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Supplemental Information

Ultrasound Neuromodulation Inhibits Seizures in Acute Epileptic Monkeys

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Transparent Methods

Experimental Animals Details

Two adult male rhesus monkeys (named “A” and “B”) participated in this study and were obtained from Guangdong Landau Biological Technology Co., Ltd., Guangzhou, China. All animals were housed individually under a 12 h/12 h light/dark cycle at 24°C ± 1°C and 55 ± 5% humidity with sufficient water and food supply. All animal protocols described in this work (Certificate number: LDACU20170306-01) were approved by the Institutional Ethical Committee of Animals Experimentation of Guangdong Landau Biological Technology Co., Ltd. All efforts were made to minimize pain and/or discomfort of the animals.

Electrode implantation and transcranial injection of penicillin

Surgery was performed under general anesthesia using isoflurane (1.5-3%, 1.5 L/min, R51022, RWD), and salivation was reduced using atropine (0.05 mg/kg, IM). The heads of the monkeys were fixed, and surgery was performed using a stereotaxic apparatus (68901, RWD) for non-human primates. Under aseptic conditions, a midline linear incision of approximately 4 cm was made, the muscle and the periosteum were separated, and the skull was exposed. The stereotactic coordinates of the right hand movement area were calculated and determined according to “A Combined MRI and Histology: Atlas of the Rhesus Monkey Brain in Stereotaxic Coordinates”(Logothetis, 2012). The right hand movement area was located at the following stereotactic coordinates: 30 mm anterior and 15 mm lateral relative to the interaural line and 3 mm dorsoventral relative to the dura. In order to accurately **position the transducer over the stimulation site,** the MRI was used to **obtain the anatomical structure**. A dental bur was used to drill 2 holes in the skull, one at the location for penicillin injection and the other at the location for electrode implantation, 5 mm anterior to the right hand movement area (Figure 1B). **At the end of the experiment, we closed these two holes with bone wax and used bone wax as a marker for our localization. Besides, in order to ensure that ultrasound stimulation is performed at the same location each time, bone wax is attached to the surface of the skull as a marker to mark the position of the ultrasonic transducer.** After the electrode implantation and EEG was recorded for 10 minutes, penicillin (2500 IU, 250 IU/μl, H44022446, Baiyunshan, Guangzhou, Guangdong province) was injected into the right hand

movement area by a microsyringe (50 μl, 1705RN, Hamilton) at 1 μl/min.

Video-EEG recording

An EEG recording system (Solar 1848, Solar) was used in these trials. Video-EEG recordings were continuously collected throughout the experiment. The EEG electrodes were fixed on the prefrontal cortex, and then the EEG signals and video recording are transmitted to the computer simultaneously. The length of each recording was approximately 8.5 hours. A portion of the 8-channel electrode was placed into the brain to record the ECOG single. The sampling rate was 500 Hz. The bandpass filter for data acquisition was set between 0.01 and 100 Hz. The number of seizures, seizure duration, inter-seizure interval time and the number of seizures per hour were calculated for trials with ultrasound stimulation and compared with trials without stimulation for each session and experimental condition explored in the study for each monkey.

Ultrasound neuromodulation

Two ultrasound transducers with fundamental frequencies of 800 kHz (IMASONIC, Voray sur l'Ognon, France) and 750 kHz (Sonic Concepts, Woodinville, CA) were used to stimulate the epileptic monkeys. A coupling cone was filled with polyvinyl alcohol (PVA). The frequency was set to 500 Hz, and the pulse duration was set to 100 ms using a first function generator, with rise and fall times set to 1 ms using a second generator (AFG3101, Tektronix) connected to the amplitude modulation entry of the first generator. A 100 W amplifier (2100L, EI, NY, USA) was used to deliver the required power to the transducer. The pressure amplitude at the focus was 1.74 MPa. An acoustic coupling device was required between the ultrasonic transducer and the monkey skull. In this study, we used a solid gel-type coupling material made of PVA(Maurice et al., 2005, Joe et al., 2019). We first designed a coupling cone based on the focal length of the ultrasonic transducer, printed it in a 3D printer, and placed the 10% PVA(363065, Sigma) solution in the coupling cone; after vacuuming, it passed a freeze-thaw cycle (12 hours of freezing and 12 hours of thawing). Finally, a fixed PVA coupling cone was obtained and stored in ultrapure water. The transducer was placed on the scalp and fixed to the mechanical arm.

Skull transmission was estimated on several clean and degassed primate skulls. These trials allowed

us to estimate the acoustic pressure at 1.74 MPa in the brains of the monkeys. The corresponding intensity spatial peak pulse average (I_{SPPA}) was 119.78 W/cm^2. By taking into account a minimum 5 s pause between each ultrasonic pulse, we also estimated the corresponding spatial peak time average intensity (I_{SPTA}) at less than 1431.71 mW/cm^2. Through a fresh monkey skull, acoustic pressure decreased about 65%. Low-intensity pulsed ultrasound was delivered for 15 minutes after penicillin injection for 30 minutes and was focused on the right hand movement area between the electrode location and the penicillin injection location. The animals were fixed on the primate chair for 7 hours after ultrasound stimulation. After each experiment, the animals were allowed to recover completely, and ceftriaxone sodium (1 g daily, IM) was administered to prevent postoperative infection for 7 days. **There are 14-day intervals in each experiment for recovery. Therefore, we considered sessions as random effects.**

Experimental process

The monkeys were anesthetized by isoflurane (1.5%, 1.5L/min) and implanted with the EEG recording electrode (Figure 1A). EEG was collected as a baseline for 10 minutes. After penicillin was injected into the prefrontal cortex with a microsyringe, spikes were recorded for 30 min. Then, the monkey received 15 min ultrasound stimulation. After 15 minutes of stimulation, the anesthesia was removed. Video-EEG was recorded for 7 hours. There are 14-day intervals in each experiment for recovery

Parameter selection

Before starting the ultrasonic stimulation experiment, we made hydrophone measurements and laser sound field measurements using the ultrasonic probe. We calibrated the energy before ultrasound and after ultrasound. These measurements showed that the ultrasound was attenuated to 65% on the monkey skull, and there was no significant change in focal position of the shape of the focal point. Our previous research have proved that ultrasound stimulation for 30min can decrease the number of spike during acute seizure(Zhengrong Lin, 2020). The monkey A and B were respectively selected to perform the experience about ultrasound stimulation time and compared the difference with the two-ultrasound transducer. There are 4 time points (5min, 15min, 30min and 60min) were set up to choose the best one.

Evaluation of temperature and safety

To evaluate the thermal effect of ultrasound in our study, the temperature in the monkey skull was recorded using a thermal infrared imager (R300, NEC Avio, Tokyo, Japan). To observe whether ultrasound stimulation can cause edema and necrosis of monkey brain tissue, T2-weighted MR imaging was performed after 15 min ultrasound stimulation.

Statistical analysis

All data are expressed as the mean ± sem. All analyses were conducted using SPSS statistics, version 22. All data were analyzed by an unpaired Student's t test. The level of statistical significance was set to a p value ≤ 0.05.

Supplemental reference lists

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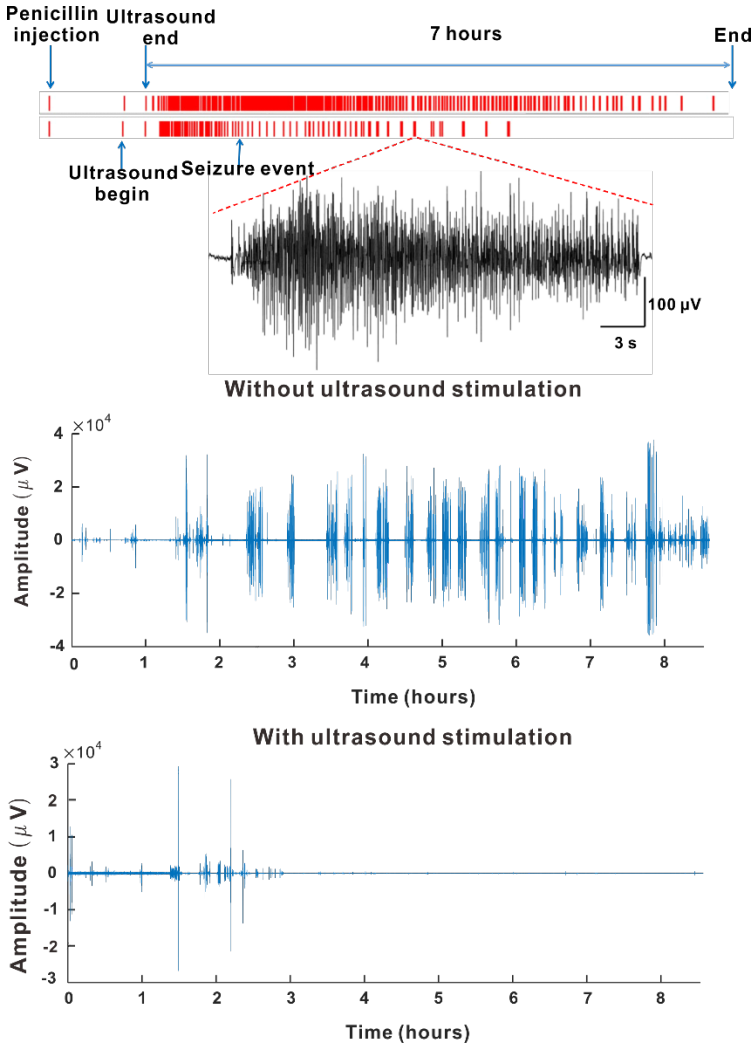
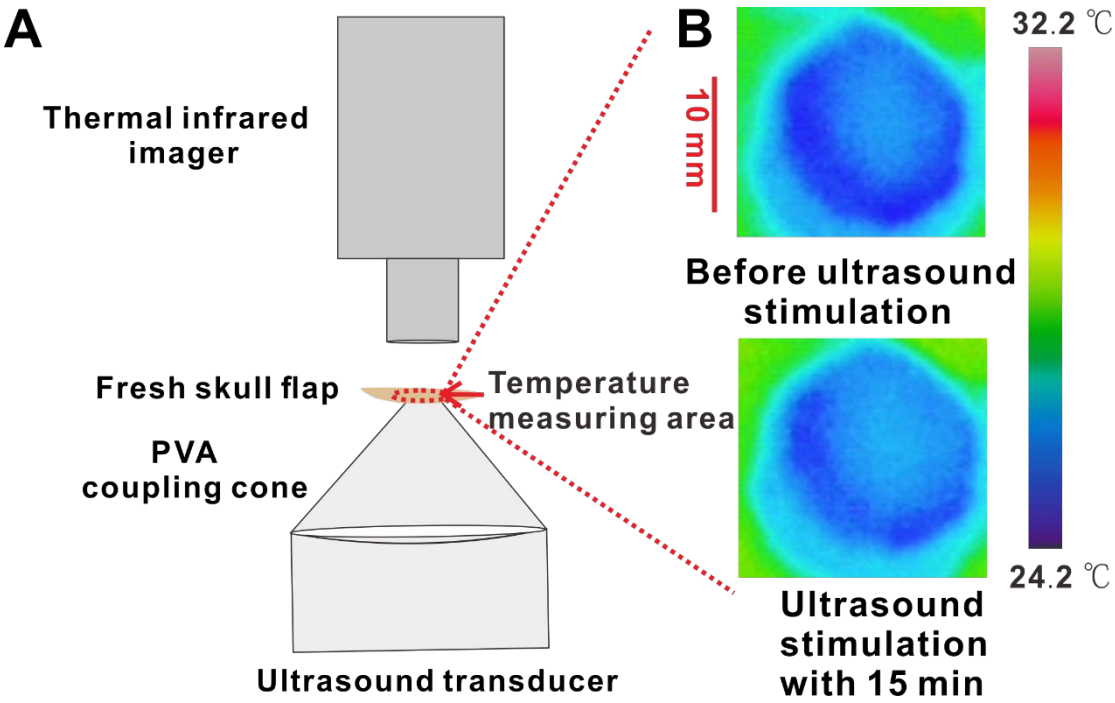
Supplementary Figure 1.



Supplementary Figure 1. The number of seizures was recorded for 7 hours after ultrasound stimulation or sham stimulation, related to Figure 2.

By quantifying the number of epileptic seizures in the EEG records, the number of episodes was reduced in monkeys after ultrasound stimulation compared with in monkeys that did not receive ultrasound stimulation.

Supplementary Figure 2.



Supplementary Figure 2. Sketch of the ultrasound stimulation site and the temperature change map after ultrasound stimulation, related to Figure 3.

A.The ultrasound transducer transmitted ultrasound stimulation, and the electroencephalogram recording electrode recorded signals from the left prefrontal cortex.

B. The temperature evaluation of the skull through which the ultrasound stimulation passed showed that, after 15 minutes of ultrasound stimulation (lower), the temperature of the skull surface did not rise more than 0.3°C above the temperature before stimulation (upper), which was within a safe temperature range.