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8-hydroxy-dipropylaminotetralin promotes neural plasticity in epileptic rats with depression★

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Abstract

Rats with chronic pilocarpine-induced temporal lobe epilepsy complicated with depression were studied. Anti-5-bromodeoxyuridine immunofluorescence staining and Timms staining showed that neurogenesis within the hippocampal dentate gyrus and mossy fiber sprouting were increased in model rats. Neurogenesis within the hippocampal dentate gyrus was further enhanced, while mossy fiber sprouting was decreased in model rats administered carbamazepine alone or in combination with the 5-hydroxytryptamine 1A receptor agonist, 8-hydroxy-dipropylaminotetralin (0.1 and 1 mg/kg). Among the groups, the effect was the most significant in rats receiving carbamazepine in conjunction with 1 mg/kg 8-hydroxy-dipropylaminotetralin. Thus, high dose 8-hydroxy-dipropylaminotetralin can improve neural plasticity in epileptic rats with depression.

Key Words: epilepsy; depression; 5-hydroxytryptamine 1A receptors; 8-hydroxy-dipropylaminotetralin; neural plasticity

Abbreviations: 8-OH-DPAT, 8-hydroxy-dipropylaminotetralin; CBZ, carbamazepine; BrdU, 5-bromodeoxyuridine

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INTRODUCTION

Recent studies have revealed that neurotransmitters such as 5-hydroxytryptamine and norepinephrine[1-2] are associated with epilepsy and depression. In particular, the 5-hydroxytryptamine 1A receptor plays an important role in depression, anxiety and epilepsy.

Epilepsy and the related depression may be linked to neural plasticity. Neural plasticity plays important roles in numerous processes in the brain, including its ability to sense, analyze and respond to various stimuli. It involves changes in neural structure and function. Neural plasticity can be divided into three stages: (1) This stage is characterized by morphological changes, including budding of collateral branches. (2) In this stage, neurotrophic factors, such as nerve growth factor and brain-derived neurotrophic factor, are produced and secreted. These help promote the growth, differentiation, regeneration and function of neural cells. (3) Stage of neuronal regeneration in the injured nervous system. Neurons have been shown to have the ability to regenerate in the injured adult nervous system[3].

Pretreatment with the 5-hydroxytryptamine 1A receptor antagonist, WAY-100635, promotes the regeneration of cells in the dentate gyrus, but it cannot inhibit mossy fiber sprouting[4]. In addition, after injection of kainic acid into the hippocampus, the 5-hydroxytryptamine 1A receptor agonist, 8-hydroxy-dipropylaminotetralin (8-OH-DPAT), can decrease the duration and frequency of kainic acid-induced epileptic discharge, and prevent transformation into generalized seizure[5]. Thus, in this study, we sought to understand the effects of 8-OH-DPAT on neural plasticity in epileptic rats with depression.

In the present study, we used chronic temporal epileptic rats with depression induced by pilocarpine to investigate the effects of 8-OH-DPAT on spontaneous seizure, depression, neurogenesis and mossy fiber sprouting in the hippocampus.

RESULTS

Animal grouping and treatments

A total of 160 Sprague-Dawley rats were used. Epilepsy was induced by pilocarpine. Thirty-two epileptic rats with depression were selected and randomly assigned to four groups (n = 8 for each): model, carbamazepine (CBZ), CBZ + low dose 8-OH-DPAT (0.1 mg/kg), and CBZ + high dose 8-OH-DPAT (1.0 mg/kg) groups. Another eigth normal rats were used as controls. Forty rats were involved in the final analysis.

Epileptic behavior following pilocarpine induction

Of the 152 rats subjected to lithium- pilocarpine, 22 did not develop epilepsy and were excluded from the experiment. Within 5 minutes after pilocarpine injection, rats developed diarrhea, piloerection and other signs of cholinergic stimulation. In the following 15 to 20 minutes, rats exhibited head bobbing, scratching, chewing and exploratory behaviors. Recurrent seizures started approximately 15 to 20 minutes after pilocarpine administration. These seizures were associated with episodes of head and bilateral forelimb myoclonus with rearing and falling, and progressed to epilepsy 35 to 40 minutes after pilocarpine administration, as described previously[6]. The epilepsy frequency in the model group was significantly increased compared with the CBZ, CBZ + low dose 8-OH-DPAT and CBZ + high dose 8-OH-DPAT groups ($P < 0.05$; Figure 1). The effect of CBZ + high dose 8-OH-DPAT was the most significant ($P < 0.05$; Figure 1). However, there were no significant differences between the CBZ group and CBZ + low dose 8-OH-DPAT group ($P > 0.05$; Figure 1).

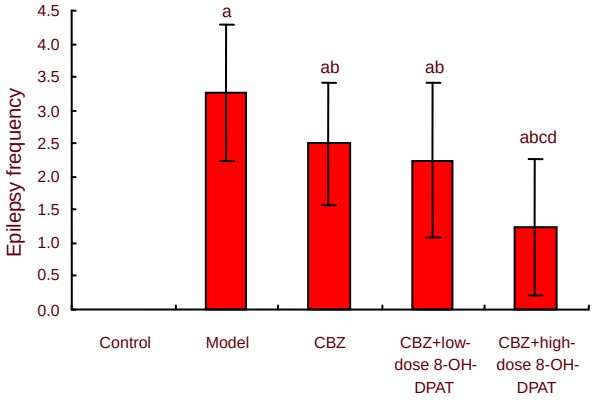

Figure 1 Comparison of behavioral differences in epilepsy frequency.

Data were expressed as mean ± SD of eight rats in each group. [a]$P < 0.05$, *vs.* control group; [b]$P < 0.05$, *vs.* model group; [c]$P < 0.05$, *vs.* carbamazepine (CBZ) group; [d]$P < 0.05$, *vs.* CBZ + low-dose 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) group using one-way analysis of variance.

CBZ and 8-OH-DPAT promote cell proliferation in the dentate gyrus

Immunofluorescence staining showed that 5-bromodeoxyuridine (BrdU)-labeled cells were located mainly in the subgranular zone and the granule cell layer (Figure 2).

The number of BrdU-positive cells was significantly increased in the hippocampal dentate gyrus in the model group compared with the control group ($P < 0.05$). In addition, the number of BrdU-positive cells was significantly higher in the CBZ, CBZ + low dose 8-OH-DPAT and CBZ + high dose 8-OH-DPAT groups ($P < 0.05$). The number of BrdU-positive cells was the greatest in the CBZ + high dose 8-OH-DPAT group ($P < 0.05$). No difference was found between the CBZ and CBZ + low dose 8-OH-DPAT groups ($P > 0.05$; Figure 3).

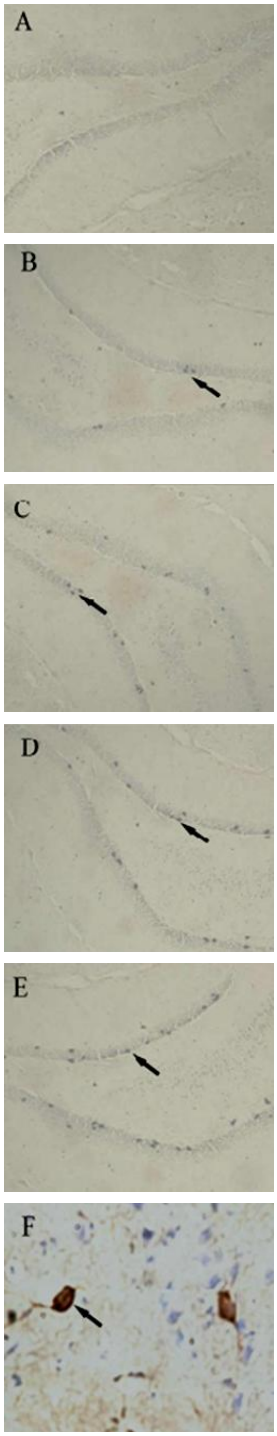

Figure 2 5-bromodeoxyuridine (BrdU) immunofluorescence of the dentate gyrus in different groups at 32 days after epilepsy induction (A–E, confocal microscopy, × 40; F, × 400).

$BrdU^+$ cells (arrows) in the granular cell layer, which represent new nerve cells.

There were more new nerve cells in the model (B, F), carbamazepine (CBZ; C), CBZ + low dose 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) (D) and CBZ + high dose 8-OH-DPAT (E) groups, compared with the control group (A).

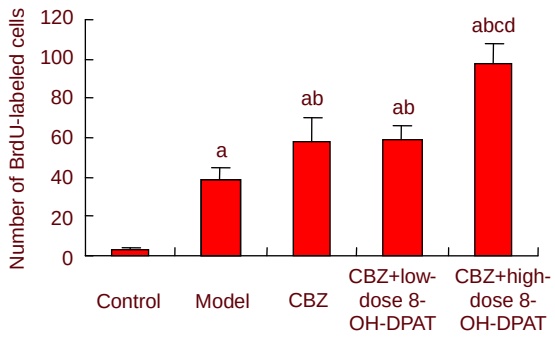

Figure 3 Mean total number of 5-bromodeoxyuridine (BrdU)-labeled cells in dentate gyrus of different groups at 32 days after epilepsy induction.

Data were expressed as mean ± SD of eight rats in each group. [a]*P* < 0.05, *vs.* control group; [b]*P* < 0.05, *vs.* model group; [c]*P* < 0.05, *vs.* carbamazepine (CBZ) group; [d]*P* < 0.05, *vs.* CBZ + low dose 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) group using one-way analysis of variance.

CBZ and 8-OH-DPAT inhibit hippocampal mossy fiber sprouting

Black Timm-stained granules were only occasionally observed in the CA3 region of the dentate gyrus in the control group. Timm scores ranged from 0–2[7]. In the model group, a dense distribution of Timm-stained granules was observed in the CA3 region. Timm scores ranged from 4–5 (Figure 4). Dentate gyrus mossy fiber sprouting was significantly higher in the model group compared with the control group (*P* < 0.05). Compared with the model group, Timm scores were significantly lower in the CBZ, CBZ + low dose 8-OH-DPAT and CBZ + high dose 8-OH-DPAT groups (*P* < 0.05), especially in the latter. There was no significant difference in mossy fiber sprouting between the CBZ and CBZ + low dose 8-OH-DPAT groups (*P* > 0.05; Figure 5).

Behavior of epileptic rats with depression

Compared with the control group, locomotion, rearing and grooming times in the open-field test were decreased remarkably, while the duration of immobility in the forced swimming test was increased significantly in the model, CBZ and two CBZ + 8-OH-DPAT groups (*P* < 0.05). However, there was no difference between the model, CBZ and two CBZ + 8-OH-DPAT groups. After 7 days of treatment, locomotion, rearing and grooming times in the open-field test were significantly decreased, while the duration of immobility in the forced swimming test was significantly increased in the model group compared with the control group (*P* < 0.05; Table 1). Locomotion, rearing and grooming times in the open-field test were significantly increased, while the duration of immobility in the forced swimming test was significantly decreased in the CBZ and two CBZ + 8-OH-DPAT groups compared with the model group (*P* < 0.05; Table 1). The effect of CBZ + high dose 8-OH-DPAT was the most significant (*P* < 0.05; Table 1). In contrast, no significant difference was found between the CBZ and CBZ + low dose 8-OH-DPAT groups (*P* > 0.05; Table 1).

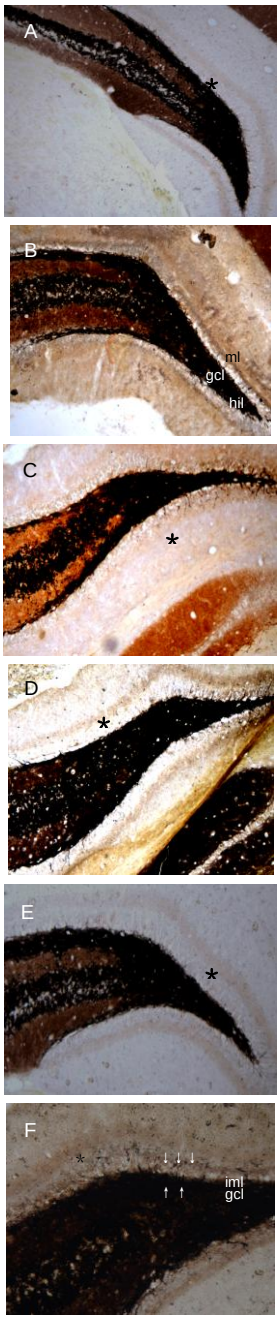

Figure 4 Timm staining in the dentate gyrus in different groups at 32 days after epilepsy induction (A–E, confocal microscopy, × 100; F, × 200).

(A) Control group; (B) model group; (C) carbamazepine (CBZ) group; (D) CBZ + low dose 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) group; (E) CBZ + high dose 8-OH-DPAT group; (F) model group.

Mossy fiber sprouting was present in the inner molecular layer in B–D, with a halo around the outside of the granule cell layer, whereas it was absent in the control group (A).

At higher magnification, mossy fiber sprouting was present in the innermost portion of the molecular layer (arrows) in the model group (B, F). Sprouting in C, D and E significantly decreased compared with B.

Asterisks (A–F) in the molecular layer represent higher magnification. *: Molecular layer; gcl: granule cell layer; hil: hilus; iml: inner molecular layer; ml: molecular layer.

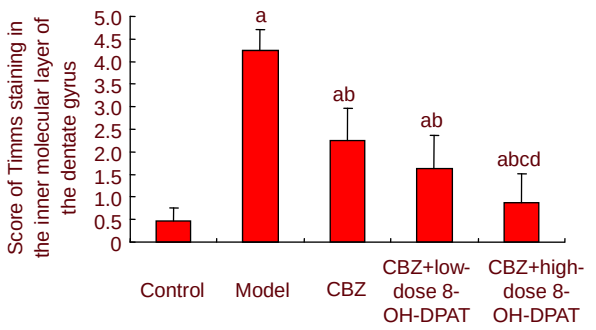

Figure 5 Scores for Timms staining in the inner molecular layer of the dentate gyrus. Data were expressed as mean ± SD of eight rats in each group. High Timm scores represent significant mossy fiber sprouting.

[a]$P < 0.05$, *vs.* control group; [b]$P < 0.05$, *vs.* model group; [c]$P < 0.05$, *vs.* carbamazepine (CBZ) group; [d]$P < 0.05$, *vs.* CBZ + low dose 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) group using one-way analysis of variance.

Table 1 Comparison of behavioral differences in open field tests (times/5 minutes) and forced swimming (duration of immobility (second))

Group	Rearing	Locomotion
Control	22.75±2.55	60.13±12.45
Model	7.50±2.51[a]	16.50±6.09[a]
CBZ	12.63±4.47[ab]	29.63±4.03[ab]
CBZ+low-dose 8-OH-DPAT	14.38±3.29[ab]	32.50±6.07[ab]
CBZ+high-dose 8-OH-DPAT	21.38±1.92[bcd]	57.00±8.40[bcd]

Group	Grooming	Duration of immobility
Control	13.38±3.02	93.75±9.44
Model	4.00±1.85[a]	196.88±14.13[a]
CBZ	7.88±2.53[ab]	170.63±10.21[ab]
CBZ+low-dose 8-OH-DPAT	8.50±2.67[ab]	160.38±10.99[ab]
CBZ+high-dose 8-OH-DPAT	12.63±1.85[bcd]	115.00±9.36[bcd]

Data were expressed as mean ± SD of eight rats in each group. [a]$P < 0.05$, *vs.* control group; [b]$P < 0.05$, *vs.* model group; [c]$P < 0.05$, *vs.* carbamazepine (CBZ) group; [d]$P < 0.05$, *vs.* CBZ + low-dose 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) group using one-way analysis of variance.

DISCUSSION

The co-occurrence of epilepsy and depression is common in neurological psychiatry. Approximately 55% of epileptic patients suffer from clinical depression[8]. It is known that the incidence of depression in patients with recurrent epileptic episodes is 10 times higher than in the general population[9]. Suicide is responsible for 10% of deaths in epileptic patients, but only 1% in the general population[10]. Kanner and Barry[11] found that epileptic seizures and depression influenced each other, exacerbating the illness.

In the present study, we used an animal model of epilepsy with coincident depression[12-15]. Rat models of epilepsy, induced by lithium chloride-pilocarpine intraperitoneal injection, have been widely used to study seizures of the limbic system. Moreover, it is easy to select rats with depression in this chronic temporal lobe epilepsy model. Treated rats exhibit slow movement, lack of appetite and irritability. While depression may occur after acute stress, and the mechanism remains unknown. After a 2-week latent period, spontaneous seizures, ranging from grades I to IV, occur at a frequency of one to three times per week[16-17]. However, in our present study, the duration of seizures was less than 50 seconds, showing that the rats had limited susceptibility to epilepsy. At 3 weeks after epilepsy induction, some rats had depression, along with low body mass and lack of appetite. The ratio reached 24%, similar to a previous study[15].

The pathogenesis of epilepsy and depression may involve a decrease in 5-hydroxytryptamine and norepinephrine signaling, including lowered affinity of the 5-hydroxytryptamine 1 receptor. Reduced 5-hydroxytryptamine, norepinephrine and dopamine signaling can initiate epileptic attacks and increase their frequency. Recent studies have shown that the 5-hydroxytryptamine 1 receptor is an important modulating factor in the 5-hydroxytryptamine system[18]. Structural and functional plasticity is an important characteristic of the nervous system, and in adults, it is related to axonal sprouting, as well as synaptic and nerve regeneration.

In the present study, 8-OH-DPAT, a 5-hydroxytryptamine 1A receptor agonist, was administered to epileptic rats with depression. Compared with traditional antiepileptic drugs, 8-OH-DPAT, at the high dose, had significant effects on epilepsy and depression in rats. In contrast, the low dose treatment had no statistically significant effect. Therefore, we hypothesize that there may be a dose-response relationship for the anti-epileptic and anti-depressive effects of 8-OH-DPAT. An extended range of doses should be utilized in future experiments. The mechanisms through which the 5-hydroxytryptamine 1A receptor agonist has anti-epileptic and anti-depressive effects are poorly understood. Some researchers have proposed the neural plasticity theory for epilepsy with coincident depression[19-20]. Additionally, previous studies have shown that the 5-hydroxytryptamine 1A receptor is associated with neural plasticity, playing a role in the development and maturation of the nervous system. The dentate gyrus has abundant 5-hydroxytryptamine 1A receptors, which mediate the neurotrophic action of 5-hydroxytryptamine in the hippocampus[21]. Therefore, we infer that the 5-hydroxytryptamine 1A receptor mediates anti-epileptic and anti-depressive effects by promoting neural plasticity.

In the present study, we observed both neuronal regeneration and mossy fiber sprouting. The process by which new neurons are produced in the brain is called neurogenesis. It includes a series of events, including birth, differentiation and migration. Research suggests

that epileptic seizures can increase neurogenesis in the subgranular layer of the dentate gyrus, which may be a protective response to brain injury[22]. Following anti-epileptic drug treatment, more than 83% of newborn neurons in the dentate gyrus of rats differentiated into adult neurons, and most of them were located in the granule cell layer. These ectopic cells may form an excitability loop that may exacerbate symptoms.

The precise mechanism of neurogenesis after status epilepticus induced by pilocarpine in the dentate gyrus is unknown. Axons of newborn dentate granule cells participate in the restructuring of abnormal mossy fiber sprouts in both the CA3 and the inner molecular layer. Radley and Tacobs[4] proposed that 5-hydroxytryptamine receptor antagonists could not protect dentate granule cells after epilepsy, but activation of the 5-hydroxytryptamine receptor may be required for the production and survival of dentate granule cells induced by epilepsy.

Mossy fiber sprouting is a process by which axons of dentate granule cells change their original direction by sending out collateral branches. These then traverse the granule cell layer and form a fibrous plexus after projecting to the inner molecular layer. They finally form new synapses with dendrites of other granule cells and associated neurons. The tips of mossy fibers are rich in Zn^{2+}, and are pale brown or black by Timms staining. Dudek and Sutula[23] believe that mossy fiber sprouting participates in the formation of new excitability loops within the dentate gyrus. The loss of pyramidal cells in the CA3 zone likely induces mossy fiber sprouting to form new synapses with granule cells, possibly forming loops[24]. Recent studies have revealed that mossy fiber sprouting is associated with spontaneous recurrent seizures. Thus, the proper coordinated expression of guidance molecules, to regulate sprouting, may protect against recurrence of seizures[25].

In the present study, neurogenesis and mossy fiber sprouting within the dentate gyrus were increased in our rat model of epilepsy with coincident depression. There was a correlation between neurogenesis and mossy fiber sprouting, which were associated with spontaneous seizures and depression. Our results indicate that plasticity of hippocampal neurons is a mechanism by which the 5-hydroxytryptamine 1A receptor mediates anti-epileptic and anti-depressive effects. Future studies should focus on the dose-response relationship of 5-hydroxytryptamine 1A receptor activation on therapeutic efficacy in this model.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

The experiment was performed at the Pharmacology Laboratory of Shanxi Medical University, China, from May 2009 to May 2010.

Materials

A total of 160 healthy, 50-day-old, female Sprague-Dawley rats, weighing 180–230 g, were provided by the Laboratory Animal Center of Shanxi Medical University (No. SCXK (Jin) 2009-001). The rats were separately housed in plastic cages at 22°C and 60% humidity under a constant light-dark cycle and were allowed free access to food and water. All procedures were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[26].

Methods

Model establishment and intervention

Adult female rats received lithium chloride (127 mg/kg, i.p.; Sigma, St. Louis, MO, USA), and methylscopolamine bromide (1 mg/kg, i.p.; TongYong, Shanghai, China) was administered on the following day to limit the peripheral effects of the convulsant. Status epilepticus was induced by injecting pilocarpine (35 mg/kg, i.p.; ABCR, Karlsruhe, Germany) 30 minutes after methylscopolamine. Animals were monitored throughout status epilepticus induction, and seizure severity was assessed according to the scale of Racine[27]. Animals that did not show obvious signs of status epilepticus were excluded, since at least 1 hour of status epilepticus is required to develop spontaneous recurrent epileptic seizures in the pilocarpine model of epilepsy. Status epilepticus occurred within 15–60 minutes and was characterized by continuous motor-limbic seizures accompanied by intermittent rearing and falling and occasional wild running spells. One hour after status epilepticus, the seizures were stopped with diazepam (4 mg/kg, i.m.)/chloral hydrate (3 mL/kg, i.p.). The rats receiving an injection of normal saline were used as the control group (n = 8). After 25 days, the rats with depression, selected from the rats with chronic epilepsy, were randomly divided into four groups(n = 8 each): model, CBZ (100 mg/kg/d, i.p.; Meitong, Jiangsu, China), CBZ (100 mg/kg per day, i.p.) + low dose 8-OH-DPAT (0.1 mg/kg per day, i.p.; Sigma) and CBZ (100 mg/kg per day, i.p.) + high dose 8-OH-DPAT (1.0 mg/kg/d, i.p.) groups. Administration was performed for 7 days.

Behavioral changes in epileptic rats

Racine 6-grade rating scale was used to evaluate behavior as follows[28]: 0, Invalid; I, facial twitching, nodding, sniffing or running about; II, forward limb clonus; III, forward limb clonus complicated with retropulsion; IV, generalized clonus complicated with retropulsion and tumble; V, sustained clonus and tumble. Successful kindling: 3 successive grade IV or V epileptic seizures. The behavioral indicators of epilepsy seizure frequency in rats were recorded after status epilepticus.

Depression detection

Open field test: Epileptic rats with depression were selected and indices of depression were compared at 25 and 32 days after status epilepticus. The open field test was performed according to a previously described

method[29] to measure spontaneous activity in rodents. Briefly, the apparatus (Shanxi Medical University, Taiyuan, China) consisting of a square arena, 100 cm × 100 cm × 40 cm, was divided into 25 cm × 25 cm equal squares on the floor. A single rat was placed in the center of the cage, and after 30 seconds of adaptation, the frequency of locomotor activity, the number of rears and the frequency of grooming were recorded manually for 5 minutes. All behaviors were recorded using a video camera (Olympus, Tokyo, Japan) located 40 cm above the arena. After each test, the arena was cleaned with 90% alcohol solution.

Forced swimming test: This test was performed immediately following the open field tests. According to Porsolt *et al* [30], rats were placed in a Plexiglas cylinder (40 cm tall, 25 cm in diameter; Shanxi Medical University, Taiyuan, China) filled with water (depth approximately 21.5 ± 1.5 cm; temperature approximately 24 ± 0.5°C) and allowed to swim. Each test session lasting for 5 minutes was videotaped from above the cylinder. The duration of immobility, which was defined as the lack of motion of the whole body except for small movements necessary to keep the animal's head above the water, was recorded. After each test, the cylinder was cleaned.

BrdU labeling and brain tissue sample preparation

The thymidine analog BrdU (Sigma) was dissolved in saline with 0.007 M NaOH in all experiments and administered intraperitoneally (50 mg/kg, twice/day, 1 day), starting on day 32 after status epilepticus. All rats were sacrificed 24 hours after the last BrdU administration. Rats were perfused transcardially with cold 0.1 M phosphate buffered saline (PBS) and 4% paraformaldehyde. Their brains were removed, post-fixed for 18 hours, and placed in 30% sucrose until they sank. Coronal cryostat sections (25 μm) were cut and stored at −20°C in cryoprotectant solution (25% ethylene glycol, 25% glycerol, 50% 0.1 M PBS, pH 7.4) for immunofluorescence.

Immunofluorescence staining for cell proliferation in the hippocampal dentate gyrus

Immunofluorescence staining was performed to observe new nerve cells. The hippocampi[31], after rinsing in PBS, were incubated in 2 M HCl at 30°C for 60 minutes to denature the DNA. The sections were subsequently incubated in 1% bovine serum albumin to block non-specific signals. Following serum blocking, the sections were incubated with mouse anti-rat BrdU monoclonal antibody (1:100; Boster, Wuhan, China) in PBS containing 0.3% Triton X-100 overnight at 4°C. Following a PBS rinse, the sections were incubated with goat anti-mouse IgG-Cy2 (1:100; Boster) for 2 hours at room temperature. The stained sections were observed by confocal microscopy (BX-51; Olympus, Tokyo, Japan)[32].

Cell quantification

Representative sections were selected to estimate the number of new nerve cells in the dentate gyrus. For immunofluorescence staining, 10 coronal sections, 25 μm thick, were selected every 210 μm at the level of the dentate gyrus, spanning from –2.56 mm to –4.52 mm of the bregma[31]. The staining was quantified using an Olympus Image System CAST program. $BrdU^+$ cells from a total of 10 sections were quantified to estimate the total cell number in the dentate gyrus. Cells with clear $BrdU^+$ nuclei were considered to be new nerve cells.

Timm staining for mossy fiber sprouting in the hippocampus

After general anesthesia with chloral hydrate, the rat thoracic cavity was dissected for transcardial perfusion with 100 mL of 4% (w/v) paraformaldehyde, followed by 100 mL of 0.1% (w/v) Na_2S and an additional 100 mL of 4% (w/v) paraformaldehyde. The brains were postfixed in 4% paraformaldehyde overnight and then placed in 30% sucrose/phosphate buffer until they sank to the bottom of the vial. The fourth coronal section of 25 μm was used for Timm staining. Sections were developed in the dark for 60 minutes in a 12:6:2 mixture of 50% gum arabic, 5.6% hydroquinone and citric acid-sodium citrate buffer with 1.5 mL 17% $AgNO_3$ solution. All images were processed with Image-pro plus 5.0 software. For Timm stained sections, the mean density (*A*) of Timm granules in the inner molecular layer of the dentate gyrus and in the stratum pyramidale and stratum oriens of the CA3 region were calculated. Changes in the number of granules were determined[33].

Statistical analysis

Statistical analysis was performed by the first author with SPSS 17.0 software (SPSS, Chicago, IL, USA). Measurement data were expressed as mean ± SD. Analysis of variance was used for intergroup comparison. A level of $P < 0.05$ was considered statistically significant.

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Author contributions: Ping Yang participated in the animal surgery and data processing. Meizhen Sun was responsible for study design, study supervision, and manuscript instruction. Liang Li participated in data collection. Yihua Shen had full access to all data and participated in data integrity and data accuracy analysis.

Conflict of interests: None declared.

Ethical approval: The study was approved by the Animal Ethics Committee of Shanxi Medical University, China.

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