents of vacuolation or nuclear budding (Figs 8A and 8B). In the forebrain cortical cells of epileptic rats, membranous structures were dissolved and destroyed, and large amounts of vacuolation and some apoptotic bodies were observed (Figs 8C1 and 8C2). In the forebrain cortical cells of CES-treated epileptic rats, the cellular degeneration caused by epilepsy was effectively inhibited. Subcellular structures were more normal, showing homogeneous cytoplasm with abundant mitochondria and

FIGURE 1: (A) An electroencephalograph (EEG) recording from six epidural stainless steel screw electrodes showing seizures induced in the rat model 3 h after injection with 5 µl ferric chloride (FeCl₃) solution (200 mM) into the right sensorimotor cortex to induce epilepsy. (B) An EEG recording showing seizures in a FeCl₃-induced epileptic rat undergoing cortical electrical stimulation (1 Hz, 0.1 mA)
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FIGURE 2: Development and progression of seizures in ferric chloride (FeCl₃)-induced epileptic rats (model), cortical electrical stimulation (CES)-treated epileptic rats and saline-treated control rats (control). CES treatment reduced the occurrence and progression of grade III/IV seizures (none exhibited grade III/IV seizures) compared with FeCl₃-induced rats; the saline-treated control group had fewer seizures compared with the other two groups. Data are mean ± SE; n = 8 animals per group. *P < 0.05, ***P < 0.001 CES-treated versus FeCl₃-induced rats, one-way analysis of variance with Dunnett’s t-test

FIGURE 3: Number of seizures per h in ferric chloride (FeCl₃)-induced epileptic rats (model) and cortical electrical stimulation (CES)-treated FeCl₃-induced epileptic rats during a 5-h recording period that started 3 h after the rats had completely recovered from the anaesthetic used during surgery. Data are mean ± SE; n = 8 animals per group. *P < 0.05 compared with FeCl₃-induced epileptic rats, one-way analysis of variance with Dunnett’s t-test
endoplasmic reticulum, and dispersed chromatin without nuclear budding. Only sparse vacuolation was found in the Golgi apparatus (Fig. 8D). Similar to what was found in the forebrain cortical cells, subcellular structures of the hippocampal cells in the normal and saline-injected control rats were normal (Figs 8E and 8F), whereas numerous vacuolations, lysosomes, swollen mitochondria and multivesicular bodies were found in the hippocampal cells of epileptic rats (Figs 8G1 and 8G2). Some cells even exhibited hyperelectron-dense cytoplasm and condensed chromatin in deformed nuclei; intact medullary sheath and distinct synaptic linkages were found. In contrast, in the CES-treated epileptic rats, hippocampal cells were relatively normal and vacuolations in the Golgi apparatus were removed (Fig. 8H).

**Discussion**

The inhibitory effects of electrical stimulation on epileptic seizures in rats have been confirmed.\(^\text{10,45}\) In one clinical study, electrical stimulation therapy for status epilepticus also resulted in a reduction in the incidence of epileptic seizures.\(^\text{46}\) One of the most serious concerns regarding epilepsy is its unpredictability: the effect that neurological damage caused by constant seizures (as well as the long-term use of antiepileptic medicines) can have on a patient’s cognitive and emotional function, can lead to great suffering for the patient and seriously impact their quality of life.\(^\text{27 – 29}\)

At present, most medicines for controlling epileptic seizures only play an inhibitory role, while there is no cure for the damage caused by seizures; in addition, some antiepileptic medications can cause

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**FIGURE 4:** Total number of seizures in ferric chloride (FeCl\(_3\))-induced epileptic rats (model) and cortical electrical stimulation (CES)-treated epileptic FeCl\(_3\)-induced rats during a 5-h electroencephalographic (EEG) recording period. There was a significant reduction in the total number of seizures in the CES group, compared with the model group. Data are mean ± SE; \(n = 8\) animals per group. **\(P < 0.01\)** compared with FeCl\(_3\)-induced epileptic rats, one-way analysis of variance with Dunnett’s \(t\)-test
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cognitive dysfunction in patients. CES treatment has brought considerable hope to people who have epileptic foci located in important functional areas and who cannot undergo resection or amputation surgery. Little research has been conducted to determine whether CES is capable of inhibiting or reversing the damage to patients’ cognitive and emotional functions. The present study was designed to evaluate this in an animal model of epilepsy, to see whether seizure-induced damage could be inhibited and what the possible mechanism might be. The low-frequency electrical stimulation of rats with focal epilepsy, such as the model induced by FeCl₃, can suppress epileptiform discharges. In the present study, continuous treatment of epileptic rats with 1 Hz electrical stimulation for 2 h effectively constrained epileptiform discharges and prevented high-grade seizures in FeCl₃-induced epileptic rats. Subsequent behavioural experiments using the Morris water maze test showed poor memory skills in the FeCl₃-induced epileptic rats compared with the saline-treated control rats. In the open-field experiments, the rearing activity and defaecation indices of the FeCl₃-induced epileptic rats were higher than those of the saline-treated control rats, and the ambulatory distances achieved by the epileptic rats was significantly reduced compared with the saline-treated control rats, indicating that the memory and emotional functions of the epileptic rats were damaged. In contrast, the epileptic rats treated with 1 Hz CES showed better behaviour in both the Morris water maze test and the open-field experiments compared with the epileptic model rats. Thus, it could be concluded that low-frequency electrical stimulation not only inhibited epileptiform discharges in epileptic rats, but also alleviated the damage to cognitive and

### Table 2: Effect of cortical electrical stimulation (CES) treatment on open-field test parameters in ferric chloride (FeCl₃)-induced epileptic rats compared with normal healthy control rats, saline-injected control rats and untreated FeCl₃-induced epileptic rats

<table>
<thead>
<tr>
<th>Behavioural measure</th>
<th>Normal healthy control rats (n = 8)</th>
<th>Saline-injected control rats (n = 8)</th>
<th>FeCl₃-induced epileptic rats (n = 8)</th>
<th>CES-treated epileptic rats (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambulatory distance, cm</td>
<td>8408.23 ± 609.63*</td>
<td>8600.84 ± 577.48*</td>
<td>4779.22 ± 1271.82</td>
<td>7763.27 ± 911.84*</td>
</tr>
<tr>
<td>Number of rearings (standing on hind limbs)</td>
<td>19.63 ± 6.84*</td>
<td>22.50 ± 4.96*</td>
<td>31.00 ± 6.55</td>
<td>22.25 ± 4.74*</td>
</tr>
<tr>
<td>Defaecation index</td>
<td>2.25 ± 1.67*</td>
<td>2.75 ± 1.28*</td>
<td>4.62 ± 2.20*</td>
<td>2.75 ± 1.28*</td>
</tr>
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Data presented as mean ± SE (n = 8 animals per group). *P < 0.05 compared with FeCl₃-induced epileptic rats; one-way analysis of variance with Dunnett’s test.
FIGURE 5: Latency period in the time taken to find a platform in normal untreated rats (normal), saline-treated control rats (control), ferric chloride (FeCl₃)-induced epileptic rats (model) and cortical electrical stimulation (CES)-treated FeCl₃-induced epileptic rats. The latency period in the time taken to find a platform decreased in all four groups as they repeated the task over a period of 4 days following surgery. The latency period on day 4 was significantly higher in the FeCl₃-induced epileptic rats, compared with all other groups. Data are mean ± SE; n = 8 animals per group; *P < 0.05 compared with FeCl₃-induced epileptic rats, one-way analysis of variance with Dunnett’s t-test.

FIGURE 6: Length of time spent on day 5 following surgery in the quadrant where the platform had previously been placed in normal untreated rats (normal), saline-treated rats (control), ferric chloride (FeCl₃)-induced epileptic rats (model) and cortical electrical stimulation (CES)-treated FeCl₃-induced epileptic rats. Data are mean ± SE; n = 8 animals per group; *P < 0.05 compared with saline-treated control rats, one-way analysis of variance with Dunnett’s t-test.
emotional function caused by the epileptic seizures induced by FeCl₃ injection.

Considerable research on the mechanisms involved in the production of epileptic seizures has highlighted the importance of neurotransmitters, ion channels and apoptosis.⁴⁸ - ⁵⁰ The reasons why BDNF protein was evaluated in the present study were based primarily on the following: (i) a correlation between epilepsy and BDNF levels has been found in previous experiments; (ii) increased BDNF protein can promote the development of epilepsy; (iii) epileptic seizures can also further enhance BDNF levels in brain tissue; and (iv) BDNF, as an important brain neurotrophic factor, is closely related to the development of learning, memory and emotional functions.⁵¹ - ⁵⁴ Western blot analysis in the present study demonstrated that the BDNF level in the forebrain tissue of the FeCl₃-induced epileptic rats was higher than that in the saline-treated control rats. After CES treatment, the BDNF protein level in the FeCl₃-induced epileptic rat forebrain tissue was reduced to a level similar to that in the saline-treated control rats, suggesting that the CES-induced decrease in BDNF protein might be beneficial for controlling epilepsy. In contrast, the BDNF level in the hippocampal tissues of the FeCl₃-induced epileptic rats was evidently lower than that in the saline-treated control rats while, after CES treatment, the BDNF level in the hippocampal tissue increased compared with the FeCl₃-induced epileptic rats.

The part of the limbic system located in the hippocampus is closely related to learning, memory and emotional functions. Missed or lost hippocampal neurons have been found

![Western blot analysis of brain-derived neurotrophic factor (BDNF) in the forebrain and hippocampal regions of the four rat groups (n = 8 animals per group): normal untreated rats (normal), saline-treated rats (control), ferric chloride (FeCl₃)-induced epileptic rats (model) and cortical electrical stimulation (CES)-treated FeCl₃-induced epileptic rats.](image)
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FIGURE 8: Transmission electron micrographs showing the neuroprotective effect of cortical electrical stimulation (CES) treatment of epileptic rats: (A) forebrain of a normal rat; (B) forebrain of a saline-treated control rat; (C1 and C2) forebrain of a ferric chloride (FeCl₃)-induced epileptic rat; (D) forebrain of a CES-treated epileptic rat; (E) hippocampus of a normal rat; (F) hippocampus of a saline-treated control rat; (G1 and G2) hippocampus of a FeCl₃-induced epileptic rat; (H) hippocampus of a CES-treated epileptic rat. CES effectively inhibited the cellular alterations associated with epilepsy. Increased cytoplasmic vacuolation, hyperelectron-dense cytoplasm and chromatin condensation were evident in the untreated FeCl₃-induced epileptic rats (G, Golgi apparatus; V, vacuolation; Ms, medullary sheath; Ab, apoptotic body; H, hyperelectron dense matter; S, synapsis).
in many epileptic rats and epileptic patients. Studies have also suggested that the occurrence and development of epilepsy is correlated with neurological deficits. BDNF can protect neurons, and promote neuronal repair and regeneration. The present study demonstrated that CES appeared to increase BDNF protein levels in the hippocampus; this suggests that CES, by regulating BDNF levels, might be able to repair the damage to learning, memory and emotional function caused by epilepsy.

Changes in neuronal degeneration have been found in epileptic models, such as epilepsy induced by kainic acid, and in neurodegenerative diseases. Consistent with these previous findings, cell necrosis and apoptosis were observed in the neuronal cells of FeCl₃-induced epileptic rats in the present study. Few studies on the potential protective effect of CES on neurons have been performed. The CES-treated FeCl₃-induced epileptic rats in the present study showed no significant differences in their cytoplasm, organelles and nuclei compared with the saline-treated control rats and the normal rats, as determined by transmission electron microscopy. Thus, a protective function of CES on neurons was shown by observations of cell ultrastructure, providing additional evidence of the beneficial effects of CES on epilepsy.

Before the advent of electrical stimulation therapy, patients whose epileptic foci were located in important functional areas could not receive surgical treatment. Although multiple subpial transection could partly inhibit epileptic seizures, it was unable to control them completely. The development of electrical stimulation treatment has brought new hope for effective epilepsy treatment for these types of patients. Without causing functional deficits when suppressing epileptic seizures, electrical stimulation could be used to personalize treatment by adjusting the stimulation parameters based on individual patient’s clinical status and seizure type. It was demonstrated in the present study that low-frequency electrical stimulation not only suppressed epileptic discharges, but it also helped to protect the cognitive and emotional functions of the rats in this animal model of epilepsy. Further research is required to determine how to select the appropriate stimulation parameters and sites, and how to use CES to treat epilepsy and its neurological complications.

Conflicts of interest
The authors had no conflicts of interest to declare in relation to this article.

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