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# Influence of hippocampal low-frequency stimulation on $GABA_A$ R α1, ICER and BNDF expression level in brain tissues of amygdala-kindled drug-resistant temporal lobe epileptic rats

Kaya Xu[a], Zhaoyang Liu[b], Likun Wang[b], Guofeng Wu[b,*,1], Tao Liu[c,*,1]

[a] Department of Neurosurgery, Affiliated Hospital of Guizhou Medical University, Guizhou, Guiyang City, PR China
[b] Department of Neurology, Affiliated Hospital of Guizhou Medical University, Guizhou, Guiyang City, PR China
[c] Guizhou Province Centre of Disease Control and Prevention, Guiyang City, PR China

## HIGHLIGHTS

- The levels of ICER and BDNF were the lowest in Hip-LFS drug-resistant epileptic rats.
- The expression of $GABA_A$ receptor subunit α1 was increased significantly post Hip-LFS.
- Seizure degree was reduced and EEGs were improved in Hip-LFS drug-resistant epileptic rats.
- ICER, BDNF signaling is a therapeutic target for the treatment of Hip-LFS.

## ARTICLE INFO

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## ABSTRACT

This study investigated the therapeutic effect of hippocampal low-frequency stimulation (Hip-LFS) and its influence on the type A γ-aminobutyric acid receptor α1 subunit ($GABA_A$ R α1 subunit), inducible cAMP early repressor (ICER) and brain-derived neurotrophic factors (BNDF). The model of epilepsy was induced by chronic electrical stimulation in amygdala. Drug-resistant and drug-sensitive epileptic rats were selected by testing their seizure response to phenytoin and phenobarbital. The changes of $GABA_A$ R α1 subunit, ICER and BDNF expression were detected via immunohistochemistry and western blot. The expression levels of ICER and BDNF were increased remarkably but the $GABA_A$ R α1 subunit decreased significantly in the drug-resistant epileptic rats. However, the expression levels of ICER, BDNF were decreased and the expression of the $GABA_A$ R α1 subunit increased significantly in the drug-resistant epileptic rats after two weeks of Hip-LFS. Meanwhile, the seizure degree was reduced and the electroencephalograms were improved. The present study demonstrated that increased ICER and BDNF might be associated with the development of drug-resistance. The effect of Hip-LFS in the treatment of drug-resistant epileptic rats might be associated with increasing the levels of the ICER and the BDNF.

## 1. Introduction

Epilepsy is one of the most common and severe disease of central nervous system. Although most of the epileptic patients could be permanently relieved with regular anticonvulsant therapy, there are still about 1/3 of epileptic patients fail to response effectively to traditional antiepileptic medications (AEDs). These patients are considered to be medically intractable or drug-resistant (Jobst and Cascino,

*Abbreviations:* Hip-LFS, hippocampal low-frequency stimulation; $GABA_A$ R, type A γ-aminobutyric acid receptor; $GABA_A$ R α1, type A γ-aminobutyric acid receptor α1; GABA, γ-aminobutyric acid; ICER, inducible cAMP early repressor; CRE, cAMP-responsive element; CREB, cAMP-response element binding protein; p-CREB, phosphorylated cAMP-response element binding protein; BNDF, brain-derived neurotrophic factors; AEDs, antiepileptic medications; DBS, deep brain stimulation; PHT, phenytoin sodium injection; PB, phenobarbital; ADT, after-discharge threshold; EEG, Electroencephalograms; SD, Sprague–Dawley; DRC, drug-resistant control; HLS, drug-resistant hippocampal low-frequency stimulation; DSC, drug-sensitive control; NRC, normal rat control; OD, average optical density; SE, status epilepticus; IOD, Integrated Optical Density

* Corresponding authors at: Department of Neurology, Affiliated Hospital of Guizhou Medical University, Guizhou Province, PR China (G. Wu).
*E-mail addresses:* wuguofeng3013@sina.com (G. Wu), 543820987@qq.com (T. Liu).

[1] Guofeng Wu and Tao Liu contributed equally to this paper. They are the co-corresponding author.

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2015; Kwan et al., 2010). Temporal lobe epilepsy is considered one of the most common types of drug-resistant epilepsy. Epilepsy surgery has become the most effective and approved treatment method for drug-resistant epilepsy (Schusse et al., 2018). Good outcome might be achieved by resecting the anterior temporal lobe in patients with TLE (Elsharkawy et al., 2009). However, only 50% of patients undergone resective surgery could be seizure free (Hirsch, 2012; Petkar et al., 2012). Recently, deep brain stimulation (DBS) has developed as a new therapeutic technique for patients with drug-resistant epilepsy (Tykocki et al., 2012). Clinical and experimental studies have verified hippocampal DBS to be effective in the treatment of drug-resistant epilepsy (Cukiert et al., 2017; McLachlan et al., 2010; Rashid et al., 2012; Wu et al., 2013; Yu et al., 2018; Zhang et al., 2009). However, the cellular mechanisms underlying the therapeutic effects of DBS remain largely undefined.

It is well known type A γ-aminobutyric acid receptor ($GABA_A$ R) is the major inhibitory neurotransmitter receptor in the brain. Previous studies suggested that the reduction in $GABA_A$ R expression level may be one of the critical mechanisms accounting for the neurobiology of drug-resistance epilepsy (Bethmann et al., 2008), with a strong association between the reduction of $GABA_A$ R α1 subunit expression with generalized epilepsy (Arain et al., 2012; Lachance-Touchette et al., 2011; Maljevic et al., 2006). ICER, a member of cAMP-response element binding protein (CREB) family, were also targeted by phosphorylated cAMP-response element binding protein (p-CREB), study shown that in epileptic rat model, p-CREB can form a dimer with ICER and bind the CRE binding site on the promoter region of $GABA_A$ R α1 subunit, modulating its expression level (Lund et al., 2008). The level of p-CREB and ICER expression in drug-resistant epileptic brain tissue was found significantly higher. This leads to enhanced binding of p-CREB and ICER to the Gabra1-p CRE, resulting in transcriptional repression and a subsequent reduction in α1 mRNA expression and the number of α1γ2-containing $GABA_A$ Rs (Lund et al., 2008). Previous studies verified that the expression level of BDNF in drug-resistant epileptic brain tissue is significantly higher than normal brain. It can activate ICER and promote its expression through specific pathway, but at the same time act as a modulator of extracellular GABA level (Martinez-Levy et al., 2016). In epileptic model, BDNF activity was found increased, affecting the expression level of $GABA_A$, inducing the occurrence of epilepsy (Martinez-Levy et al., 2016). Our previously published studies have demonstrated that the $GABA_A$ α1 subunit decreased and p-CREB, CREB increased remarkably in drug-resistant epilepsy. And the Hip-LFS could increase the $GABA_A$ R α1 subunit and decrease the CREB, p-CREB (Wu et al., 2017). However, little information regarding the changes of ICER and BDNF in drug-resistant epileptic rats undergone Hip-LFS are reported. In the present study, we reported for the first time that Hip-LFS might decrease the expression of the ICER, BDNF and their relationship with the $GABA_A$ R α1 subunit in amygdala-kindled drug-resistant epileptic rats. This line of investigation might provide valuable data toward a novel understanding of the molecular mechanism underlying the antiepileptic effects of DBS in the treatment of drug-resistant epilepsy.

## 2. Results

### 2.1. Changes of EEG in different groups

To determine the effect of Hip-LFS on seizure activity, the mean frequency of EEG across animals was calculated. The normal EEG displayed a lower frequency and lower amplitude base wave (Fig. 1A). Compared with HLS, DSC, NRC group, there were interictal spikes, high frequency, and high amplitude single peak of epileptic discharge in the DRC group. All animals showed a decrease in mean spike frequency post-HLS and all animals spike reductions were statistically significant ($P < 0.05$). The EEG frequency in the DRC group (19.8 ± 1.686 Hz) was significantly higher than the NRC and the DSC group. In the HLS group, the frequency of EEG (15.800 ± 1.033 Hz) decreased remarkably compared to the DRC group. The frequency in the occurrence of seizures of the DRC group was the highest, compared to the other three groups ($P < 0.05$) (Fig. 1B). These results indicated that Hip-LFS could generate a significant decrease in the frequency of seizure

### 2.2. Effects of the Hip-LFS on seizure degree and duration

Seizure duration was also evaluated during the experimental periods. Hip-LFS showed effects on decreasing the duration of the seizures (mean seizure duration in seconds. Seizure duration after Hip-LFS was reduced to 39.500 ± 3.894 s in the HLS group compared to the DRC group (82.100 ± 9.158 s, $P < 0.05$). Therefore, Hip-LFS resulted in a statistically significant reduction in duration of seizures. Significant differences were observed among the four groups. ($F = 138.782$, $P < 0.05$). Duration of seizures in the DSC group was 36.100 ± 6.557 s, no significant difference was noted as compared with the HLS group (Fig. 2). There were significant differences in the seizure degree between the HLS group and the DRC group as well as the DSC group ($P < 0.05$), no significant difference was noted as compared with the DSC group ($P > 0.05$) (Table.1).

### 2.3. Changes in BDNF, proBDNF and ICER levels

BDNF expression increased markedly after status epilepticus (SE) (Roberts et al., 2006). To confirm whether Hip-LFS could change the BDNF expression, the BDNF was immunostained with specific antibodies, moreover we measured the abundance of proBDNF by Western blot. The positive cells of BDNF were detected in the CA1 and CA3 regions of hippocampus. A little amount of the brown-yellow granules were observed in the CA1 and CA3 regions of NRC group. A large number of brown-yellow granules stained darker were noted in the hippocampal CA3 and CA1 regions, compared with the DSC group and the NRC group. In the HLS group, the number of brown-yellow granules decreased, compared with the DRC group (Fig. 3A). The average light density of BDNF positive cells in the DRC group was significantly higher than the NRC and the DSC group ($P < 0.05$). In the HLS group, the average light density of BDNF positive cells decreased compared to the DRC group ($P < 0.05$). There were no significant difference between the NRC and the DSC group ($P > 0.05$) (Fig. 3B).

The study further found the proBDNF abundance decreased remarkably in the HLS group as measured by Western blotting, and a significant difference was observed among the four groups ($F = 135.368$, $P < 0.05$). The proBDNF abundance was the highest in the DRC group, compared with the other three groups. There was no significant difference in the abundance of proBDNF between the HLS group and the DSC group ($P > 0.05$) (Fig. 3C, D).

Experimental data have shown that the expression of BDNF in drug-resistant epileptic brain tissue was significantly increased. It could activate the ICER and promote its expression through specific pathway (Martinez-Levy et al., 2016). To elucidate the changes in BDNF expression in different groups, the hippocamal tissues were immunostained with anti-ICRE antibodies, and then the abundance of the ICRE was determined by Western blot analysis. In the DRC group, the number of the brown-yellow granules was significantly increased compared to and the NRC group and the DSC group (Fig. 4.A). The average light density of ICER positive cells in the DRC group was significantly higher than the NRC and the DSC group ($P < 0.05$). In the HLS group, the average light density of ICER positive cells decreased compared to the DRC group ($P < 0.05$). There were no significant difference between the NRC and the DSC group ($P > 0.05$) (Fig. 4B). Western blot method also demonstrated results similar to the proBDNF abundance, which showed a significant difference among the four groups ($F = 30.228$, $P < 0.05$). The abundance of the ICER was increased in the DRC group as compared with the NRC group as well as the DSC groups. The ICER levels decreased in the HLS group