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Ultrasound stimulation inhibits recurrent seizures and improves behavioral outcome in an experimental model of mesial temporal lobe epilepsy

Hilola Hakimova [a], Sangwoo Kim [b], Kon Chu [b], Sang Kun Lee [b], Bumseok Jeong [c,*], Daejong Jeon [a,b,**]

[a] Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea
[b] Department of Neurology, Comprehensive Epilepsy Center, Biomedical Research Institute, Seoul National University Hospital (SNUH), Seoul, Republic of Korea
[c] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

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ABSTRACT

Current therapies for epilepsy consist mostly of pharmacological agents or invasive surgery. Recently, ultrasound (US) stimulation has been considered a promising tool for the noninvasive treatment of brain diseases, including epilepsy. However, in temporal lobe epilepsy (TLE), a common form of epilepsy, neurophysiological and functional outcomes following US stimulation are not well defined. To address this, we developed a paradigm of transcranial pulsed US stimulation to efficiently suppress seizure activity in the initial/acute period in a kainate (KA)-induced mouse model of mesial TLE. Pulsed US stimulation inhibited acute seizure activity and either delayed the onset of or suppressed status epilepticus (SE). Kainate-treated mice that had received US stimulation in the initial period exhibited fewer spontaneous recurrent seizures (SRSs) and improved performance in behavioral tasks assessing sociability and depression in the chronic period of epilepsy. Our results demonstrate that US stimulation in the acute period of epilepsy can inhibit SRSs and improve behavioral outcomes in a mouse model of mesial TLE. The present study suggests that noninvasive transcranial pulsed US stimulation may be feasible as an adjuvant therapy in patients with epilepsy.

This article is part of a Special Issue entitled "Status Epilepticus".

1. Introduction

Epilepsy is one of the most common neurological disorders worldwide [1] and can be caused by various precipitating events, such as an initial/acute prolonged seizure (status epilepticus, SE), stroke, head trauma, or infection [2]. After a latent seizure-free period, spontaneous recurrent seizures (SRSs) develop and lead to chronic epilepsy. Thus, it may be important to suppress or inhibit SE to reduce SRSs or prevent the development of epilepsy. Many patients with epilepsy are also afflicted with psychiatric comorbidities, including social dysfunction, cognitive impairment, and anxiety/mood disorders [3–6]. To date, various anticonvulsants (commonly known as "antiepileptic drugs," AEDs) are widely available and are the most commonly used therapy for epilepsy [7]. However, about 30% of patients have seizures that are resistant to pharmacological treatment [8–12]. Although surgery in epileptic zones and electrical stimulation of the brain have palliative effects in many patients with intractable and pharmacoresistant epilepsy, these invasive surgical approaches are accompanied by inevitable risks [13–16]. Thus, additional noninvasive curative therapeutic approaches are needed.

Ultrasound is a mechanical pressure wave with a frequency above the threshold for human hearing (>20 kHz) [17]. Over the last decade, many studies have shown that transcranial ultrasound (US) stimulation at low intensities for short exposure times can excite or inhibit neuronal activity in the brain without observable neural damage; thus, transcranial US stimulation has been considered as therapy for neurological disorders, including epilepsy [18–27]. The neuromodulatory potential of transcranial US stimulation in epilepsy was suggested by a few laboratory studies showing that US stimulation attenuates acute seizure activity [28,29]. However, recurrent seizure severity and behavioral outcomes in the chronic period were not examined.

Mesial temporal lobe epilepsy (TLE) is the most common form of TLE in humans and is also one of the most difficult-to-treat types of epilepsy. It is characterized by partial seizures that may generalize secondarily

Abbreviations: US, ultrasound; TLE, temporal lobe epilepsy; kainate, KA; SRS, spontaneous recurrent seizure; EEG, electroencephalography; SE, status epilepticus.

* Correspondence to: B. Jeong, Laboratory of Clinical Neuroscience and Development, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea.
** Correspondence to: D. Jeon, Laboratory for Neurotherapeutics, Department of Neurology, Comprehensive Epilepsy Center, Biomedical Research Institute, Seoul National University Hospital (SNUH), 101 Daehak-ro, Jongno-gu, Seoul 110-744, Republic of Korea. Tel.: +82 2 2072 0121; fax: +82 2 2072 7424.
E-mail addresses: bs.jeong@kaist.ac.kr (B. Jeong), clark.jeon@gmail.com (D. Jeon).

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and by localized ictal and interictal electroencephalographic (EEG) abnormalities, behavioral dysfunction, and hippocampal sclerosis [30–32]. An experimental model of TLE induced by a single kainate (KA) injection into the hippocampus has been recognized as a good model of mesial TLE [32–35]. Kainate injection induces several convulsive seizures just before the KA-induced SE, followed by a seizure-free latent period and then a chronic period involving SRSs [33,34].

In this study, we developed a stimulation protocol with pulsed US stimulation to efficiently suppress acute seizure activity and the subsequent SE in the KA model of mesial TLE. Consequently, we attempted to investigate functional outcomes following the US stimulations by measuring the number of SRSs and by conducting several behavioral tasks in the chronic period.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (5–6 weeks old) were used. Mice were housed under a 12-h light/dark cycle and provided ad libitum access to food and water. Animal care and handling were conducted according to the guidelines approved by the Institutional Animal Care and Use Committee at the Korea Advanced Institute of Science and Technology.

2.2. Generation of KA model and electrode implantation

Kainate microinjection and EEG surgery were performed as described previously [36–40]. All animals were anesthetized with 1.5–2% isoflurane (5% for initial induction), and surgery was performed using a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). Kainate (0.3 μg/0.3 μl in saline, Tocris) was unilaterally microinfused into the CA3 area (AP = −2.0 mm, ML = −2.5 mm, and DV = 2.0 mm from the bregma) via a needle (33 gauge, NanoFil, WPI) connected to a 25-μl Hamilton syringe; the flow rate (0.04 μl min^{-1}) was regulated by a syringe pump (SP101i, WPI). After KA microinjection, a skull screw electrode was positioned at AP = −2.0 mm and ML = −2.5 mm from the bregma. A reference screw electrode was inserted into the skull above the cerebellum. The electrodes were secured onto the skull via dental acrylic.

2.3. EEG recordings and analysis of behavioral seizure severity

Electroencephalographic recording in vivo was performed as described previously [36–40]. Immediately after brain surgery, animals were placed in a small acrylic cage (15 × 20 × 15 cm) and then allowed to freely move in the cage. Electroencephalographic recordings combined with video monitoring were conducted right after the surgery. The video-EEG signals were recorded for 5 h after the KA injection for acute seizure activity and US stimulation. In addition, the video-EEG signals were continuously recorded 24 h per day from days 21 to 35 after the KA injection for measuring SRSs. The EEG experimental schedule is outlined in detail in Fig. 1A. Electrical activities were recorded after being amplified (×1200), bandpass-filtered at 0.1–70 Hz, and digitized with a 400-Hz sampling rate using a digital EEG system (Comet XL, Astro-Med, Warwick, RI, USA). Electrographic seizures were defined as the EEG signals showing the changes in the amplitude (>3× background) and frequency of the EEG activity (repetitive spiking with a frequency of 4–12/s and lasting for at least 10 s). Status epilepticus was defined as continuous or subcontinuous epileptiform spikes on EEG and tonic–clonic behavioral seizures. The averaged number of spikes in SE was analyzed for 1 min at 10 min after the SE onset. Behavioral seizure severity was rated according to Racine's scale [41]: stage 1: immobility and rigid posture; stage 2: mouth movements, head nodding, and repetitive movements; stage 3: forelimb clonus; stage 4: severe seizures with rearing and falling; stage 5: severe seizures with loss of posture or jumping; and stage 6: tonic–clonic seizures. Seizures characterized as stage 1, 2, or 3 were considered partial seizures, whereas those categorized as stage 4, 5, or 6 were considered generalized convulsive seizures.

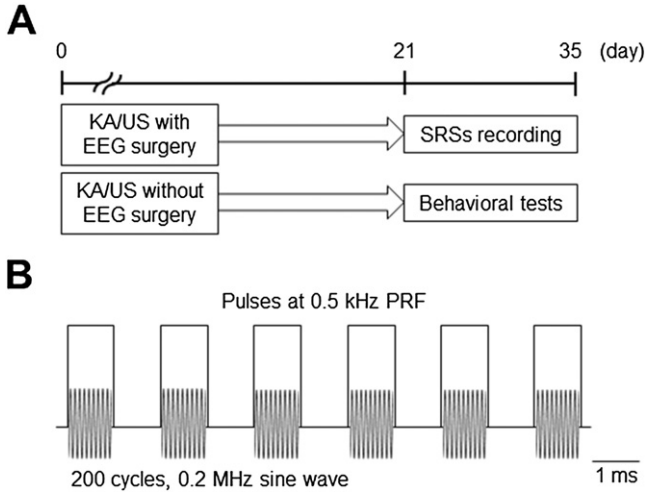

Fig. 1. Experimental design. (A) Time schedule of experiments, including behavioral testing and EEG recording. (B) Schematic illustration of US stimulation. The parameters were regulated by two function generators. A single US pulse contained 200 acoustic sine waves at 0.2 MHz, repeated at a pulse repetition frequency (PRF) of 0.5 kHz.

2.4. US stimulations

Ultrasound stimulations were conducted as described previously [24,29]. To optimize US stimulation conditions to effectively inhibit seizure activity, we performed pilot experiments with different protocols. Consequently, the following pulsed US parameters were chosen and used in the present study: (1) single 1-ms US pulse (5 Vpp) containing 200 acoustic cycles (0.5 Vpp) of 0.2 MHz and (2) single US pulses were repeated at pulse repetition frequencies (PRFs) of 0.5 kHz. Ultrasound stimulation was conducted immediately after animals began to display ictal spikes on EEG recording and concomitant convulsive seizure behaviors (stage 4, 5, or 6) following KA microinjection. Ultrasound (30 s) was administered by placing a transducer (19-mm diameter, Ultran Group) on their heads (just over the hippocampus). The transducer was induced by a function generator program (Agilent 33522A 2-Ch, 250 MSa/s; 30 MHz Function/Arbitrary Waveform Generator), which was amplified by a power generator (240L RF Power Amplifier). Ultrasonic gel was used to coat the surface of the transducer and the animal's head during each stimulation. For behavioral studies, animals received US stimulations for 30 s without EEG recording whenever they started to display convulsive seizures (stage 4, 5, or 6). The number of convulsive seizures before SE differed in each mouse (from 6 to 30); thus, the number of US stimulations was different in each mouse. The experimental protocol of US stimulations is illustrated in Fig. 1A.

2.5. Behavioral tasks

All behavioral procedures were video recorded, and all mice performed behavioral tasks without EEG surgery. Behaviors of three groups of mice (subjects) were compared: (1) nonepileptic control group (n = 11), with no treatment; (2) KA group (n = 10), treated with KA alone; and (3) KA/US group (n = 13), treated with KA and US stimulation. The same mice were used in all behavioral tasks; light/dark transition, sociability, and forced swim tasks were performed at 3-day intervals in order.