Neurosci Bull October 1, 2013, 29(5): 565–575. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1326-y

·Original Article·

BDNF–TrkB signaling pathway is involved in pentylenetetrazole-evoked progression of epileptiform activity in hippocampal neurons in anesthetized rats

Xu Liu[1], Jia Liu[1], Juan Liu[1], Xiao-Ling Liu[2], Ling-Yan Jin[2], Wei Fan[1], Jin Ding[1], Li-Chao Peng[2], Yun Wang[1], Xin Wang[1]

[1]*Neurology Department, Zhongshan Hospital, Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200032, China*

[2]*Department of Anaesthesia, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai 200032, China*

Corresponding authors: Xin Wang and Yun Wang. E-mail: wang.xin@zs-hospital.sh.cn; yunwang@fudan.edu.cn

ABSTRACT

Pentylenetetrazole (PTZ) is a widely-used convulsant used in studies of epilepsy; its subcutaneous injection generates an animal model with stable seizures. Here, we compared the ability of PTZ *via* the intravenous and subcutaneous routes to evoke progressive epileptiform activity in the hippocampal CA1 neurons of anesthetized rats. The involvement of the BDNF–TrkB pathway was then investigated. When PTZ was given intravenously, it induced epileptiform bursting activity at a short latency in a dose-dependent manner. However, when PTZ was given subcutaneously, it induced a slowly-developing pattern of epileptogenesis; first, generating multiple population-spike peaks, then spontaneous interictal discharge-like spike, leading to the final ictal discharge-like, highly synchronized bursting firing in the CA1 pyramidal layer of the hippocampus. K252a, a TrkB receptor antagonist, when given by intra-cerebroventricular injection, significantly reduced the probability of multiple population spike peaks induced by subcutaneous injection of PTZ, delayed the latency of spontaneous spikes, and reduced the burst frequency. Our results indicate that PTZ induces a progressive change of neuronal epileptiform activity in the hippocampus, and the BDNF–TrkB signaling pathway is mainly involved in the early phases of epileptogenesis, but not the synchronized neuronal burst activity associated with epileptic seizure in the PTZ animal model. These results provide basic insights into the changing pattern of hippocampal neuronal activity during the development of the PTZ seizure model, and establish an *in vivo* seizure model useful for future electrophysiological studies of epilepsy.

Keywords: epilepsy; pentamethylenetetrazole; hippocampus; BDNF; TrkB

INTRODUCTION

In the central nervous system, pentylenetetrazole (PTZ) acts as a cerebral stimulant; it counteracts the action of the γ-aminobutyric acid type A ($GABA_A$) receptor by interacting with the picrotoxin-barbiturate binding site, closing Cl^- channels, thereby interfering with $GABA_A$ receptors and provoking seizures[1]. Despite the fact that PTZ has been widely used to induce seizures in rodent models in epilepsy studies[2,3], the mechanisms underlying PTZ-induced epileptogenesis are still not fully understood.

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein belonging to the nerve growth factor family of neurotrophins, and is widely expressed in several areas of the adult brain, including the hippocampus[4,5]. BDNF is mainly secreted by astrocytes[6], while neuronal

activity affects BDNF release[7,8]. Studies have shown that BDNF plays a key role in epileptogenesis[9-12]. It binds to TrkB receptors and activates down-stream protein kinases to phosphorylate substrates, inducing an increased presynaptic release probability or changes in the postsynaptic functions of receptor such as the $GABA_A$ receptor[5,13]. Neurons are also directly depolarized or fire action potentials with rapid application of BDNF[13,14]. In addition, epileptiform activity increases the expression levels of both BDNF mRNA and protein[15-17]. Early transgenic study showed that, in BDNF-heterozygous mice, compared to the wild-type controls, the kindling rate of the epilepsy was decreased by two-fold[18]. Moreover, a recent study showed that when the TrkB receptor is knocked out (but not BDNF itself), the epileptiform activity in the kindling model of epilepsy is suppressed[19]. Our previous *in vivo* electrophysiological study also demonstrated that blockade of TrkB receptors inhibits epileptogenesis in an animal model with cyclothiazide-induced seizures[20]. Thus, these results suggest that BDNF–TrkB signaling is pro-epileptic[16,17,21].

Animal models of seizures, including those induced by kindling, kainic acid (KA), pilocarpine, and cyclothiazide, have all shown an association with the BDNF–TrkB signaling pathway[20,22-25]. However, the role of the BDNF–TrkB signaling pathway in PTZ-induced seizures is still not clear. Early studies showed that PTZ causes epileptic seizures and changes the expression of BDNF mRNA and protein, while other experiments suggested that PTZ does not alter the level of BDNF protein[26,27]. Thus, it is worthwhile to further study the involvement of the BDNF–TrkB signaling pathway in the PTZ model.

Hippocampal neurons play a key role in epileptogenesis, particularly in temporal lobe epilepsy[28], and hippocampal neuronal electrical activity has a tight relationship with epileptogenesis[29]. However, little is known about the direct effect of PTZ on hippocampal neuronal activity in intact animals. In the current study, the anesthetized rats were used to first compare the effects of PTZ, given either intravenously (i.v.) or subcutaneously (s.c.), on the activity of hippocampal CA1 pyramidal neurons, and the involvement of the BDNF–TrkB signaling pathway in PTZ-induced epileptogenesis was investigated.

MATERIALS AND METHODS

Drugs and Solutions

The following drugs were freshly made before each experiment: pentylenetetrazole salt in 1 mL distilled water and K252a in DMSO for intracerebroventricular (i.c.v.) injection (from Tocris, Northpoint, Bristol); and urethane (25%; Sigma Aldrich Chemical Co., Poole, UK) was dissolved in distilled water.

Animal Preparation

All experiments were carried out in urethane-anesthetized Sprague-Dawley rats (male, 280–350 g). All the rats were kept in a controlled animal facility (21 ± 1°C) under a 12-h light/dark cycle, with food and water available *ad libitum*. All the experimental procedures were carried out in accordance with the Chinese National Science Foundation Animal Research Regulations and approved by the Ethic Committee on Laboratory Animals, Fudan University. At the end of the experiments, the rats were euthanized by anesthetic overdose.

The rats were prepared as previously described[20,29,30]. Briefly, rats were first anesthetized with urethane (1.2 g/kg, s.c.). The absence of a withdrawal reflex was used to judge the anesthesia level during the whole experimental period, and additional anesthetic (urethane, 0.2–0.6 mg/kg, s.c.) was administered when necessary. During the experiment, body temperature was maintained at 37.0 ± 0.5°C with a heating blanket (Harvard Apparatus Limited, Edenbridge, UK). In order to expose the top part of the skull, an incision was made in the midline of the head after the animal was mounted in a stereotaxic frame. For i.c.v. cannula implantation, a hole was drilled in the skull above the left lateral ventricle (0.3 mm posterior to bregma, 1.3 mm lateral to the midline). A guide cannula (Bilaney Consultants Ltd., Sevenoaks, UK), pre-cut to extend 4 mm below the surface, was then placed into the drilled hole for later i.c.v. drug delivery, and secured with the dental cement.

Recording and Data Acquisition

To insert the recording and stimulating electrodes, a large burr hole was drilled in the left side of the skull above the hippocampal area. Both the dura and pia were then pierced and removed. The electrode positions were determined according to the stereotaxic atlas of the rat brain[31]. A

concentric bipolar electrode (Harvard Apparatus Ltd.) was placed near the CA3 region (3.8–4.5 mm posterior to bregma, 3.5–4.0 mm lateral to the midline, and 3.0–3.8 mm below the brain surface) to stimulate the Shaffer collateral pathway and/or the CA3 pyramidal cell layer. A tungsten electrode (0.5 MΩ; WPI, Stevenage, UK) was placed 3.5–4.2 mm posterior to bregma, 2.0–3.0 mm lateral to the midline, and 2.0–2.5 mm below the brain surface in order to record from the CA1 pyramidal neuron layer. During stimulation, a square-wave pulse at 0.2 ms in duration was generated using a constant-current pulse generator (Digitimer, Welwyn Garden City, UK). The test pulses evoked an upward excitatory postsynaptic field potential (fEPSP) in CA1 with a population spike (PS) superimposed on its rising phase. During recordings of evoked responses, the fEPSP-PS of CA1 pyramidal neurons was sampled every 60 s by a test pulse. The baseline activity was recorded for evidence of spontaneous activity. The electrophysiological signals were amplified 200 times and filtered (0.3–3 kHz) using a NeuroLog System (Digitimer). The signals were then visualized and stored in a PC computer through an A–D converter (CED 1401 micro; Cambridge Electronic Design, Cambridge, UK). Once both the fEPSPs and PSs reached a maximum, by adjusting both electrodes, the baseline activity was first monitored for at least 30 min until a stable recording was achieved. After 30 min of baseline recording of all responses, K252a or its vehicle was administered by i.c.v. injection (5 μL) through the pre-implanted guide cannula into the lateral ventricle at a slow injection rate. Either 30 or 60 min after K252a injection, PTZ was administered to evoke seizure activity either by i.v. or s.c. injection. Hippocampal neuronal responses were recorded either for 1 h (i.v.) or 3 h (s.c.) after either PTZ or vehicle injection by recording the transformation of the evoked potentials from a single PS peak into a multi-peaked PS and spontaneous epileptiform burst activity in CA1 pyramidal neurons[32].

Data Analysis

The recorded electrophysiological signals were analyzed offline using the Spike2 program (Cambridge Electronic Design). Since the PS peaks typically represent epileptiform evoked responses[29] the latency for evoking the second and third PS peaks was calculated and analyzed. Spiking events were defined as being spontaneously-generated with high amplitude (>0.5 mV) and containing one or two spikes occurring at a low frequency (<1 Hz). The latency for these spikes was analyzed from the usual 'silent' baseline. Highly-synchronized bursting activity was defined, in distinguishing from spontaneous spiking events, as having high-frequency multiple high-amplitude spikes (>0.5 mV) with an initial inter-spike interval of <0.2 s, a minimum of 5 spikes, and a burst duration >1 s, as previously reported. Group data are presented as mean ± SEM. Across the data groups, statistical significance between means was determined using one-way ANOVA with Tukey HSD *post hoc* analysis (GraphPad Prism, GraphPad Software Inc., San Diego, CA). Comparisons within a group used the two-tailed *t*-test. Significance level was set at $P < 0.05$.

RESULTS

Intravenous PTZ Induced Epileptiform Bursting Activity in CA1 Pyramidal Neurons of Anesthetized Rats in a Dose-dependent Manner

In a previous study, we demonstrated that s.c. injection of PTZ at a high dose induced progressive epileptiform activity in the CA1 pyramidal layer[3], but not i.c.v. injection. Here we first studied the epileptiform bursting activity induced in the CA1 pyramidal layer of anesthetized rats after bolus i.v. injection of PTZ.

Intravenous injection of saline did not induce any change on the baseline firing. However, i.v. injection of cumulative PTZ at doses of 55, 110 and 220 mg/kg dose-dependently evoked epileptiform burst activity in hippocampal CA1 neurons (Fig. 1). In total of eight rats tested, 25% (1/8), 75% (6/8) and 100% (8/8) of rats were induced to exhibit epileptiform burst(s) when PTZ was given at cumulative doses of 55, 110 and 220 mg/kg, respectively (Fig. 1Ba). Intravenous PTZ-induced bursting activity occurred normally in a very short latency, even during the drug injection period (50 s) (Fig. 1A). The average evoked burst numbers (within 1-h recording time) were 0.4 ± 0.2 (n = 8), 1.5 ± 0.7 (n = 8) and 10.6 ± 2.0 (n = 8), at the doses of 55, 110 and 220 mg/kg, respectively (Fig. 1Bb). The corresponding burst frequencies were 0.0030 ± 0.0003 Hz (n = 3) at 110 mg/kg and 0.0054 ± 0.0007 Hz (n = 8) at 220 mg/kg (Fig. 1Bc). Further statistical analysis

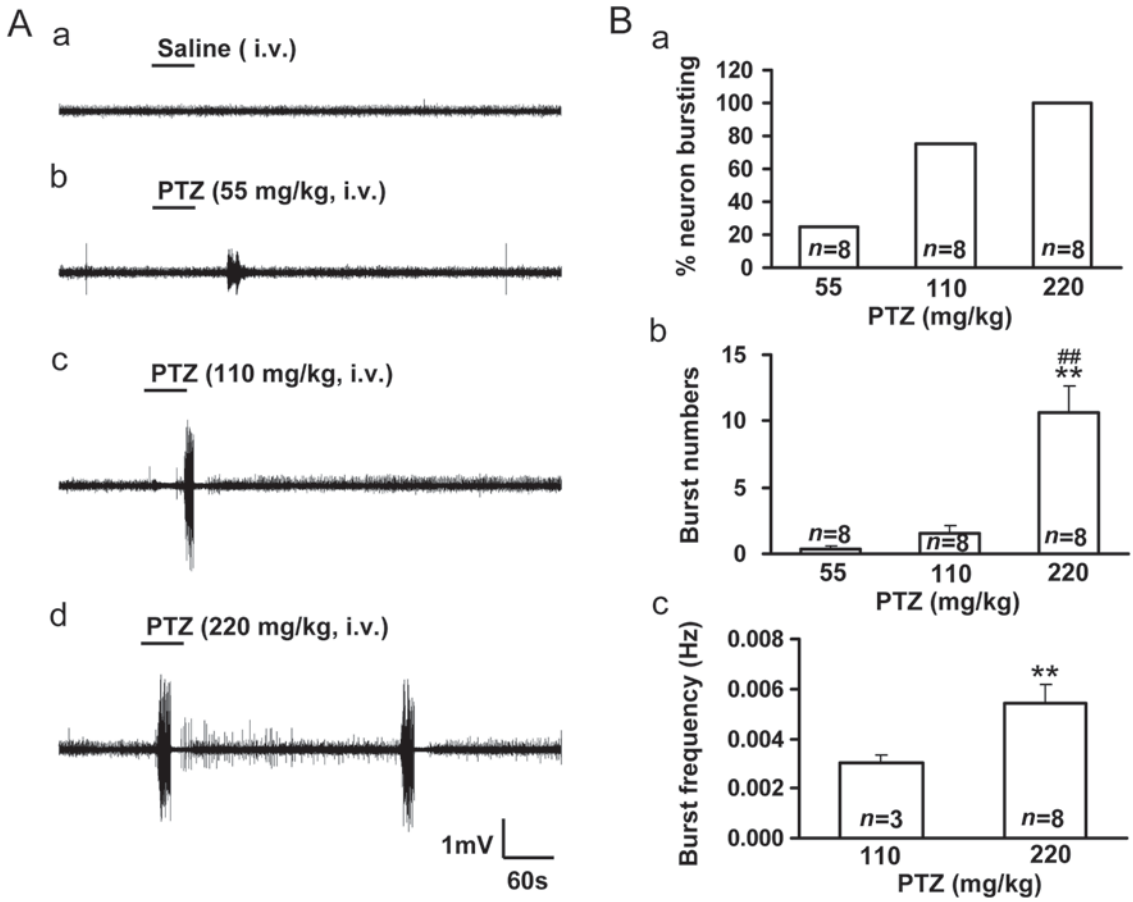

Fig. 1. Intravenous (i.v.) PTZ dose-dependently induced epileptiform bursting activity in hippocampal CA1 pyramidal neurons in anesthetized rats. A: Traces showing raw recordings of spontaneous activity in hippocampal CA1 pyramidal neurons with i.v. injection (bar above the trace) of cumulative PTZ (55, 110, and 220 mg/kg). B: Bar histograms showing the group data on the probability of evoked bursts (a), burst number (b) and burst frequency (c). *****P* **<0.01 *versus* 110 mg/kg group, ##*P* <0.01 *versus* 55 mg/kg group.**

showed that the high dose of PTZ (220 mg/kg, i.v.) evoked more epileptiform bursts and a higher bursting frequency in hippocampal CA1 neurons (P <0.01) (Fig. 1B).

Subcutaneous PTZ Induced Progressive Epileptogenesis in CA1 Pyramidal Neurons in Anesthetized Rats in a Dose-dependent Manner

A previous study demonstrated that s.c. PTZ at 383 mg/kg induces epileptiform burst activity in anesthetized rats[3]. We therefore investigated the dose-dependent effect of s.c. PTZ on the induction of progressive epileptogenesis in the CA1 pyramidal layer.

In anesthetized rats, recordings were made from the CA1 pyramidal cell layer while stimulating the CA3 area once every 60 s. In the control condition, a downward single PS peak normally appeared on the upward evoked EPSP and there was no spontaneous activity (Fig. 2Aa, Ba). PTZ at doses of 55, 165 and 550 mg/kg (s.c.) dose-dependently induced progressive epileptiform activity, similar to a previous study in the cyclothiazide-induced epilepsy model[30]. First, PTZ dose-dependently induced multiple PS peaks in the stimulus-evoked EPSP-PS complex field potential in CA1 pyramidal neurons in a time-dependent manner (Fig. 2Ab, c). At all three doses tested, a second PS-peak was induced in all rats tested at latencies of 60.6 ± 7.3 (55 mg/kg; n = 5), 11.7 ± 0.8 (165 mg/kg; n = 7) and 8.2 ± 1.1 min (550 mg/kg; n = 5). The latency for the second peak showed significant dose-dependent decreases (P <0.05

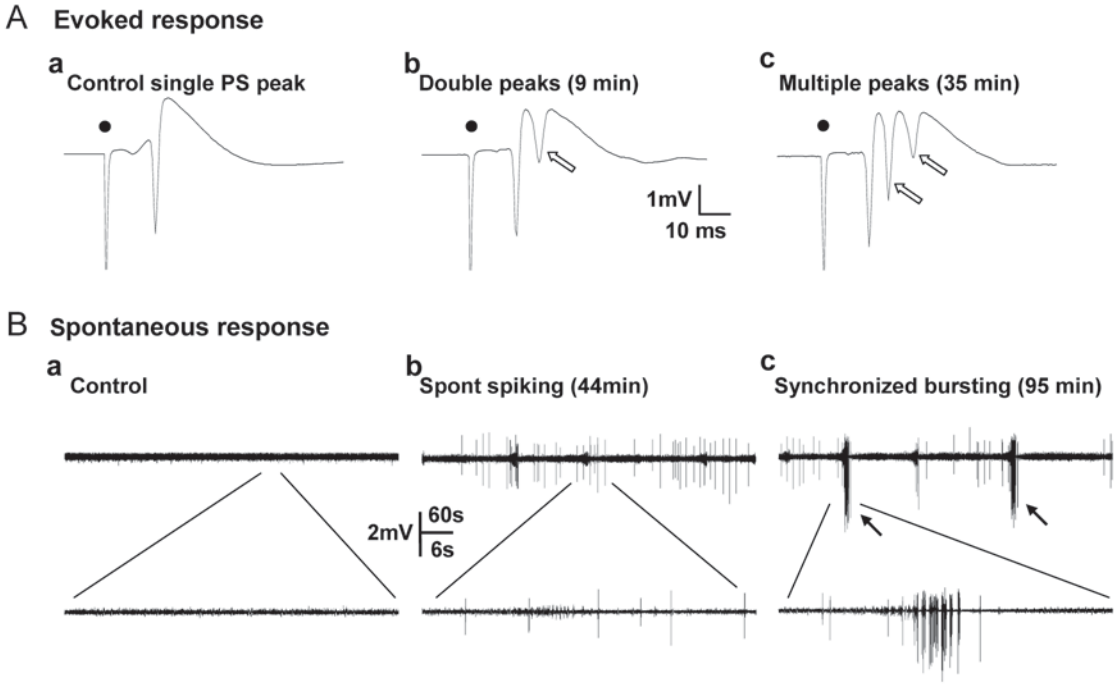

Fig. 2. Typical progress of PTZ-evoked epileptiform activity in CA1 neurons in an anesthetized rat. A: Recordings showing that the evoked CA1 pyramidal neuron PS peak transformed from a single peak (a) under control conditions to double (b) and multiple peaks (c) (arrows) after PTZ injection (165 mg/kg, s.c.). Filled circle indicates stimulus artifact. The time in parenthesis is the latency of the multiple PS peaks after PTZ injection. B: Spontaneous discharges recorded in the same rat as in (A). Before PTZ injection, the baseline activity was usually silent in CA1 pyramidal cells (a). After PTZ injection, some spontaneous spiking activity appeared (b) and finally formed highly-synchronized epileptiform bursts (c) (arrows).

to $P < 0.001$; Fig. 3Ab). The third peak was induced only in 1 out of 5 rats when PTZ was applied at 55 mg/kg dose, but was induced in all rats tested with PTZ at 165 and 550 mg/kg doses (Fig. 3Aa). The latencies were 24.7 ± 6.3 (n = 7) and 22.8 ± 2.3 min (n = 5) for PTZ doses of 165 and 550 mg/kg, respectively, and they were not statistically different (Fig. 3Ab). The second events following PTZ injection were spontaneous high-amplitude spikes, which were induced in all rats at all 3 doses of PTZ (Fig. 3Ba). The latencies for these spontaneous high- amplitude spikes were 103.6 ± 4.3 (n = 5), 41.2 ± 3.3 (n = 7) and 6.4 ± 2.9 min (n = 5), respectively, for PTZ doses of 55, 165 and 550 mg/kg. The differences in the latencies for these spikes showed significant dose-dependent decreases (Fig. 3Bb). The final events following PTZ injection were highly synchronized bursts. The burst activity was induced only by PTZ at doses of 165 mg/kg (6/7 rats) and 550 mg/kg (5/5 rats) (Fig. 3Ba). The latencies for inducing synchronized epileptiform bursts were 65.3 ± 4.6 min (165 mg/kg; n = 6) and 32.8 ± 3.8 min (550 mg/kg; n = 5) (Fig. 3Bb), a significant dose-dependent decrease. Then we further analyzed the physical properties of the bursts at PTZ doses of 165 and 550 mg/kg, both of which induced epileptiform bursts. The number of bursts during the 3-h recording period after PTZ injection increased dose-dependently, 42 ± 10 (n = 6) at 165 mg/kg, and 96 ± 15 (n = 5) at 550 mg/kg (Fig. 3Ca). However, the burst frequency did not differ between 165 and 550 mg/kg PTZ. The mean epileptiform burst frequencies were 0.0064 ± 0.0028 Hz (n = 6) and 0.0082 ± 0.0022 Hz (n = 5), respectively for PTZ doses of 165 and 550 mg/kg (Fig. 3Cb). This progressive pattern of epileptogenesis in hippocampal neurons after PTZ injection is similar to those reported in the cyclothiazide-induced epilepsy model[30].

Effect of K252a on PTZ-induced Epileptiform Activity in CA1 Pyramidal Neurons

In order to determine the involvement of the BDNF–TrkB

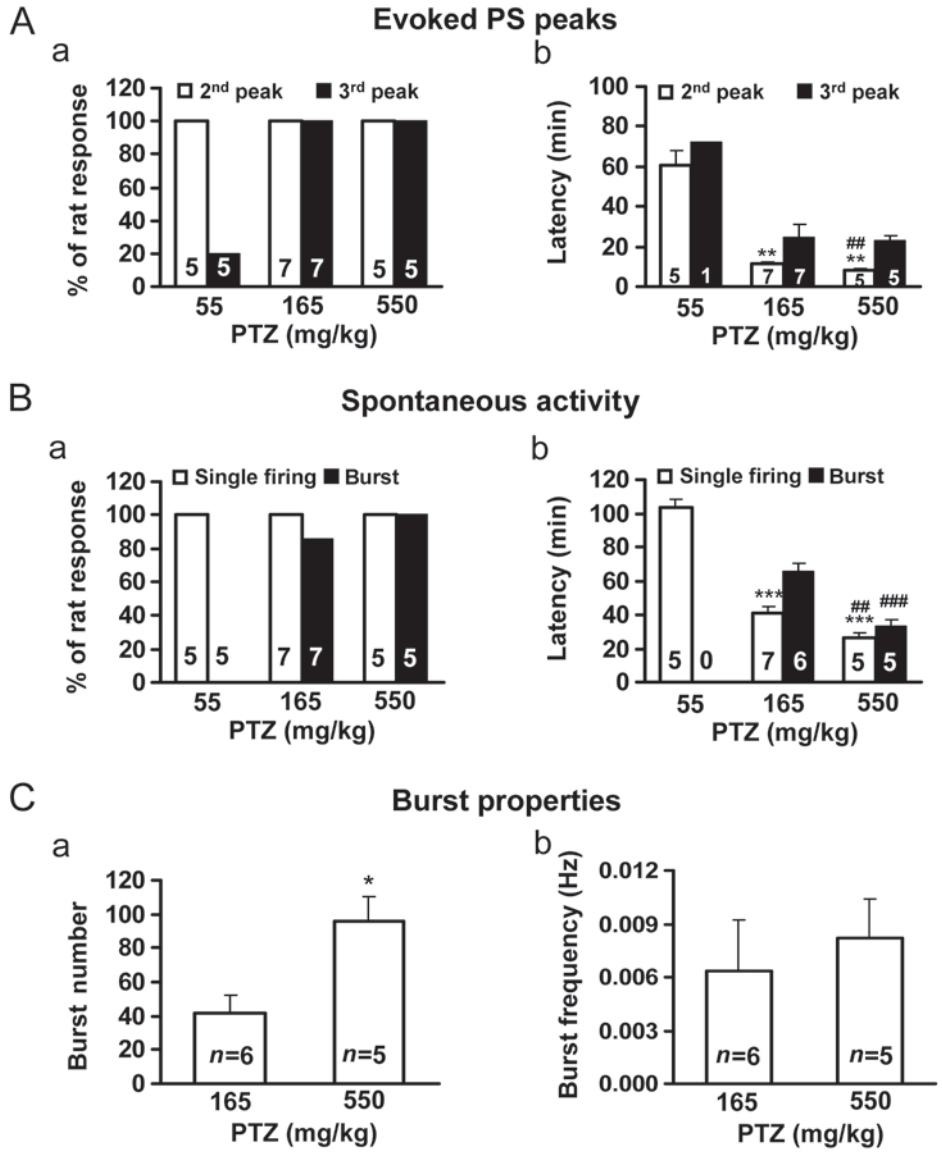

Fig. 3. Effect of subcutaneous PTZ on the progressive change of epileptiform activity in CA1 pyramidal neurons in anesthetized rats. Histograms show that PTZ (55, 165, and 550 mg/kg) dose-dependently induced stimulation-evoked CA1 PS peak progression from single to double and multiple peaks (***P*** **<0.01 *vs* 55 mg/kg group; ##*P* <0.01 *vs* 165 mg/kg group, respectively) (A), spontaneous activity progression from 'silent' to high-amplitude spikes and highly-synchronized bursts (******P*** **<0.001 *vs* 55 mg/kg; ##*P* <0.01, ###*P* < 0.001 *vs* 165 mg/kg group) (B); and burst number and burst frequency changes (****P*** **<0.05 *vs* 165 mg/kg) (C).**

receptor signaling pathway in PTZ-induced epileptogenesis, we used K252a, a non-competitive inhibitor for tyrosine kinase receptors including TrkB receptors. K252a was directly administered into the cerebral fluid by i.c.v. injection (250 μmol/L, 5 μL) either 30 min (n = 5) or 1 h (n = 5) prior to PTZ injection (165 mg/kg). In all rats, i.c.v. injection of K252a alone for 3 h induced neither changes in the PS peak nor spontaneous activity in the hippocampal CA1 layer. However, pretreatment of K252a (250 mol/L, i.c.v.) significantly delayed the latency to stimulus-evoked multiple peaks and spontaneous high-amplitude spikes after PTZ injection at 165 mg/kg (s.c.).

K252a pretreatment for 60 min significantly delayed the onset latency of the second and third evoked peaks from 11.7 ± 0.8 min (n = 7) to 30.6 ± 3.8 min (n = 5, P <0.01) and 24.7 ± 6.3 min (n = 7) to 57.6 ± 8.3 min (n = 5, P <0.05); however, K252a pretreatment for 30 min had no effect on either of these peaks (Fig. 4Ab). In addition, K252a pretreatment for either 30 or 60 min also significantly delayed the onset latency to spontaneous high-amplitude spikes induced by PTZ (165 mg/kg, s.c.) from 41.2 ± 3.3 min (n = 6) to 53 ± 2.2 min (n = 5, P <0.01) and 58.8 ± 4.9 min (n = 4, P <0.05), respectively. In contrast, pretreatment with K252a for either 30 or 60 min had no effect on the latency for the burst occurrence and the total burst number (Fig. 4Bb, *C*a). However, K252a pretreatment for 60 min, but not for 30 min, significantly suppressed the burst frequency from 0.0092 ± 0.0016 Hz (n = 6) to 0.0035 ± 0.0005 Hz (n = 4, P <0.05). These results indicate that K252a, a TrkB receptor inhibitor, has a suppressive effect

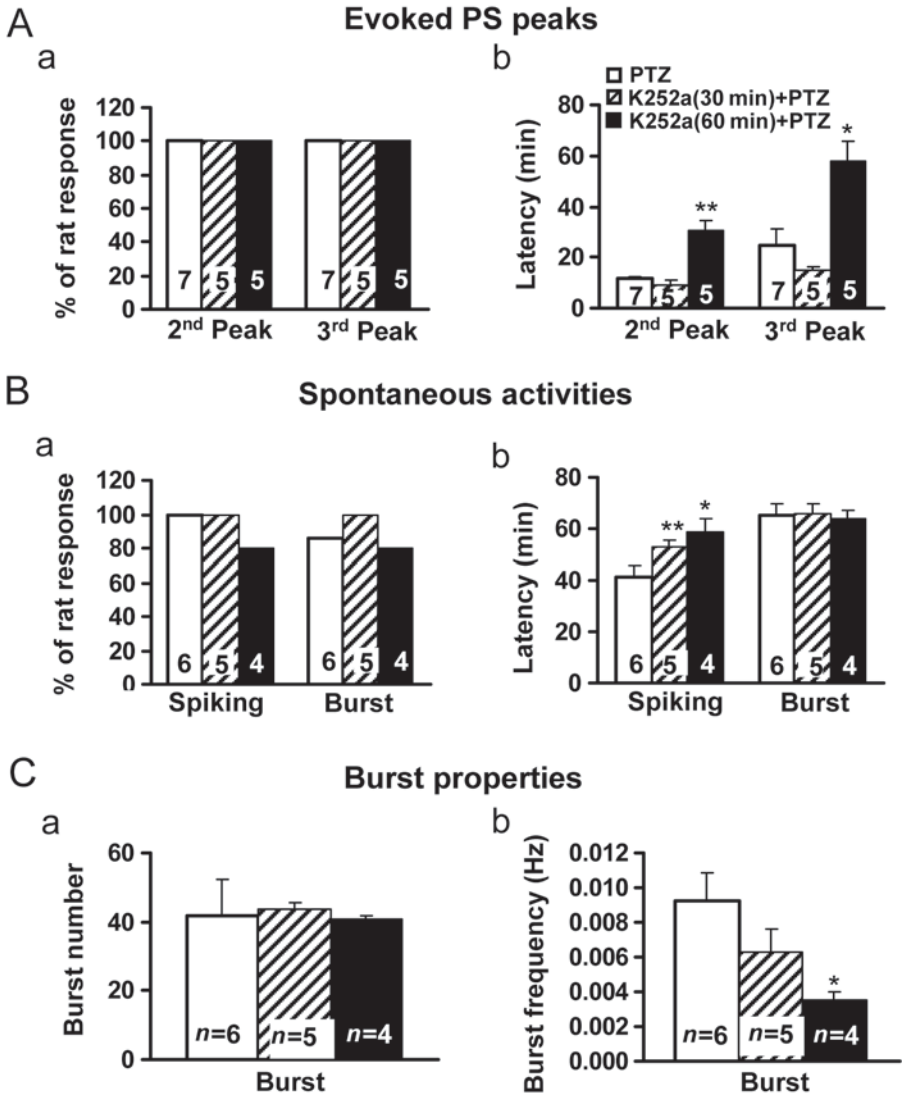

Fig. 4. K252a suppressed PTZ-induced epileptiform activity in hippocampal CA1 in anesthetized rats. Bar histograms showing that K252a (250 μmol/L, 5 μL, i.c.v.) delayed PTZ-evoked (165 mg/kg, s.c.) CA1 pyramidal neuron PS peak transformation from a single to epileptiform multiple peaks (A), and the PTZ-induced epileptiform spontaneous high-amplitude spike activity (B). But K252a did not affect the bursts induced by PTZ (C). *P <0.05, **P <0.01 *versus* the PTZ control.

on the epileptiform activity progression induced by PTZ.

DISCUSSION

In the current study, by using the PTZ model, we demonstrated that PTZ induces the progressive development of the epileptiform activity in CA1 pyramidal neurons in a dose-dependent manner, given either i.v. or s.c. Blocking BDNF–TrkB signaling with the TrkB receptor antagonist K252a significantly suppressed the occurrence of multiple PS peaks and the interictal-like spontaneous high-amplitude spike firing induced by PTZ, but had minor effects on the PTZ-induced highly-synchronized bursting activity. These results suggested that the BDNF–TrkB signaling pathway is involved in the early phases of PTZ-induced epileptogenesis, but has minor effects on the epileptic seizure-related synchronized neuronal burst activity.

PTZ is widely used to generate seizure animal models for epilepsy studies[2,3], which have shown that PTZ-kindling in rats induces moderate neuronal loss in hippocampal CA1, CA3, CA4 and the dentate gyrus[3,33], and increases the proliferation of granule cell precursors in the adult brain[34]. A PTZ-induced generalized seizure in immature rats provokes endogenous ACh-induced interictal-like discharges in adult hippocampal CA3[35]. An *in vitro* study also showed that perfusion of cell cultures with PTZ (10 mmol/L) results in the generation of ictal-like epileptiform activity[36]. Our previous *in vivo* study also preliminarily showed that systemic (s.c.) application of PTZ induces epileptiform activities in the hippocampus[3]. In the current study, we used *in vivo* electrophysiological techniques to record hippocampal CA1 neuronal activity when PTZ was given either i.v. or s.c. Our results demonstrated that PTZ had dose-dependent effects on the induction of epileptiform activity in CA1 hippocampal neurons in anesthetized rats. When either saline or a low dose of PTZ (55 mg/kg) was injected i.v., no epileptiform activity was evoked. However, when the PTZ dose was increased to 110 and 220 mg/kg, the hippocampal neurons exhibited bursting activity at a very short latency. In contrast, when PTZ was given s.c., the burst activity had a long latency to the initiation of stimulus-evoked PS peak transformation from single to multiple peaks and spontaneous high amplitude interictal-like spikes. The induction of different patterns of CA1 neuronal epileptic responses when PTZ was administered either i.v. or s.c. was probably due to different concentrations of PTZ (or its metabolites) in the brain. The concentration of PTZ by i.v. injection rises quickly and reaches the maximal level within a few minutes or even seconds. Indeed, the burst-firing responses of CA1 neurons to PTZ occurred even when the PTZ was being injected (by slow injection, see Methods). However, the CA1 neuronal response to s.c. injection of PTZ was much slower, with a pattern similar to that induced by the convulsant cyclothiazide[20,30]: an initial transformation of the stimulus-evoked PS from a single peak to epileptiform multiple peaks, and then the occurrence of interictal-like high-amplitude spontaneous spike firing, to the final ictal-like highly-synchronized bursting. It should be noted that the current study was performed using the anesthetized animal, in which the anesthetic would affect the response to convulsant stimulation. Thus, the dose of the convulsant used to evoke the epileptiform activity and the latency of the neurons to respond to the convulsant stimulation *per se* were certainly different from those of animals in a conscious state. Indeed, our previous studies on cyclothiazide-induced seizures showed that the doses needed to evoke epileptic seizures in awake rats (Racine score III and above as well as bursting EEG) were much lower than those in anesthetized rats (hippocampal bursting activity)[20,29]. However, since in the current study we compared the response patterns of hippocampal neurons to the PTZ administered by either the i.v. or s.c. route, and then studied the effect of blockade of the BDNF–TrkB signaling pathway on the PTZ-induced epileptic activity, we believe that use of the anesthetized rats, as in a previous study[29], did not affect the conclusions drawn from the results.

In this study, our results demonstrated that, when the animals were given PTZ by s.c. injection, 100% of the rats showed, at the first stage, multiple peaks of the PS, a traditional determinant of epileptogenesis. Spontaneous epileptic seizure-like bursts occurred in 100% of the rats given either 165 or 550 mg/kg PTZ at a relatively long latency. Such ictal-like activity was composed of high-amplitude and high-frequency synchronized bursting, similar to both the cyclothiazide and KA models[3,20] as well as human temporal lobe epilepsy[37,38]. Between the the evoked response change and the spontaneous bursting

activity, low-frequency, interictal-like high-amplitude individual spikes occurred. This progressive pattern of the epileptogenesis induced by PTZ suggests that the epileptiform neuronal activity has to reach a certain threshold before becoming abnormally synchronized. Indeed, in our current experiment, low dose of PTZ (55 mg/kg) only evoked spontaneous interictal-like spike activity, but no transformation to bursting (at least in our 3-h recording period), further supporting the notion that the formation of epileptiform bursts depends on an activity threshold. Once the threshold is exceeded, the network activity is synchronized. The reason for the network activity to surpass the threshold for inducing ictal-like bursting activity remains to be investigated.

BDNF is a secreted protein[39] that, in human, is encoded by the BDNF gene[40,41], which belongs to the neurotrophin family of growth factors. BDNF plays important roles in the CNS: promoting the growth and differentiation of new neurons and synapses, supporting the survival of existing neurons[42,43], and enhancing hippocampal learning and memory[44,45]. BDNF binds to at least two receptors, TrkB and TrkA, on the surface of cells capable of responding to it[46]. BDNF–TrkB signaling is likely a common pathway involved in many different epilepsy models[9], including KA, kindling, pilocarpine and cyclothiazide models[20,22-25]. BDNF potentiates glutamatergic neurotransmission as well as inhibiting GABAergic neurotransmission[47,48]. Thus, the excessive activity evoked by a convulsant, such as PTZ, may induce BDNF release to further enhance neuronal excitability and thus promote the occurrence of epilepsy.

However, previous results on the relationship between BDNF and PTZ are contradictory. Some studies showed that PTZ-induced seizures strongly increase BDNF mRNA levels without corresponding increases of the protein[27]. Other studies showed that in a PTZ model, astrocytes may participate in epilepsy through a marked but transient decrease in BDNF expression[26]. Thus, the relationship between BDNF and PTZ is unclear. In the current study, we used the progressive pattern of hippocampal neuronal epileptogenesis evoked by PTZ *in vivo* to address the relationship between PTZ-induced seizures and the BDNF–TrkB signaling pathway. Our results demonstrated that, by using the TrkB receptor inhibitor K252a, BDNF–TrkB signaling pathway was directly involved in the progressive development of the interictal-like spontaneous spiking activity, but with little effect on the ictal-like bursting discharges. The reason why K252a had only a minor effect on the PTZ-induced bursting activity, but significantly affected the progression of epileptogenesis (i.e. latency to both the PS peaks and spontaneous spike firing), is likely due to the induction mechanism of PTZ. PTZ is mainly considered to be a non-selective $GABA_A$ receptor inhibitor, which reduced GABAergic function in the CNS. Epileptic-seizure, which is directly related to the synchronized neuronal bursting activity, involves complex changes of cell membrane and intracellular signaling pathways. Our recent study on the mechanism of interaction between the $GABA_A$ receptor and K^+-Cl^+ co-transporter 2 in synchronizing neuronal epileptic bursting (unpublished data) suggested that reduced GABA inhibitory function alone is not sufficient to generate this synchronization. In addition, the current result is different from a previous study showing that BDNF–TrkB pathway is a major signaling pathway in mediating epileptic-seizure induction in hippocampus in the cyclothiazide model[20]. Cyclothiazide is a dual-action convulsant acting by enhancing AMPA receptor transmission and inhibiting $GABA_A$ receptor function, while PTZ mainly acts as a $GABA_A$ receptor inhibitor[1,20]. This may explain the different involvement of the BDNF–TrkB signaling pathway in these two seizure models.

In conclusion, we demonstrated here that PTZ stimulates hippocampal neuronal epileptogenesis initially by evoking multiple PS peaks, towards spontaneous high-amplitude spike firing and to the final highly-synchronized burst firing a time-dependent manner, and that the BDNF–TrkB signaling pathway is involved in this PTZ-induced progressive change of neuronal epileptiform activity. These results provide basic insights into the pattern of change in hippocampal neuronal activity during the development of the PTZ seizure model, and this *in vivo* electrophysiological seizure model could be used for future epilepsy studies.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (31129003, 81171224, and 31271188), Shanghai Municipal Science and Technology Commission, China (10JC1402800) and Shanghai Municipal Health Bureau, China (20114218).

Received date: 2012-10-12; Accepted date: 2012-11-28

REFERENCES

[1] Qu H, Eloqayli H, Sonnewald U. Pentylenetetrazole affects metabolism of astrocytes in culture. J Neurosci Res 2005, 79: 48–54.

[2] Zhao R, Weng CC, Feng Q, Chen L, Zhang XY, Zhu HY, *et al.* Anticonvulsant activity of BmK AS, a sodium channel site 4-specific modulator. Epilepsy Behav 2011, 20: 267–276.

[3] Qian B, Sun Y, Wu Z, Wan L, Chen L, Kong S, *et al.* Epileptiform response of CA1 neurones to convulsant stimulation by cyclothiazide, kainic acid and pentylenetetrazol in anaesthetised rats. Seizure 2011, 20: 312–319.

[4] Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J 1982, 1(5): 549–553.

[5] Binder DK. Neurotrophins in the dentate gyrus. Prog Brain Res 2007, 163: 371–397.

[6] Cardile V, Pavone A, Gulino R, Renis M, Scifo C, Perciavalle V. Expression of brain-derived neurotrophic factor (BDNF) and inducible nitric oxide synthase (iNOS) in rat astrocyte cultures treated with Levetiracetam. Brain Res 2003, 976: 227–233.

[7] Goodman LJ, Valverde J, Lim F, Geschwind MD, Federoff HJ, Geller AI, *et al.* Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. Mol Cell Neurosci 1996, 7: 222–238.

[8] Balkowiec A, Katz DM. Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA *in situ*. J Neurosci 2000, 20: 7417–7423.

[9] Binder DK, Croll SD, Gall CM, Scharfman HE. BDNF and epilepsy: too much of a good thing? Trends Neurosci 2001, 24: 47–53.

[10] Croll SD, Suri C, Compton DL, Simmons MV, Yancopoulos GD, Lindsay RM, *et al.* Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and *in vitro* hyperexcitability in the hippocampus and entorhinal cortex. Neuroscience 1999, 93(4): 1491–1506.

[11] Papaleo F, Silverman JL, Aney J, Tian Q, Barkan CL, Chadman KK, *et al.* Working memory deficits, increased anxiety-like traits, and seizure susceptibility in BDNF overexpressing mice. Learn Mem 2011, 18(8): 534–544.

[12] Kotloski R, McNamara JO. Reduction of TrkB expression de novo in the adult mouse impairs epileptogenesis in the kindling model. Hippocampus 2010, 20(6): 713–723.

[13] Kafitz KW, Rose CR, Thoenen H, Konnerth A. Neurotrophin-evoked rapid excitation through TrkB receptors. Nature 1999, 401: 918–921.

[14] Rose CR, Blum R, Kafitz KW, Kovalchuk Y, Konnerth A. From modulator to mediator: rapid effects of BDNF on ion channels. Bioessays 2004, 26: 1185–1194.

[15] Gall C, Lauterborn J, Bundman M, Murray K, Isackson P. Seizures and the regulation of neurotrophic factor and neuropeptide gene expression in brain. Epilepsy Res Suppl 1991, 4: 225–245.

[16] Elmer E, Kokaia Z, Kokaia M, Carnahan J, Nawa H, Lindvall O. Dynamic changes of brain-derived neurotrophic factor protein levels in the rat forebrain after single and recurring kindling-induced seizures. Neuroscience 1998, 83: 351–362.

[17] He XP, Minichiello L, Klein R, McNamara JO. Immunohistochemical evidence of seizure-induced activation of trkB receptors in the mossy fiber pathway of adult mouse hippocampus. J Neurosci 2002, 22: 7502–7508.

[18] Kokaia M, Ernfors P, Kokaia Z, Elmér E, Jaenisch R, Lindvall O. Suppressed epileptogenesis in BDNF mutant mice. Exp Neurol 1995, 133: 215–224.

[19] Bergami M, Berninger B, Canossa M. Conditional deletion of TrkB alters adult hippocampal neurogenesis and anxiety-related behavior. Commun Integr Biol 2009, 2: 14–16.

[20] Wang Y, Qi JS, Kong S, Sun Y, Fan J, Jiang M, *et al.* BDNF-TrkB signaling pathway mediates the induction of epileptiform activity induced by a convulsant drug cyclothiazide. Neuropharmacology 2009, 57: 49–59.

[21] Scharfman HE, Goodman JH, Sollas AL, Croll SD. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor. Exp Neurol 2002, 174: 201–214.

[22] Lahteinen S, Pitkanen A, Saarelainen T, Nissinen J, Koponen E, Castrén E. Decreased BDNF signaling in transgenic mice reduces epileptogenesis. Eur J Neurosci 2002, 15: 721–734.

[23] Gleeson LC, Ryan KJ, Griffin EW, Connor TJ, Harkin A. The beta2-adrenoceptor agonist clenbuterol elicits neuroprotective, anti-inflammatory and neurotrophic actions in the kainic acid model of excitotoxicity. Brain Behav Immun 2010, 24: 1354–1361.

[24] Paradiso B, Zucchini S, Su T, Bovolenta R, Berto E, Marconi P, *et al.* Localized overexpression of FGF-2 and BDNF in hippocampus reduces mossy fiber sprouting and spontaneous seizures up to 4 weeks after pilocarpine-induced status epilepticus. Epilepsia 2011, 52: 572–578.

[25] Tongiorgi E, Armellin M, Giulianini PG, Bregola G, Zucchini S, Paradiso B, *et al.* Brain-derived neurotrophic factor mRNA and protein are targeted to discrete dendritic laminas by events that trigger epileptogenesis. J Neurosci 2004, 24: 6842–6852.

[26] Nanda SA, Mack KJ. Seizures and sensory stimulation result in different patterns of brain derived neurotrophic factor protein expression in the barrel cortex and hippocampus. Brain Res Mol Brain Res 2000, 78: 1–14.

[27] Zhu H, Chen MF, Yu WJ, Wang WJ, Li F, Liu WC, *et*

al. Time-dependent changes in BDNF expression of pentylenetetrazole-induced hippocampal astrocytes *in vitro*. Brain Res 2012, 1439: 1–6.

[28] Behr J, Lyson KJ, Mody I. Enhanced propagation of epileptiform activity through the kindled dentate gyrus. J Neurophysiol 1998, 79(4): 1726–1732.

[29] Kong S, Qian B, Liu J, Fan M, Chen G, Wang Y. Cyclothiazide induces seizure behavior in freely moving rats. Brain Res 2010, 1355: 207–213.

[30] Qi J, Wang Y, Jiang M, Warren P, Chen G. Cyclothiazide induces robust epileptiform activity in rat hippocampal neurons both *in vitro* and *in vivo*. J Physiol 2006, 571: 605–618.

[31] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates, seconded. New York: Academic Press, 1986.

[32] Wheal HV, Bernard C, Chad JE, Cannon RC. Pro-epileptic changes in synaptic function can be accompanied by pro-epileptic changes in neuronal excitability. Trends Neurosci 1998, 21: 167–174.

[33] Pavlova T, Stepanichev M, Gulyaeva N. Pentylenetetrazole kindling induces neuronal cyclin B1 expression in rat hippocampus. Neurosci Lett 2006, 392: 154–158.

[34] Jiang W, Xiao L, Wang JC, Huang YG, Zhang X. Effects of nitric oxide on dentate gyrus cell proliferation after seizures induced by pentylenetrazol in the adult rat brain. Neurosci Lett 2004, 367: 344–348.

[35] Meilleur S, Aznavour N, Descarries L, Carmant L, Mamer OA, Psarropoulou C. Pentylenetetrazol-induced seizures in immature rats provoke long-term changes in adult hippocampal cholinergic excitability. Epilepsia 2003, 44: 507–517.

[36] Raza M, Shaheen F, Choudhary MI, Rahman AU, Sombati S, DeLorenzo RJ. *In vitro* inhibition of pentylenetetrazole and bicuculline-induced epileptiform activity in rat hippocampal pyramidal neurons by aqueous fraction isolated from Delphinium denudatum. Neurosci Lett 2002, 333: 103–106.

[37] Avoli M, D'Antuono M, Louvel J, Köhling R, Biagini G, Pumain R, *et al.* Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system *in vitro*. Prog Neurobiol 2002, 68: 167–207.

[38] Avoli M, Louvel J, Pumain R, Köhling R. Cellular and molecular mechanisms of epilepsy in the human brain. Prog Neurobiol 2005, 77: 166–200.

[39] Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors 2004, 22: 123–131.

[40] Jones KR, Reichardt LF. Molecular cloning of a human gene that is a member of the nerve growth factor family. Proc Natl Acad Sci U S A 1990, 87: 8060–8064.

[41] Maisonpierre PC, Le BMM, Espinosa R 3rd, Ip NY, Belluscio L, de la Monte SM, *et al*. Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. Genomics 1991, 10: 558–568.

[42] Acheson A, Conover JC, Fandl JP, DeChiara TM, Russell M, Thadani A, *et al.* A BDNF autocrine loop in adult sensory neurons prevents cell death. Nature 1995, 374: 450–453.

[43] Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 2001, 24: 677–736.

[44] Yamada K, Nabeshima T. Brain-derived neurotrophic factor/TrkB signaling in memory processes. J Pharmacol Sci 2003, 91: 267–270.

[45] Lee MC, Okamoto M, Liu YF, Inoue K, Matsui T, Nogami H, *et al.* Voluntary resistance running with short distance enhances spatial memory related to hippocampal BDNF signaling. J Appl Physiol 2012,113(8): 1260–1266.

[46] Patapoutian A, Reichardt LF. Trk receptors: mediators of neurotrophin action. Curr Opin Neurobiol 2001, 11: 272–280.

[47] Kang H, Schuman EM. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science 1995, 267: 1658–1662.

[48] Zhu WJ, Roper SN. Brain-derived neurotrophic factor enhances fast excitatory synaptic transmission in human epileptic dentate gyrus. Ann Neurol 2001, 50: 188–194.