

# Inhibitory effects of noninvasive pulse transcranial magnetic stimulation on cerebral cortex epileptiform discharges in epileptic rat models

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Received: 2003-02-26 Accepted: 2003-07-20 (04/SH)

Hu QS, Chen JF, Tang JQ. Inhibitory effects of noninvasive pulse transcranial magnetic stimulation on cerebral cortex epileptiform discharges in epileptic rat models. *Zhongguo Linchuang Kangfu* 2003;7(25):3468-9

## Abstract

**AIM:** To observe the effect of pulse transcranial magnetic stimulation (PTMS) on the cerebral cortex epileptiform discharges (CCED) induced by penicillin sodium in rats and to investigate its possible mechanisms.

**METHODS:** Epileptic rat models were established in 48 Wistar rats by penicillin, in which the frequency and amplitude of the epileptiform discharges were used as indexes to assess the effects of PTMS of the unilateral cerebral cortex and cerebellar vermis on CCED.

**RESULTS:** After PTMS of the unilateral cerebral cortex, CCED frequency was significantly decreased by  $(36 \pm 10)\%$  ( $t = 3.873$ ,  $P < 0.01$ ), with the amplitude decreased by  $(21 \pm 8)\%$  ( $t = 2.984$ ,  $P < 0.05$ ). The lasting time of inhibitory effect produced by PTMS of the cerebellar vermis was longer than that generated by stimulation of the unilateral cerebral cortex. Thirty minutes after the termination of PTMS, the CCED frequency decreased by  $(63 \pm 7)\%$  ( $t = -3.497$ ,  $P < 0.001$ ). The inhibitory effect produced by PTMS were partially reversed by naloxone injected into the lateral ventricles.

**CONCLUSION:** PTMS of the cerebral cortex and cerebellar vermis may have significant inhibitory effects on CCED.

## INTRODUCTION

Approximately 25% of the epileptic patients do not respond to drugs or suffer unacceptable side effects to result in discontinuation of medications. As epilepsy is associated with the imbalance between the excitation and inhibition of the central nervous system at the cortical level, and external direct current fields may modulate and suppress low-calcium activity by directly polarizing CA1 pyramidal cells<sup>[1]</sup>. Transcranial magnetic stimulation (TMS) provides a noninvasive evaluation of separate excitatory and inhibitory functions of the cerebral cortex. In addition, repetitive transcranial magnetic stimulation (rTMS) can modulate the excitability of cortical networks<sup>[2]</sup>. Electrical stimulation of the cerebellar cortex can inhibit paleocerebellum refractory epilepsy, and a magnet outside the cerebral dura mater of the cerebral cortex can also inhibit epileptic seizures; noninvasive impulse magnetic stimulation of the unilateral cerebellar cortex or two magnets on the cephalic and caudal of the rat head respectively to generate constant magnetic field has proved to significantly inhibit cerebral cortex epileptiform discharges (CCED) in epileptic rat model induced by penicillin. It has been found that low-frequency (0.5 Hz) rTMS effectively prevented the development or status epileptics of pentylenetetrazol-induced seizures. TMS prolonged cortical silent period in patients with untreated idiopathic generalized epilepsy<sup>[3, 4]</sup>. In this study, we observed the effects of pulse transcranial magnetic stimulation (PTMS) of unilateral cerebral cortex and cerebellar vermis on CCED in epileptic rat models induced by penicillin sodium and to investigate the possible mechanisms of these effects.

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## MATERIALS AND METHODS

### Materials

Forty-eight Wistar rats weighing 200 to 300 g were provided by the Experimental Animal Center, Medical College of Wuhan University. Penicillin sodium was manufactured by Huabei Pharmaceutical Co, and naloxone hydrochloride by Beijing Sihuan Pharmaceutical Co. HP-1 pulse magnetic stimulator was designed and assembled by our department, with the diameter of the coils being 28 mm.

### Methods

#### CCED rat model

The rats were anesthetized with 100 g/L ethylurethane (10 mL/kg, i. p.) and a tracheal cannulation was performed. A hole was drilled 3 mm posterior to the anterior fontanelle and 3 mm on the left to the sagittal suture to expose the recording area (2 mm × 2 mm) on the cerebral cortex. A piece of gelatin sponge immersed in penicillin sodium solution (200 U/mm<sup>3</sup>) was applied on the surface of the unilateral cerebral cortex to induce CCED. Once the epileptiform discharge frequency and amplitude were stabilized, the gelatin sponge was removed and warm saline tampon was used to cover the wound. Recording of the electric activities of the ampicerebellar cortex was performed using silver-ball electrode and LMS-2B two-channel physiological recorder, monitored at the same time with SR12 oscilloscope.

#### Noninvasive PTMS technique

The pulse magnetic stimulation generated by HP-1 impulse magnetic stimulator with the stimulation amplitude of 10 000 G (approximately 1 T), frequency of 10 Hz, plateau time of 0.5 ms was imposed at the site 0.2 to 0.3 cm from the left cerebral or cerebellar vermis for 30 min.

#### Intracerebroventricular injection naloxone

Naloxone (1 g/L, 20 μL) were slowly injected into the lateral ventricle within 2 min, and at the end of the experiment, 2 μL blue ink was injected to verify the position of the syringe.

Statistical analysis: The differences between the groups were analyzed with paired *t* test by SAS software, and all data were expressed as Mean ± SD.

## RESULTS

### Effects of PTMS on normal electrical activities in the unilateral cerebral cortex and cerebellar vermis (*n* = 5)

After PTMS of the unilateral cerebral cortex and cerebellar vermis for 30 min, the frequency and amplitude of electroencephalogram (EEG) in rat exhibited no significant changes.

### Epileptic rat model induced by penicillin (*n* = 6)

Two or three minutes after the application of the penicillin sodium solution-immersed gelatin sponge on the sensorimotor area of the cerebral cortex, paroxysmal CCED occurred in the unilateral or bilateral of cerebral cortex, with the frequency and amplitude increased with time and stabilized at about 30 min. When the gelatin sponge was removed 30 min later, the frequency of CCED was  $34 \pm 15$ /min with the amplitude of 0.2 to 2.0 mV. Most of the CCED were represented in sharp waves, spike waves and spike-slow waves or other

erwise, and steady CCED could last for 5 to 6 h (Figure 1).

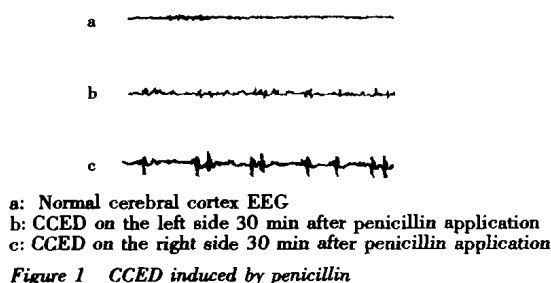


Figure 1 CCED induced by penicillin

#### Effects of PTMS of the unilateral cerebral cortex on CCED ( $n = 13$ )

5 min after the start of PTMS of the cerebral cortex with penicillin treatment, the frequency of CCED decreased by  $(36 \pm 10)\%$  ( $t = 3.873$ ,  $P < 0.01$ ) and the amplitude reduced by  $(21 \pm 8)\%$  ( $t = 2.984$ ,  $t < 0.05$ ). At 15 min, the frequency decreased by  $(12 \pm 5)\%$  but recovered to the control level at 25 min.

#### Effects of PTMS of the cerebellar vermis on CCED ( $n = 9$ )

At 5 min during PTMS of the cerebellar vermis, the frequency of CCED decreased by  $(15 \pm 9)\%$  ( $t = 2.649$ ,  $P < 0.05$ ) and the amplitude was also decreased significantly. At 30 min, the frequency had decreased by  $(32 \pm 9)\%$  ( $t = 2.649$ ,  $P < 0.05$ ) and further reduced by  $(63 \pm 7)\%$  ( $t = -3.497$ ,  $P < 0.01$ ) even at 30 min after the termination of PTMS, showing no tendency for recovery.

#### Effects of naloxone (1 g/L, i. c. v) + PTMS of unilateral cerebral cortex on CCED ( $n = 5$ )

After intracerebroventricular injection of naloxone and PTMS of the unilateral cerebral cortex for 5 min, the frequency of CCED only decreased by  $(2 \pm 4)\%$ , and had recovered the control level by 15 min.

#### Effects of naloxone (1 g/L, i. c. v) + PTMS of cerebellar vermis on CCED ( $n = 10$ )

After intracerebroventricular injection of naloxone and PTMS of the cerebellar vermis for 5 min, the frequency of CCED did not decrease but increased by  $(25 \pm 4)\%$  ( $t = 4.019$ ,  $P < 0.05$ ) instead and by  $(72 \pm 3)\%$  ( $t = 3.128$ ,  $P < 0.01$ ) at 30 min. Thirty minutes after the termination of PTMS, the frequency of CCED showed the tendency to decrease but was still  $(52 \pm 6)\%$  ( $t = 2.986$ ,  $P < 0.01$ ) higher than the control level (Figure 2).

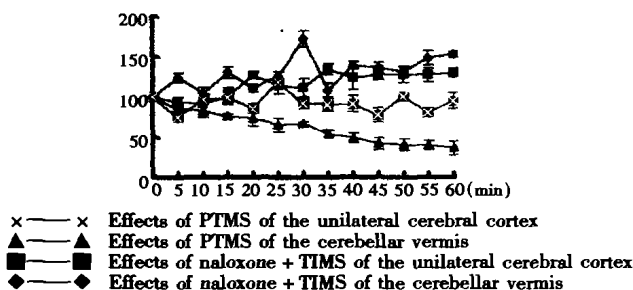


Figure 2 Effects of PTMS of the cerebral cortex and cerebellar vermis on CCED

## DISCUSSION

Magnetic stimulation is noninvasive due to the homogeneous distribution of the magnetic permeability in the tissues that enables the magnetic field to easily penetrate the skin and skull to reach the deep layers of the tissues. The coil 90 mm in diameter generates 1.4 T magnetic flux at 5 mm under the coils and 1.1 T at the axial core. The specific resistance of epicranium and skull are large and inversely proportional to the inductive current that can be generated, therefore magnetic stimulation provides better safety. It was reported that single-pulse TMS used to examine the motor conduction pathway did not elicit seizure in any of the 35 epileptic patients<sup>[5-7]</sup>.

TMS has significant inhibitory effects of seizure in both human and animal subjects<sup>[3, 4]</sup>, and chronic cerebellar stimulation can re-

duce refractory seizures by activating Purkinje cells. Our previous study demonstrated that PTMS of the cerebellum cortex or constant magnetic field generated by two magnets fixed on the cephalic and caudal of the rat head significantly inhibited CCED in penicillin-induced epileptic rat models.

Penicillin can induce epileptiform activity by blocking the  $\gamma$ -aminobutyric acid (GABA)-mediated inhibitory pathway<sup>[1]</sup>. In the event of epileptic seizure onset, the epileptic focus may produce a rapidly changing magnetic field, which can be significantly inhibited by the application of a constant external magnetic field outside the cerebral cortex. Acute exposure of electrically kindled rats to low-intensity magnetic fields (60 Hz) might reduce the electric discharge frequency, possibly through the mechanism of activating endogenous opiate-like substances. PTMS of both the cerebral cortex and cerebellar vermis produces inhibitory effects on CCED, with more obvious inhibitory effects by the stimulation of the latter. Intracerebroventricular injection of naloxone, an opiate receptor antagonist, may reverse the effects of PTMS of the cerebral cortex and cerebellar vermis, with longer time needed for reversing the effect by stimulation of the cerebellar vermis. These findings indicate that PTMS of the cerebral cortex and cerebellar vermis can inhibit the amplitude and frequency of penicillin-induced CCED and this effect may be related to endogenous opiate peptide in the central nervous system. As for the different inhibitory effects of PTMS of the cerebral cortex and cerebellar vermis, it needs further research.

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## 经颅脉冲磁刺激对大鼠癫痫模型痫样放电的抑制作用

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### 摘要

目的:观察无创经颅脉冲磁刺激(PTMS)对青霉素所致大鼠皮质痫样放电(CCED)的作用,进一步探讨PTMS作用于大鼠皮质及小脑蚓部后,对CCED作用的可能机制。

方法:选用Wistar大鼠48只,以CCED频率和幅度为指标,观察PTMS一侧大鼠大脑皮质、小脑蚓部对青霉素所致CCED的影响。

结果:PTMS一侧大脑,CCED频率明显降低了 $(36 \pm 10)\%$  ( $t = 3.873$ ,  $P < 0.01$ ),其幅度也平均降低了 $(21 \pm 8)\%$  ( $t = 2.984$ ,  $P < 0.05$ );PTMS小脑蚓部的抑制效应持续的时间较PTMS大脑皮质的作用时间长,停止PTMS小脑蚓部30 min后,CCED频率仍降低了 $(63 \pm 7)\%$  ( $t = -3.497$ ,  $P < 0.001$ );侧脑室注射纳洛酮可部分翻转PTMS的抑制作用。

结论:PTMS大脑皮质和小脑蚓部可明显抑制CCED频率和幅度。

关键词:经颅脉冲磁刺激;纳洛酮;大脑皮质

中图分类号:R741 文献标识码:A 文章编号:1671-5926(2003)25-3468-02  
胡祁生,陈静芳,汤剑青. 经颅脉冲磁刺激对大鼠癫痫模型痫样放电的抑制作用[J]. 中国临床康复, 2003, 7(25): 3468-9

http://www.zglckf.com/2003ml/03-25xy.htm

(Edited by Li CH)