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Low-frequency stimulation of the hippocampal CA_3 subfield is anti-epileptogenic and anti-ictogenic in rat amygdaloid kindling model of epilepsy

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ABSTRACT

Neuromodulation with low-frequency stimulation (LFS), of brain structures other than epileptic foci, is effective in inhibiting seizures in animals and patients, whereas selection of targets for LFS requires further investigation. The hippocampal CA_3 subfield is a key site in the circuit of seizure generation and propagation. The present study aimed to illustrate the effects of LFS of the CA_3 region on seizure acquisition and generalization in the rat amygdaloid kindling model of epilepsy. We found that LFS (monophasic square-wave pulses, 1 Hz, 100 μA and 0.1 ms per pulse) of the CA_3 region significantly depressed the duration of epileptiform activity and seizure acquisition by retarding progression from focal to generalized seizures (GS). Moreover, GS duration was significantly shortened and its latency was significantly increased in the LFS group demonstrating an inhibition of the severity of GS and the spread of epileptiform activity. Furthermore, LFS prevented the decline of afterdischarge threshold (ADT) and elevated GS threshold indicating an inhibition of susceptibility to GS. These results suggest that LFS of the hippocampal CA_3 subfield is anti-epileptogenic and anti-ictogenic. Neuromodulation of CA_3 activity using LFS may be an alternative potential approach for temporal lobe epilepsy treatment.

Neuromodulation with brain stimulation is emerging as a promising alternative approach for the treatment of intractable epilepsy due to its titratability, reversibility, and low risk of complications [16]. A large body of evidence has demonstrated that low-frequency stimulation (LFS) can produce long-lasting suppression of epileptiform activity. The targets of LFS in most studies were epileptic foci [9,11,12,26,28]. We first reported that LFS (1 Hz) of the central piriform cortex, which is a brain region outside the site of kindling, exerts inhibitory effects on amygdaloid kindling seizures in rats [29,30], and LFS of the cerebellar fastigial nucleus produces a similar but weaker effect [24]. However, we also found LFS of some structures, such as the tuberomammillary [27] and the mediodorsal thalamic nucleus, induces aggravation of kindling seizures (unpublished data). Therefore the target selection is still crucial for the therapeutic effects of LFS.

The hippocampal CA_3 subfield is a key site in the circuit of seizure generation and propagation. Prominent sclerosis is observed in the CA_3 region in patients with mesial temporal epilepsy [10]. Many types of epileptiform activity have been observed to propagate from the CA_3 to CA_1 [15,17,21] and the CA_3 region was reported as the primary driving region for the seizures and the pathway for longitudinal [6] and contralateral [3] seizure propagation. Moreover, it was reported that under epileptic conditions *in vitro*, CA_3 networks emit an interictal discharge at 0.5–1 Hz to prevent the occurrence of ictal events generated by entorhinal cortex networks and depress the ability of the monosynaptic path to activate CA_1-subiculum networks [2]. Epileptiform synchronization could be reduced by mimicking CA_3 interictal firing activity [1,5]. These results suggest that functional integrity of CA_3 neurons may represent a critical control point in mesial temporal lobe epilepsy.

Additionally, our preliminary data showed that lesion of the CA_3 region abolishes the suppressive effect of LFS of the cerebellar fastigial nucleus on seizure acquisition in amygdaloid kindling rats, indicating that the CA_3 region is involved in the inhibitory mechanisms or circuits of LFS in kindled seizures (unpublished data). These results lead us to hypothesize that neuromodulation of CA_3 activity by LFS might be useful for treatment of epilepsy. Therefore, the present study was designed to investigate the effect of LFS on amygdaloid kindling seizures by employing the hippocampal CA_3 subfield as the target of LFS in rats.

Animals used in this study were male Sprague–Dawley rats (280–300 g, Grade II, certificate no. SCXK2003-0001; provided by the Experimental Animal Center, Zhejiang Academy of Medical Science, Hangzhou, China). All experiments were in accordance with the ethical guidelines 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 (NIH publication no. 80–23) revised in 1996.

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Efforts were made to minimize the number of animals used in the study and their suffering.

Under chloral hydrate anesthesia (400 mg/kg, i.p.), rats were mounted in a stereotactic apparatus. Electrodes were implanted into the right basolateral amygdala (AP: −2.4 mm, *L*: −4.8 mm, *V*: −8.8 mm) and the right CA_3 (AP: −5.3 mm, *L*: −5 mm, *V*: −6 mm). The electrodes were made of twisted stainless steel wires with a diameter of 0.2 mm that was Teflon-coated except for 0.5 mm at the tip. The tip separation was 0.7–0.8 mm. The electrodes were connected to a miniature receptacle, which was embedded in the skull with dental cement. Animals were allowed to recover from surgery for 10 days.

Kindling stimulation of the amygdala consisted of 1 s trains of monophasic, 1 ms square-wave pulses at 60 Hz. Electroencephalograms (EEGs) of the amygdala were recorded. Afterdischarge threshold (ADT) was determined on day 0 as previously reported [26,27,29,30]. All animals were subjected to kindling stimulation with the same current intensity as their own ADT once daily for 30 days. In the LFS group ($n = 10$), from the first day after ADT was determined to day 30, the CA_3 was subjected to LFS (monophasic square-wave pulses, 1 Hz, 100 μA and 0.1 ms per pulse) for 15 min immediately after cessation of the kindling stimulation in the amygdala. Control rats ($n = 12$) were also connected to the stimulator for 15 min, but no current was delivered.

Seizure severity during kindling was classified according to the modification of Racine [20]: (1) facial movement; (2) head nodding; (3) unilateral forelimb clonus; (4) bilateral forelimb clonus (BFC) and rearing; and (5) BFC and rearing and falling. Stages 1–3 were considered focal seizures, while stages 4 and 5 were considered generalized seizures (GS) [13,19]. Afterdischarge duration (ADD) and GS duration (GSD), which was defined as the duration of BFC, were additionally recorded. We also recorded the latency from the cessation of kindling stimulation to onset of GS (GS latency) if GS was evoked. The animals were considered fully kindled when they exhibited three consecutive stage 5 seizures. ADT and GS threshold (GST) were detected when the animal was fully kindled and at the end of behavioral testing on day 30. At the end of the experiments, electrodes placements were histologically verified. Only animals with electrodes correctly implanted in both the basolateral amygdala and the CA_3 were included in the statistical analysis. In this experiment, 22 out of 28 rats fulfilled this criterion.

All data were presented as mean ± SEM. Statistical analysis was carried out by SPSS v13.0 for Windows. Analysis of group progression of seizure stages and ADD during kindling acquisition was performed by two-way ANOVA for repeated measures. Comparison of the cumulative number of stimulations needed in each seizure stage and to each seizure stage during kindling acquisition was done with nonparametric Mann–Whitney *U* test. One-way ANOVA was used for the comparison of other indices. For all analyses, the tests were two-sided and a $P < 0.05$ was considered significant.

As shown in Fig. 1A, the progression of behavioral seizure stages in animals receiving LFS of the CA_3 at 1 Hz was significantly slower than those in the control group ($P < 0.001$). The average stimulation required for control animals to be fully kindled was 16.6 ± 0.9, with the last animal being fully kindled on day 20. By contrast, the average seizure stage of the LFS group on day 20 was 3.5 ± 0.3 and only 30% of the animals were fully kindled. At the end of behavioral testing on day 30, 20% of the animals receiving LFS treatment were still not fully kindled. Meanwhile, the increase of ADD along with seizure acquisition was significantly inhibited by LFS treatment ($P < 0.001$; Fig. 1B). Representative ictal EEGs recorded from the right amygdala on day 0, 5, 10, 20 and 30 are shown in Fig. 2.

To further evaluate the effect of LFS of the CA_3 on stepwise progression of kindling, the number of stimulations needed to reach and remain in each seizure stage was calculated. The cumulative number of stimulations required to reach each seizure stage in the

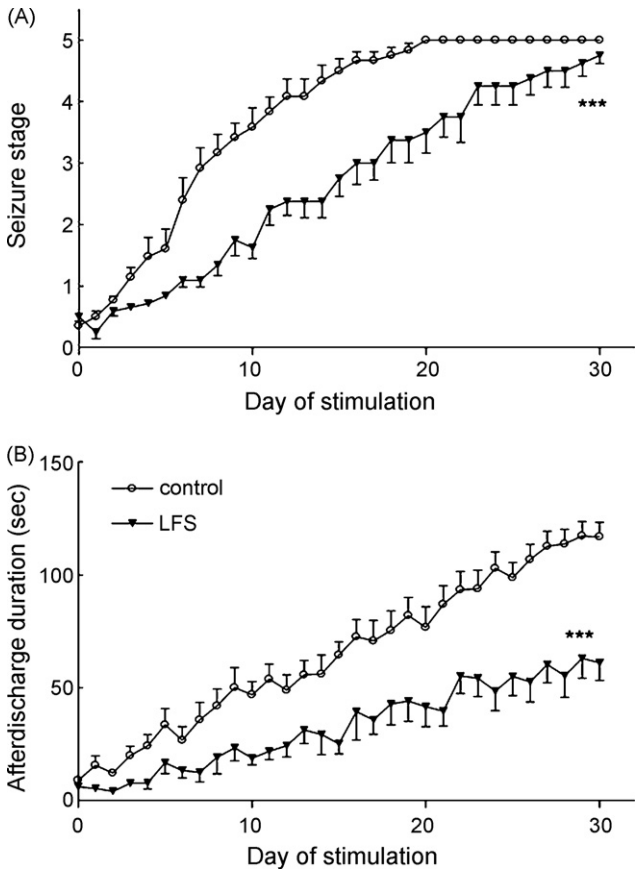

Fig. 1. Inhibitory effects of LFS of the hippocampal CA_3 subfield on (A) behavioral stage progression of seizures and (B) afterdischarge duration in amygdaloid kindling rats (control group, $n = 12$; LFS group, $n = 10$). Data were shown as mean ± SEM. Asterisks show significant differences from the control group (***$P < 0.001$).

LFS group was markedly increased compared with that in controls (Fig. 3A), and rats in the LFS group retained seizures mainly in stages 0–3, whereas no effect on staying time in stage 4 was observed (Fig. 3B).

Once the animal was fully kindled, i.e. exhibited three consecutive stage 5 seizures, the average duration of these three GS in the LFS group was significantly shorter ($P < 0.05$; Fig. 4A) with remarkably prolonged latency compared with that in the control group ($P < 0.01$; Fig. 4C). In addition, GS duration in the LFS-treated group on days 20 and 30 was significantly shortened than in the control group on the same days ($P < 0.01$ and 0.001, respectively; Fig. 4B). Moreover, GS appeared without latency in control animals on days 20 and 30, whereas the onset of GS in LFS group was markedly postponed on the same days ($P < 0.001$ and 0.01, respectively; Fig. 4D).

ADT decreased by about 60% in fully kindled control animals. However, it only decreased by approximately 30% in the group receiving LFS of the CA_3 and was significantly different from controls ($P < 0.01$; Fig. 5A). Similarly, on day 30 when the behavior testing was terminated, ADT in the LFS group was also significantly higher than that of the control group ($P < 0.001$; Fig. 5A). Additionally, GST in the LFS-treated group was significantly elevated either when the animal was fully kindled or on day 30 ($P < 0.05$; Fig. 5B).

In the present study, we found that LFS at 1 Hz of the CA_3 is anti-epileptogenic and anti-ictogenic, supporting the suggestion that neuromodulation of CA_3 activity using LFS may be a potential alternative approach for epilepsy treatment, especially for patients with complex partial seizures and secondarily generalized seizures. The progression of behavioral seizures as well as the ADD in the LFS group was markedly inhibited, and cumulative days of stimula-