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Wide therapeutic time-window of low-frequency stimulation at the subiculum for temporal lobe epilepsy treatment in rats

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ABSTRACT

Low-frequency stimulation (LFS) has been considered as an option for the treatment of intractable epilepsy. However, previous data showed that LFS of certain brain regions only exerts its effect within a very narrow therapeutic time window, which lasts from seconds to tens of seconds, thus restricting its clinical application. The present study was designed to determine whether there exists a target with a wider therapeutic window for LFS treatment. Therefore, evoked seizures in the rat were induced by amygdala kindling and spontaneous seizures were induced by pilocarpine. The effects of different modes of LFS at the subiculum on the progression and severity of evoked seizures and the frequency of spontaneous seizure were evaluated. We found that (i) LFS at 1 Hz delivered to the subiculum before and immediately after the kindling stimulations, or after the cessation of afterdischarge (afterdischarge duration, ADD) decreased the seizure stages and shortened the ADD both in seizure acquisition and expression in amygdaloid-kindled seizures. In addition, even LFS delivered after duration of double the ADD prolonged the kindling progression. (ii) LFS delivered at 1 Hz, but not 0.5, 3 or 130 Hz, immediately after the cessation of kindling stimulations retarded the progression of kindling seizures. (iii) Pilocarpine-induced spontaneous seizures were completely inhibited by 1 Hz LFS. Thus, these results demonstrated that LFS of the subiculum has a wide therapeutic time-window for temporal lobe epilepsy treatment in rats, suggesting that the subiculum may be a promising and suitable target for clinical application.

Introduction

Low-frequency stimulation (LFS, 1–3 Hz) has been an alternative option in deep brain stimulation (DBS) treatments for intractable epilepsy because of its relatively lower risk of complications than high-frequency stimulation (HFS) (Burbaud et al., 2002; Feddersen et al., 2007; Grill et al., 2004; Moss et al., 2004). Evidence from both clinical and experimental studies demonstrated that LFS, applied either to epileptic foci (Gaito et al., 1980; Goodman et al., 2005) or to brain structures outside of the foci, acts against epileptic seizures or epileptogenesis (Sun et al., 2010; Yamamoto et al., 2002, 2006; Yang et al., 2006; Zhang et al., 2009; Zhu-Ge et al., 2007). These studies indicated that LFS may be a promising approach for clinical anti-epileptic treatment. However, so far many crucial problems, such as the selection of targets, the optimal stimulation parameters, and the underlying mechanism, remain unclear and worthy of research.

Recently, we first reported that only LFS delivered to the cerebellar fastigial nucleus (Wang et al., 2008), amygdala (Wu et al., 2008) and entorhinal cortex (Xu et al., 2010) within several seconds after the appearance of seizure discharges rather than after the cessation of afterdischarges (AD) suppresses amygdaloid-kindling seizures, which demonstrates a narrow "therapeutic time-window" for LFS treatment. Because the time-window is so short (seconds to tens of seconds), requiring an implanted stimulating device coupled with real-time signal analysis techniques using sensitive and specific seizure-detection algorithms, it increases the difficulty of LFS treatment. Thus, it is valuable to look for targets that have wider time-windows or are not time-dependent.

Abbreviations: LFS, low-frequency stimulation; DBS, deep brain stimulation; HFS, high-frequency stimulation; AD, afterdischarge; TLE, temporal lobe epilepsy; EEG, electroencephalogram; ADT, afterdischarge threshold; ADD, afterdischarge duration; GS, generalized seizure; GST, generalized seizure threshold; GSD, generalized seizure duration; SE, status epilepticus; ANOVA, analysis of variance.

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The subiculum is the major input and output gateway of the hippocampal CA1–subiculum–entorhinal cortex pathways (Stafstrom, 2005). It is an important structure in the generation and maintenance of epileptic activity (Fabo et al., 2008). The subiculum also exhibits hyperexcitability and excessive synchrony in human temporal lobe epilepsy (TLE) (de Guzman et al., 2006). In human TLE patients, interictal synchronous spikes (~1 Hz) originate from the subiculum (Cohen et al., 2002) and further studies found that multiple forms of interictal spike activity are generated from the subiculum (Fabo et al., 2008). Because interictal synchronous spikes are thought to exert an anticonvulsant effect (Avoli, 2001; de Curtis et al., 2001; Janszky and Ebner, 2002), it is intriguing to consider whether LFS of the subiculum, mimicking the frequency of interictal synchronous spikes, may suppress seizures. Furthermore, because interictal synchronous spikes may exert their influence interictally, LFS of the subiculum may have a wider time-window or may not show time-dependent characteristics.

The present study was designed to determine whether LFS of the subiculum has an anticonvulsive effect on kindling seizures and whether it has a wide therapeutic time window. The kindling model was used to control the occurrence of seizures and to readily assess the time-dependent effect in detail. The pilocarpine-induced spontaneous model was used to analyze the effect of LFS at the subiculum on spontaneous seizures.

Materials and methods

Animals

All experiments were approved by the Zhejiang University Animal Experimentation Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats (260–300 g, Grade II, Certificate No. SCXK2008-0033, Experimental Animal Center, Zhejiang Academy of Medical Science, Hangzhou, China) were maintained in individual cages with a 12-h light–dark cycle (lights on from 08:00 to 20:00). Water and food were given ad libitum. Experiments were carried out each day between 10:00 and 17:00.

Surgery

Under chloral hydrate anesthesia (400 mg/kg, i.p.), rats were mounted in a stereotaxic apparatus (512600, Stoelting, USA). Electrodes were implanted into the right basolateral amygdala (AP: −2.4 mm, L: −4.8 mm, V: −8.8 mm) and the right subiculum (AP: −6.0 mm, L: −3.0 mm, V: −3.0 mm). In experiment 2, electrodes were also implanted into the entorhinal cortex (AP: −6.8 mm, L: −4.9 mm, V: −8.0 mm) (Paxinos and Watson, 2005). The electrodes were made of twisted stainless steel Teflon-coated wires (diameter 0.2 mm, A.M. Systems, USA) insulated except at the tip (0.5 mm); the tip separation was about 0.7 mm. The electrodes were connected to a miniature receptacle, which was attached to the skull with dental cement. After surgery, rats were allowed 7–10 days of recovery.

Amygdaloid-kindling seizures

Stimulation of the amygdala was delivered by a constant-current stimulator (SEN-7203, SS-202 J; Nihon Kohden, Japan), and electroencephalograms (EEGs) at the amygdala and the subiculum were recorded with a Neuroscan system (Compumedics, Melbourne, Australia). On the first day of stimulation, afterdischarge threshold (ADT) was determined with a 1 s stimulus of 60 Hz monophasic square-waves at 1 ms per pulse. The stimulus intensity began at 60 μA, and was subsequently increased in 20 μA steps every 30 min until at least 5 s of AD was elicited. The minimal intensity that produced an AD was designated as the ADT for that animal, and was used for daily stimulation. The ADT intensity ranged from 100 to 300 μA in each group. From the next day, the right amygdala was subjected to once-daily kindling stimulation and EEGs at the amygdala and the subiculum were recorded through the respective electrodes to analyze the AD duration (ADD) and the EEG power.

Seizure severity was classified according to Racine (1972): (1) facial movement; (2) head nodding; (3) unilateral forelimb clonus; (4) bilateral forelimb clonus and rearing; and (5) rearing and falling. Stages 1–3 were considered as focal seizures and stages 4–5 as generalized seizures (GS) (Racine, 1972). When animals had three consecutive stage 5 seizures, they were regarded as fully kindled.

Experiment 1: Effects of LFS at the subiculum on kindling acquisition

In experiment 1, rats were divided into 2 groups matched for their ADTs. In group 1, the right subiculum was subjected to LFS (monophasic square-wave pulses, 1 Hz, 0.1 ms per pulse, 300 μA) for 15 min, immediately after the cessation of the kindling stimulation in the amygdala (immediate LFS, $n=9$). Meanwhile, the other group was given sham LFS (control, $n=9$): the rats were left in the chamber and connected to the apparatus for 15 min but no current was delivered.

Experiment 2: Effects of LFS at various targets on kindling acquisition

In experiment 2, rats were divided into 4 groups matched for their ADTs. In groups 1–3, LFS was delivered immediately after the cessation of the AD (ADD-delayed LFS) at the subiculum ($n=8$), the amygdala ($n=5$) or the entorhinal cortex ($n=5$), while the control group ($n=5$) was given sham LFS after the cessation of AD.

Experiment 3: Effects of LFS at the subiculum within a wide time-window on kindling acquisition

In experiment 3, rats were divided into 5 groups matched for their ADTs. In the control group ($n=9$), sham LFS was delivered as described in experiment 1. In the pre-treatment group ($n=9$), 15 min LFS at the subiculum was delivered before the kindling stimulation in the amygdala. In the other three groups, delivery of LFS was delayed by double the duration of the AD, i.e. 12–186 s (double ADD-delayed LFS, $n=8$), 0.5 h (0.5 h-delayed LFS, $n=8$) or 2 h (2 h-delayed LFS, $n=5$) after cessation of the AD in the amygdala.

Experiment 4: Effects of DBS at different frequencies at the subiculum on kindling acquisition

In experiment 4, rats were divided into 4 groups: control ($n=6$), 0.5 ($n=5$), 3 ($n=6$) and 130 Hz ($n=5$) groups. The control group received sham LFS as described above. Animals in the other groups were subjected to 0.5 or 3 Hz LFS (other parameters as described in experiment 1) or 130 Hz high-frequency stimulation (HFS; monophasic square-wave pulses, 130 Hz, 0.1 ms per pulse, 300 μA) for 15 min immediately after cessation of the kindling stimulation.

Experiment 5: Effects of LFS at the subiculum on fully kindled seizures

In experiment 5, after the rats were fully kindled (they were not treated with LFS during kindling), the post-kindled ADT was determined by the same procedure used for kindling ADT, and the GS threshold (GST) was determined by continuously increasing the current intensity in steps until a GS was elicited.

On the next day, rats were divided into 3 groups: a group where immediate LFS was delivered for 15 min ($n=6$), another group where LFS was delivered for 15 min after a delay double the ADD ($n=5$), and a control group ($n=5$) with sham LFS. All rats were stimulated with the GST as the kindling current daily for 10 days. The seizure stage, ADD and GS duration (GSD) were measured by the same procedures used for kindling seizures. In addition, both ADT and GST were determined again on day 10.