

## Effects of Chronic Stress and Phenytoin on the Long-term Potentiation (LTP) in Rat Hippocampal CA1 Region

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**Abstract** To investigate the changes of LTP in hippocampal CA1 region induced by chronic stress and the effect of phenytoin on them, thirty-two adult male Sprague-Dawley rats were randomly divided equally into four groups: control group, control-phenytoin group, stress-saline group and stress-phenytoin group. Isolated hippocampal slices of rats were used to observe the changes of long-term potentiation (LTP) in hippocampal CA1 field using electrophysiological technique. Amplitude of population spike (PS) and field excitatory postsynaptic potentials (fEPSPs) slope were used to indicate the changes of LTP. High-frequency stimulation (HFS) was applied to Schaffer collaterals of hippocampal CA3 field, and the changes of PS amplitude and fEPSPs slope in CA1 field were observed. The results showed that the LTP induction rate, the increases of PS amplitude and fEPSPs slope after HFS in control and stress-phenytoin groups were significantly greater than those in stress-saline group ( $P < 0.05$ ). There were no significant differences between control group and stress-phenytoin group or between control and control-phenytoin groups in these indexes ( $P > 0.05$ ). It is suggested that chronic stress can damage the development of LTP in hippocampal CA1 field, while phenytoin can protect the LTP of stressed hippocampal slices in normal state.

**Key words** phenytoin; long-term potentiation; hippocampus; stress

Stress is the response to stimulation from inside and outside with complicated effects on organisms. Appropriate stressful reactions are helpful in resisting diseases by activating unspecific modulation system, while severe or prolonged stresses are harmful and even induce mental and physical disorders such as recurrent depression, post-traumatic stress disorder (PTSD), Alzheimer's disease and epilepsy [1]. Hippocampus, a main brain region of key importance for learning, memory and emotion, is the target of stressful hormone and mediates stress response. It has plasticity and is liable to be impaired during chronic stress and participates in the pathology of some stress-related diseases that markedly affect memory and other cognitive functions [2,3]. Exploring the effects of stress on hippocampal functions can help to reveal pathogenesis of stress-related disorders.

Long-term potentiation (LTP), a persistent increase in the efficacy of synaptic connections induced by high-frequency

stimulation (HFS), has been regarded as a model of synapse plasticity. LTP in the hippocampus is especially related to learning and memory. Most scholars consider that  $Ca^{2+}$  entering into the postsynaptic cells triggers the development of LTP [4–6]. Excitation of presynapses induces release of excitatory amino acid (EAA), which facilitates the  $Ca^{2+}$  influx into postsynapses by combining with *N*-methyl-*D*-aspartic acid (NMDA) receptors. The increase of intracellular  $Ca^{2+}$  provokes a series of physiochemical reactions and induces LTP. It has been reported that exposure to stressful event can impair the induction of LTP in the hippocampus [7–9]. However, the mechanisms of LTP suppression under stress have not been fully elucidated.

Phenytoin, an anti-epileptic drug, can decrease the  $Ca^{2+}$  influx, reduce the EAA release, and enhance presynaptic and postsynaptic inhibition. It was reported that phenytoin could also prevent the stress-induced reductions in CA3 apical dendritic length and branch point numbers [10, 11]. It was unknown whether phenytoin could protect hippocampal cognitive functions from stress damage.

In our recent study, we investigate the changes of LTP

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in hippocampal CA1 region caused by chronic stress and the effect of phenytoin on them.

## Materials and Methods

### General methods

Thirty-two adult male Sprague-Dawley rats (Shanghai Sippr-Bk Lab Animal Co., Ltd.), weighing 200–300 g each, were used. The rats were allowed for a week recovery from transport before they were used in the experiment. Rats were maintained at  $23 \pm 0.5$  °C in a light controlled environment (light turned on at 7 am and off at 7 pm), and supplied with food and water. Thirty two rats were divided equally into four groups. Rats in control group were untreated. Rats in phenytoin control group were injected intraperitoneally with phenytoin (Sigma) (40 mg/kg body weight) everyday. Rats in stress-saline group and stress-phenytoin group were injected intraperitoneally with saline (10 ml/kg body weight) and phenytoin (40 mg/kg body weight) respectively before they were forced to swim in a rectangular glass container made by ourselves (80 cm×40 cm×40 cm) for 15 min each day in 4 weeks. Water temperature was ( $24 \pm 1$ ) °C.

### Slice preparation

After four weeks, all rats were lightly anaesthetized with ether and decapitated. The brain was quickly removed and placed in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) for about 2 min. The two hippocampi were dissected transversely and mid-dorsally, and hippocampal slices (500  $\mu$ m thick) were prepared using a tissue chopper (Americal TEYLER Lab). The slices were kept in ACSF at room temperature in a continuously oxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub>) chamber. Four to five slices were placed on nylon net in an interface chamber (Americal TBYLER Lab) and incubated for 90 min. The slices bathed constantly with humidified 95% O<sub>2</sub> and 5% CO<sub>2</sub> were superfused (2 ml/min) continuously with oxygenated ACSF at 32–33 °C. The ACSF consisted of (in mM) NaCl 124.0, KCl 3.5, NaH<sub>2</sub>PO<sub>4</sub> 1.25, MgSO<sub>4</sub> 1.5, NaHCO<sub>3</sub> 25, CaCl<sub>2</sub> 2.0 and 10.0 glucose. The time between tissue preparation and recording was less than 9 h.

### Stimulation and recording

Extracellular field potentials were recorded using 2 M NaCl-filled glass microelectrodes with tip resistances 1–2 M $\Omega$ . Field excitatory postsynaptic potentials (fEPSPs) and

population spikes were recorded by electrodes placing in the CA1 pyramidal cell layer. Polar stimulating electrodes were positioned slightly below the surface of the Schaffer collaterals. Field potentials were filtered, amplified and simultaneously displayed on a VC-11 storage oscilloscope. fEPSPs slope and PS amplitude were measured.

Two slices were selected from each rat for the final analysis of data according to three criteria: (1) the baseline of the field potentials should be stable for at least 15 min; (2) at half maximum intensity, at least 1 mV PS must be evoked; (3) ratio of PS<sub>post HFS</sub> vs. PS<sub>Baseline</sub> amplitude representing induction of LTP should be higher than 150% and persisted for at least 30 min. After the baseline was recorded, HFS (100 Hz, 100 pulses) was applied and recording was continued for at least 30 min to determine the effects of HFS. PS amplitude and fEPSPs slope were expressed in percent changes from baseline.

### Data analysis and statistics

Data were expressed as mean  $\pm$  SD. PS amplitude and fEPSPs slope changes were analyzed by one-way ANOVA followed by post hoc comparison. The LTP induction rate was analyzed by Chi-square.

## Results

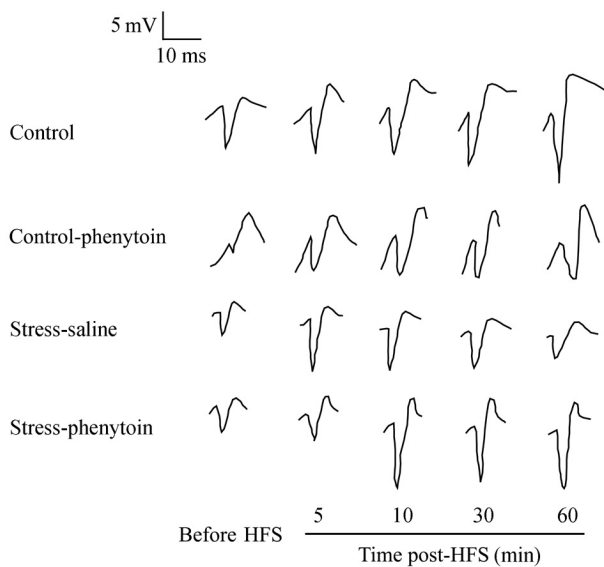
### LTP induction rate

LTP induction rates after HFS in control group (56%) and in stress-phenytoin group (50%) were significantly higher than those in stress-saline group (19%) ( $P < 0.01$ , Chi-square test). There was no significant difference between control group and stress-phenytoin group, or control group and control-phenytoin group (62%) ( $P > 0.05$ ).

Fig. 1 illustrated typical PS and fEPSPs recorded on slices in CA1 pyramidal cells from control, control-phenytoin, stress-saline and stress-phenytoin groups. The increases of PS amplitude and fEPSPs slope on slices after HFS in stress-saline group were smaller than those in control group or stress-phenytoin group. The slices of stress-phenytoin group exhibited normal increases of PS amplitude and fEPSPs slope compared with control group. The slices of control and control-phenytoin groups showed similar increases of PS amplitude and fEPSPs slope after HFS.

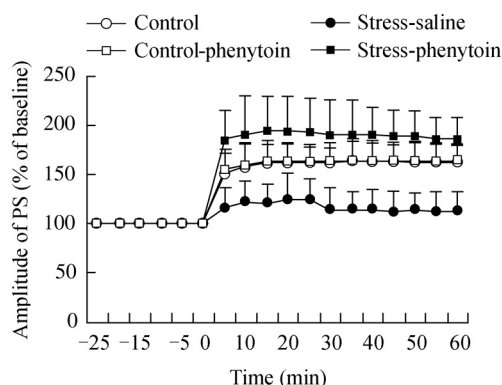
### Amplitude increase of PS

Our results showed that 4-week forced-swimming treatment suppressed the increase of PS amplitude after HFS



**Fig. 1** Schaffer collateral/commissural-evoked extracellular PS and fEPSPs recorded on slices in field CA1 from control group, control-phenytoin group, stress-saline group and stress-phenytoin group

in rats. As shown in Fig. 2, HFS in control group or in stress-phenytoin group elicited a robust increase in PS amplitude, which was significantly different from that in stress-saline group ( $P < 0.01$ ). In comparison with the control group, similar HFS in slices from stress-phenytoin rats produced greater, but insignificant ( $P > 0.05$ ) amplitude of PS. There was no significant difference between control group and control-phenytoin group ( $P > 0.05$ ).

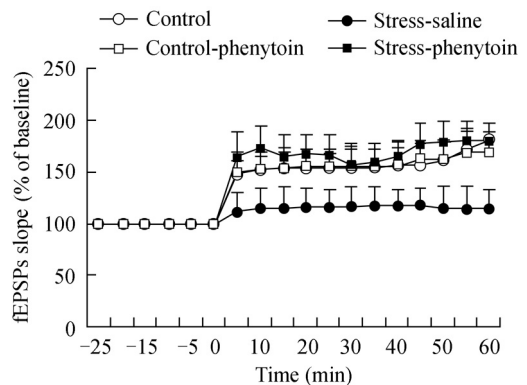


**Fig. 2** Effects of stress and phenytoin on the amplitude of PS in the CA1 region ( $n=8$ )

**fEPSPs slope**

Our results also showed that 4-week forced-swimming suppressed the increase of fEPSPs slope in hippocampus after HFS in rats (Fig. 3). In comparison with control group

or stress-phenytoin group, significantly smaller fEPSPs slope at all post-HFS was found in stress-saline group ( $P < 0.05$ ). There was no significant difference between control group and stress-phenytoin group ( $P > 0.05$ ) or between control and control-phenytoin groups ( $P > 0.05$ ).



**Fig. 3** Effects of stress and phenytoin on the fEPSPs slope in the CA1 region ( $n=8$ )

**Discussion**

Our present study revealed that chronic forced-swimming suppressed LTP induction in the hippocampal CA1 field as shown in other studies [7–9]. Foy and his colleagues [12] reported that behavioral stress inhibited the development of LTP in CA1 region of hippocampal slice, and other stress paradigms (for example, exposure to brightly chamber) also impaired LTP and primed burst potentiation—a low-threshold form of LTP. The underlying mechanisms of the stress damage to LTP are unclear. Previous studies in our laboratory showed that forced-swimming for 4 weeks induced atrophy of the apical dendrites and loss of CA3 pyramidal neurons in rats [13,14]. Other researchers also found that stress could damage CA3 pyramidal neurons [15,16]. As dendrites are crucial structures for signal transmission of neural system, we presume that the loss of neurons and changes of synapse structure induced by stress reduce the synaptic message transmission and depress the development of LTP ultimately.

Our results showed clearly that phenytoin could keep LTP from being inhibited by stress, but not affect normal LTP development. Compared with the control group, HFS in slices from stress-phenytoin rats produced greater, though not significant, PS amplitude and fEPSPs slope. These results suggested that phenytoin had protective actions on long-term synaptic plasticity. Although phenytoin was reported to protect neurons in hippocampal CA3

region from atrophy induced by stress, the effect of phenytoin on hippocampal functions is still unknown. Our current finding is the first demonstration of functional protective action of phenytoin to keep hippocampal LTP from damage by stress.

Calcium is important in a variety of neurotoxic systems. Stress-induced metaplasticity can disrupt  $\text{Ca}^{2+}$  homeostasis and thus endanger hippocampal neurons [3]. A number of studies reported that phenytoin inhibited  $\text{Ca}^{2+}$  influx [17] and diminished EAA release [18]. This may be critical for protective effects of phenytoin against stress.

Our studies showed that protective effects of phenytoin not only promoted cell survival, but also improved cellular function during stress. Up to now, there is no effective medication in certain stress-related disorders. We expect that our studies could offer a theoretical basis to improve the therapy of these disorders.

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