EBioMedicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Entorhinal Principal Neurons Mediate Brain-stimulation Treatments for Epilepsy

Zhenghao Xu [a,b,1], Yi Wang [a,1], Bin Chen [a], Cenglin Xu [a], Xiaohua Wu [c], Ying Wang [a], Shihong Zhang [a], Weiwei Hu [a], Shuang Wang [c], Yi Guo [c], Xiangnan Zhang [a], Jianhong Luo [a], Shumin Duan [a], Zhong Chen [a,c,*]

[a] Department of Pharmacology, Key Laboratory of Medical Neurobiology of The Ministry of Health of China, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, China
[b] Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China
[c] Epilepsy Center, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

ARTICLE INFO

Article history:
Received 19 August 2016
Received in revised form 22 November 2016
Accepted 22 November 2016
Available online xxxx

Keywords:
Epilepsy
Neuronal circuit
Optogenetics
Entorhinal cortex
Brain stimulation
Kindling

ABSTRACT

Brain stimulation is an alternative treatment for epilepsy. However, the neuronal circuits underlying its mechanisms remain obscure. We found that optogenetic activation (1 Hz) of entorhinal calcium/calmodulin-dependent protein kinase II α (*CaMKIIα*)-positive neurons, but not GABAergic neurons, retarded hippocampal epileptogenesis and reduced hippocampal seizure severity, similar to that of entorhinal low-frequency electrical stimulation (LFES). Optogenetic inhibition of entorhinal *CaMKIIα*-positive neurons blocked the antiepileptic effect of LFES. The channelrhodopsin-2-eYFP labeled entorhinal *CaMKIIα*-positive neurons primarily targeted the hippocampus, and the activation of these fibers reduced hippocampal seizure severity. By combining extracellular recording and pharmacological methods, we found that activating entorhinal *CaMKIIα*-positive neurons induced the GABA-mediated inhibition of hippocampal neurons. Optogenetic activation of focal hippocampal GABAergic neurons mimicked this neuronal modulatory effect and reduced hippocampal seizure severity, but the anti-epileptic effect is weaker than that of entorhinal LFES, which may be due to the limited spatial neuronal modulatory effect of focal photo-stimulation. Our results demonstrate a glutamatergic-GABAergic neuronal circuit for LFES treatment of epilepsy, which is mediated by entorhinal principal neurons.

1. Introduction

Temporal lobe epilepsy (TLE) is one of the most common types of human epilepsy. The seizures associated with TLE typically arise in the hippocampus, and they are often resistant to antiepileptic drugs (Bialer and White, 2010; Schwartzkroin, 1994). Recurrent uncontrolled hippocampal seizures can result in learning and memory impairments (Lin et al., 2012) as well as sudden unexpected death in TLE patients (Espinosa et al., 2009). Although surgical resection of the epileptic focus may abolish hippocampal seizures, this approach is limited by the strict requirements for surgical selection, the risk of cognitive impairment (Bonelli et al., 2013), and the high recurrence rate for seizures after several years (Berg, 2011; Thom et al., 2010). Recently, brain stimulation has been proposed as an alternative option for treating epilepsy with advantages of reversibility and controllability (Fisher and Velasco, 2014). However, clinical translation of brain stimulation techniques requires an understanding of its underlying mechanisms, especially because electrical stimulation at specific brain areas can induce memory impairment (Coleshill et al., 2004), undesirable emotional responses (Lanteaume et al., 2007; Liu et al., 2012), and neuroendocrine disorders (Fink and Jamieson, 1974).

Low-frequency electrical stimulation (≤5 Hz, LFES) is a promising brain stimulation strategy for treating epileptic seizures. LFES that targeted the epileptic focus (Yamamoto et al., 2006) and the areas outside of the focus, such as the piriform cortex (Yang et al., 2006; Zhu-Ge et al., 2007), cerebellum (Wang et al., 2008), or white matter (Koubeissi et al., 2013), reduced seizure severity in TLE. Recently, increasing evidence has suggested that LFES has a short-term or even instantaneous antiepileptic effect during seizures; and it may therefore be possible that using "closed-loop" or seizure-triggered LFES (delivering LFES in response to seizure-related electroencephalographic activity) in a specific brain region could inhibit epileptic seizures while leaving other aspects of brain function less affected (Berenyi et al., 2012). Indeed, closed-loop optogenetic modulation of neuronal spiking in the epileptic focus was also shown to suppress seizures (Krook-Magnuson et al., 2013; Lopez-Meraz et al., 2004). Thus, increased attention is being paid to exploring epilepsy-related neuronal circuits to identify the

Abbreviations: AAV, adeno-associated virus; ADD, afterdischarge duration; ADT, afterdischarge threshold; ANOVA, analysis of variance; AP, antero-posterior; CaMKIIα, Calcium/calmodulin-dependent protein kinase II alpha; ChR2, Channelrhodopsin 2; EC, entorhinal cortex; eYFP, enhanced Yellow Fluorescent Protein; GST, generalized seizure threshold; HFES, High-frequency electrical stimulation; IN, interneuron; LFES, Low-frequency electrical stimulation; LMOL, lacunosum-moleculare layer; ML, mediolateral; PN, principal neuron; SNR, substantia nigra pars reticulate; TLE, Temporal lobe epilepsy; V, ventral; VGAT, vesicular GABA transporter; WT, wild-type.

* Corresponding author.
E-mail address: chenzhong@zju.edu.cn (Z. Chen).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ebiom.2016.11.027

optimal brain targets of brain stimulation (Krook-Magnuson and Soltesz, 2015; Paz et al., 2013).

The entorhinal cortex (EC) projects to nearly all parts of the hippocampus and may play important roles in both seizure initiation and seizure propagation in TLE (McIntyre and Gilby, 2008). By serving as a gateway, the EC may modulate the balance between inhibition and excitation in the hippocampus. Dysfunctions of the EC are frequently observed in epileptic brains, including atrophy (Bartolomei et al., 2005), hypometabolism (Goffin et al., 2009; Guo et al., 2009; Wang et al., 2014) and cell loss (Du et al., 1995). Each of these could potentially contribute to epileptogenesis and chronic seizures in the hippocampus. Our previous study showed that LFES at the EC had an antiepileptic effect when delivered during an epileptic afterdischarge duration (ADD) (Xu et al., 2010), suggesting that the EC may be a potential target for brain stimulation treatment for TLE. However, the neuronal circuitry underlying the mechanisms relevant to this process remains unclear. Therefore, in the present study, we focused on the classical but still mysterious entorhinal-hippocampal circuit. By using optogenetic techniques (Deisseroth, 2011), we demonstrate that an endogenic antiepileptic neuronal circuit, which is mediated by entorhinal *CaMKIIα*-positive neurons, is involved in the entorhinal LFES treatments for hippocampal seizures.

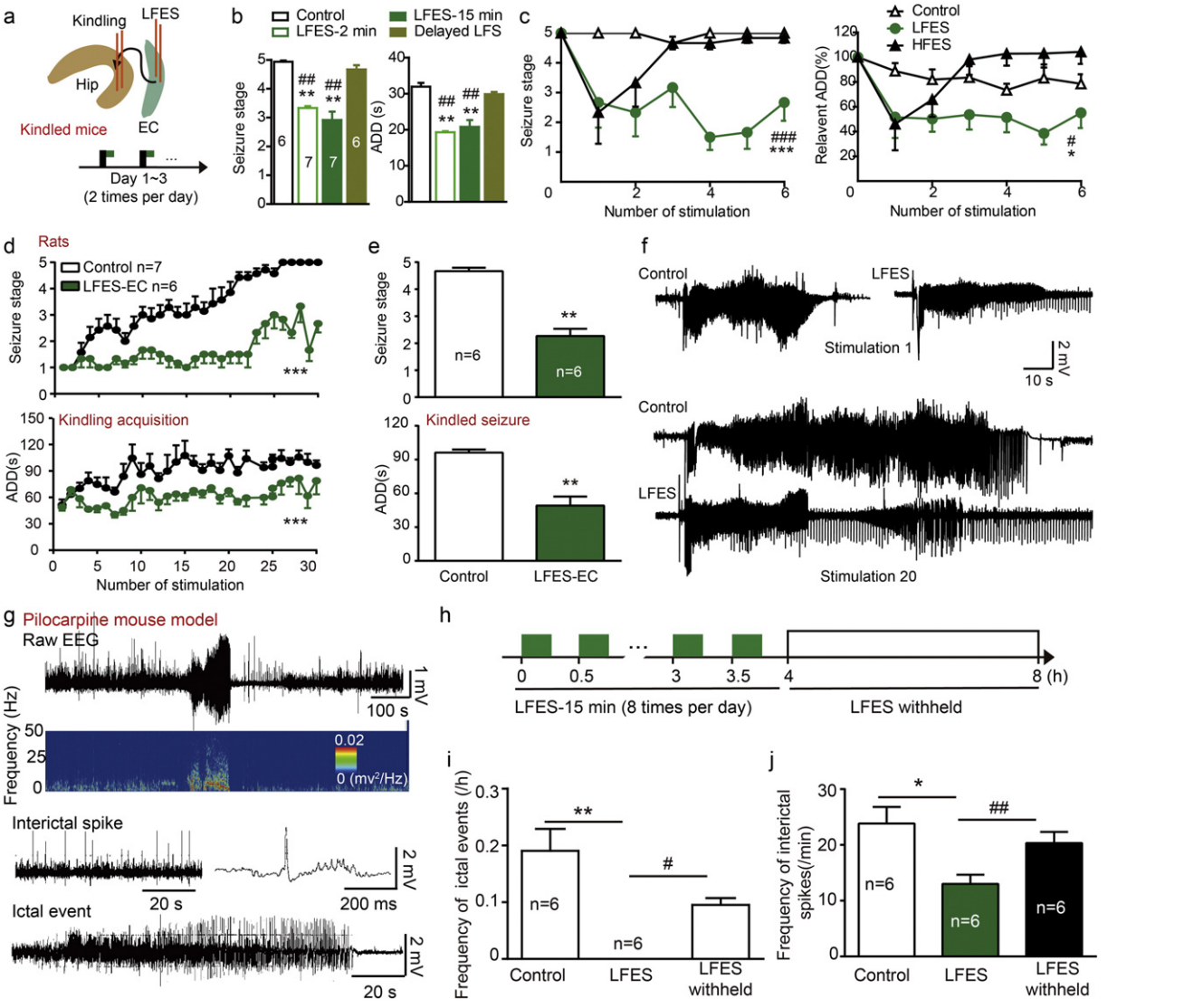

Fig. 1. Entorhinal LFES reduced the severity of hippocampal seizures. a) Schematic diagram for kindling stimulation and LFES (low-frequency stimulation, 1 Hz, 2 or 15 min) delivery in kindled mice. Black rectangle indicates kindling stimulation while the green rectangle indicates LFES. b) The effect of entorhinal LFES on the seizure stage and ADD of hippocampal kindled seizures. LFES delivered 4 s after the kindling stimulation, while delayed LFES means LFES (1 Hz, 15 min) delivered after the termination of ADDs (about 30 s after the kindling stimulation). c) The effect of repeated entorhinal LFES (1 Hz, 0.3 mA) and high-frequency electrical stimulation (HFES, 50 Hz, 0.1 mA, 5 s on/5 s off) in kindled mice (two-way ANOVA for repeated measures followed by LSD *post hoc* test). d) Entorhinal LFES retarded the hippocampal kindling acquisition in rats (two-way ANOVA for repeated measures followed by LSD *post hoc* test). e) Entorhinal LFES reduces the severity of hippocampal kindled seizures in rats (one-way ANOVA for repeated measures followed by LSD *post hoc* test). f) Representative EEG showing entorhinal LFES shortened the ADD during kindling acquisition in rats. g) Representative EEG showing interictal and ictal events in a chronic established mouse pilocarpine model of epilepsy. Ictal events were defined as regular spike clusters with a duration of ≥20 s, spike frequency of ≥2 Hz and amplitude at least three times of the baseline EEG. Interictal spikes were defined as regular spikes with a spike frequency <2 Hz and an amplitude at least three times of that of the baseline EEG. h) Schematic describing the delivery of entorhinal LFES. Green rectangle: LFES (1 Hz, 15 min); White rectangle: LFES withheld. i) Entorhinal LFES reduced the frequency of ictal events. (Wilcoxon signed rank test or Wilcoxon matched-pairs signed rank test for comparing to the LFES; Mann-Whitney *U* test was used for comparing LFES withheld to control). k) Entorhinal LFES reduced the frequency of interictal spikes (Student's *t*-test for comparing to the controls, and paired *t*-test for comparing to the LFES withheld). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the baseline or control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ compared to HFES or LFES withheld. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article as: Xu, Z., et al., Entorhinal Principal Neurons Mediate Brain-stimulation Treatments for Epilepsy, EBioMedicine (2016), http://dx.doi.org/10.1016/j.ebiom.2016.11.027