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Communication

Intracranial Monitoring to Verify Novel Transcranial Electric Stimulation in an Epileptic Swine Model

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Abstract: Invasive deep brain stimulation has proven to be clinically therapeutic for patients with drug-refractory epilepsy. The aim of this study was to develop a novel transcranial electrical device as a noninvasive stimulation modality for seizure treatment. We fabricated a novel transcranial electrical device and tested it in four swine brains with depth electrodes surgically implanted under neuro-navigation. Stimulation with two high-frequency alternating currents was used to cause an interference envelope. Acute focal epilepsy was induced by a subcortical injection of penicillin and specific anesthesia protocol. The frequency and electric field of the stimulation in the hippocampus were investigated. The two frequencies (2 k and 2.14 kHz) of stimulation successfully caused an envelope of 140 Hz. With 1 mA stimulation, the electric field degraded gradually and induced an in situ electric field of 0.68 mV/mm in the hippocampi. The interference mode transcranial electric stimulation attenuated the originally induced epileptic form discharges. No neuronal or axonal injuries were noted histopathologically after the stimulation. The feasibility and biosafety of our proposed device were preliminarily verified. Future translational research should focus on the electrode deposition and stimulation parameters for a quantitative therapeutic effect.

Keywords: epilepsy; swine; transcranial electric stimulation; transcranial alternating current stimulation

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1. Introduction

With modern technologies, forced electric fields can be used to stimulate the brain, probe neural patterns, and treat brain diseases [1]. Regarding neuroprostheses translation, intracortical microstimulation via the acoustoelectric transduction of ultrasonic signals was used to investigate brain plasticity in a rat model [2]. Clinically, neuromodulation such as deep brain stimulation (DBS) therapy for drug-resistant epilepsy has shown therapeutic effectiveness [3,4]. Since electrical conduction within the brain can be modulated by electrical stimulation through intracranial electrodes, these fields can be triggered via transcranial electrical stimulation (tES), which is given noninvasively through the scalp [5]. The advantages of tES include a low cost, portability, and potential at-home applications, fueling a proliferation of human trials [6]. Previous studies demonstrated that tES is able to increase or decrease the regulation of neuronal excitability and entrain spontaneous oscillatory activity. Through simultaneous entrainment to the rhythm of external electric fields,

the phase co-alignment of intrinsic oscillations can be manipulated, and their functional importance can be studied [7–10].

The transcranial alternating current stimulation (tACS), one of the tES approaches, represents an exciting tool for causally probing the physiological and behavioral roles of brain rhythms and their synchronization [11]. When a transcranial alternative current provides electrical stimulation at a frequency that is synchronized with the brain's oscillation, a rhythm resonance occurs and can influence the activity of the brain [5,12,13]. In addition, a noninvasive strategy using two tACS with time interference to electrically stimulate neurons at depth has also been reported [14].

Over the last decade in neurology and neurosurgery, the implantation of depth electrodes has increasingly been used as the method of choice for invasive localization in refractory focal epilepsy [15–17]. Depth electrodes enable the precise recording of brain waves from deep cortical and subcortical structures in refractory epileptic patients who are considering surgery [18]. Recent developments are gradually enabling intracranial monitoring to be used within an electric field investigation setting. The implantation of depth electrode arrays has been used to measure the spatial and temporal characteristics of electrical fields within primate brains generated by tES [19]. Furthermore, an intracranial electroencephalogram platform was built to measure the shunt-current conduction out of the brain after kainic acid-induced seizures [20].

In this study, we aimed to explore electric field distribution with a newly developed device. We also investigated the effect of the interference mode tACS on the epileptiform activity in a swine model.

2. Materials and Methods

2.1. Protocol of the Swine Model

In the swine model, four pigs (Lanyu 400, Taiwan) aged 2–4 months old with a body weight around 15 kg were used. All procedures were approved by the Institutional Animal Care and Use Committee of the National Laboratory Animal Center (approval no: NLAC-109-M-027).

After the induction of anesthesia with an intramuscular injection of Zoletil (Virbac, Carros, France, 5 mg/kg) and xylazine (2 mg/kg), each animal was given a bolus dose of fentanyl (100 μg) during the initial craniotomy, 75–125 μg/h continuously, and maintained on 0.5% isoflurane. The protocol was designed to induce an epileptic discharge [21].

Epileptiform activity was induced by a subcortical injection (5 mm below the cortical surface) of 5 μL benzylpenicillin (PCN, PENNA, Sigma, St. Louis, MO, USA) 2 h after the induction of anesthesia. An epileptiform discharge was interpretated by an epilepsy surgeon (Y.C.W) and defined as a high-voltage polyspike >1 Hz or paroxysmal sharp waves lasting over 3 s detected by the depth electrodes.

2.2. Transcranial Electric Stimulation

The self-fabricated tES device is a current-controlled, multi-electrode system. The multi-mode transcranial stimulator works via a microcontroller (STM32F104, STMicroelectronics, EU) and an analog-to-digital converter (Texas Instruments, Dallas, TX, USA). The device has a sampling rate of up to 2000 Hz and a signal resolution of up to 0.5 μV. The resistance can be adjusted to change the alternating current that was transmitted through the electrodes between 0.5 mA and 5 mA. A sensing device monitor INA226 (Texas Instruments, USA) was used to constantly monitor the current, and an automatic circuit breaker ensured that the current remained below the set limit (Figure 1A,B).

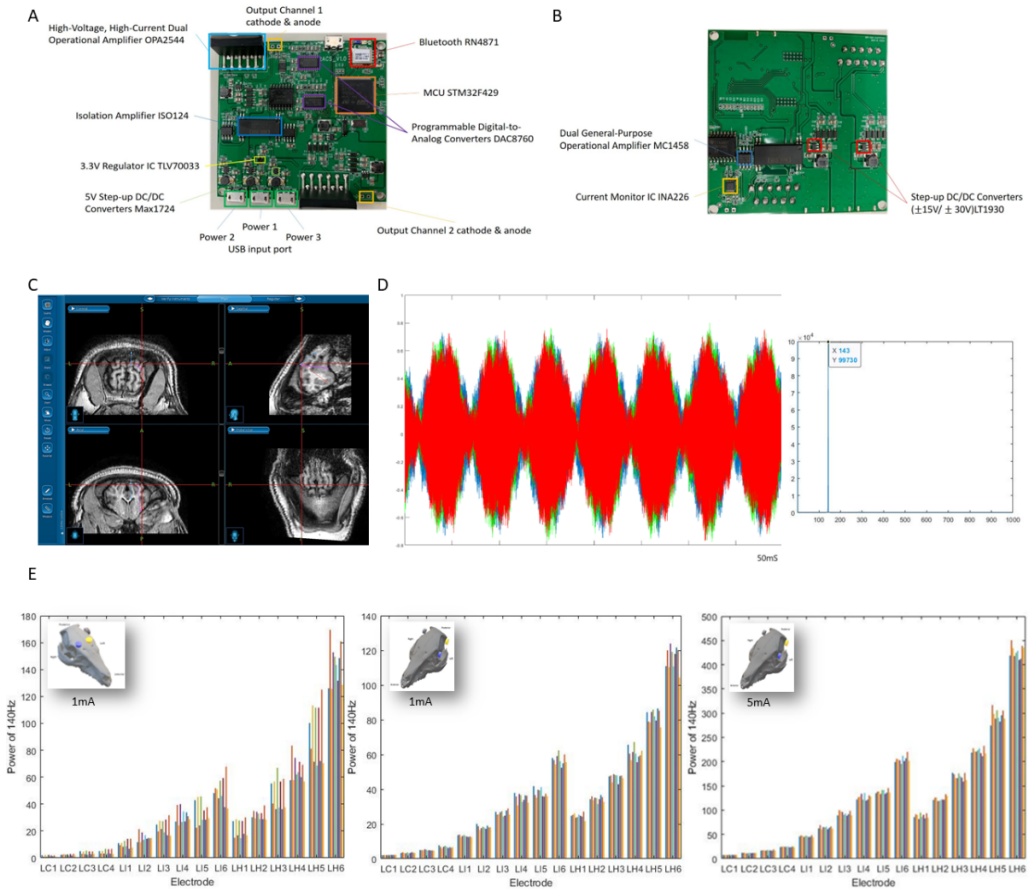

Figure 1. The front view (**A**) and back view (**B**) of the electronic component and circuit board of the novel transcranial electric stimulation device. (**C**) Three trajectories for depth electrodes planned with neuro-navigation, targeting left cingulate gyrus, insula, and hippocampus. (**D**) The envelope created by the tACS on 2 K and 2.14 K Hz. The signal captured from depth electrode at hippocampus shows that the electric field was 0.68 mv/mm, and frequency was 140 Hz. (**E**) The schematic view of the electrodes deployment and the power of 140 Hz signal. The power increased when the electrodes were shifted closer to hippocampus or when the current elevated. LC, left cingulate gyrus; LI, left insula; LH, left hippocampus.

Stimulation was applied via three round Ag/AgCl electrodes measuring 0.79 cm^2 on each surface, and the electrodes were put on the top or side of the skull. One of the electrodes was the return electrode to pick up the remaining current.

2.3. Depth Electrode Implantation with Neuro-Navigation

Anatomical brain imaging involved routine T1 magnetization-prepared rapid gradient-echo (MP-RAGE) and T2-weighted imaging for targeting purposes. We used a neuro-navigation system (Medtronic, Minneapolis, MN, USA) to deploy three depth electrodes (Adtech Co., Fond du Lac, WI, USA) with four and six contacts, each spaced apart by 5 mm. The electrodes were surgically implanted through a skull bone burr hole and durotomy over the sensorimotor cortex. The covering targets were the anterior cingulate gyrus, insula cortex, and hippocampus (Figure 1C).

2.4. Histopathological Examination

The acute procedures were performed in one day, after which the pigs were euthanized. The brains were removed and perfused after a 24 h recovery period to allow for the detection of the persistent effects after a bout of stimulation. Hematoxylin and eosin (HE) staining,

Luxol Fast Blue (LFB) staining, Fluoro-Jade C (FJC) staining, and Bielschowsky silver (BSK) staining were performed.

3. Results

The reliability of the electric current output was verified using a DAQ Pad 6259 acquisition card (National Instrument, Austin, TX, USA), which showed that the 1 mA current applied was actually 0.9821 $\pm$ 0.0438 mA. When the current was set at 5 mA amplitude, the actual value was 5.0060 $\pm$ 0.0432 mA. The frequency of the two tACS were set at 2 kHz and 2.14 kHz. Frequency domain analysis shows that the frequency after interference was 140 Hz, and that the peak electric field was 0.68 mV/mm, as captured by the depth electrodes in situ in the hippocampi of the first two pigs (Figure 1D). Electric fields had a more dominant distribution at the surface contacts compared to the deep contacts. The electrodes were then shifted to near the bottom of the skull (temporal side), and the power of 140 Hz increased at the deep contacts (Figure 1E).

In the other two pigs, we recorded 5 min epileptic form discharges, which were induced around 30 min after the PCN injection (Figure 2A). After the epileptic spike occurrence, the two pairs of tACS electrodes delivered 1 mA at the temporal side. The originally induced high voltage spikes (Figure 2B) were attenuated after 10 min of stimulation (Figure 2C). In one of the pigs, the polyspikes were nearly eliminated after 10 min of stimulation at 5 mA.

Histopathology Changes after Electrical Stimulation

Acute neutrophil infiltration and the hemosiderin deposition on HE staining were due to the surgical implantation itself. No acute neuronal injury was noted at the surface sensorimotor cortex or hippocampus after electrical stimulation, since there was no demyelination or coagulation necrosis on LFB staining around the trajectory, and no acute necrosis of neurons on FJC staining. The alignment of nerve fibers was not compromised as determined by BSK staining. Acute focal seizures within 1 h after the PCN injection did not cause any diffuse neuronal injuries (Figure 2D,E).

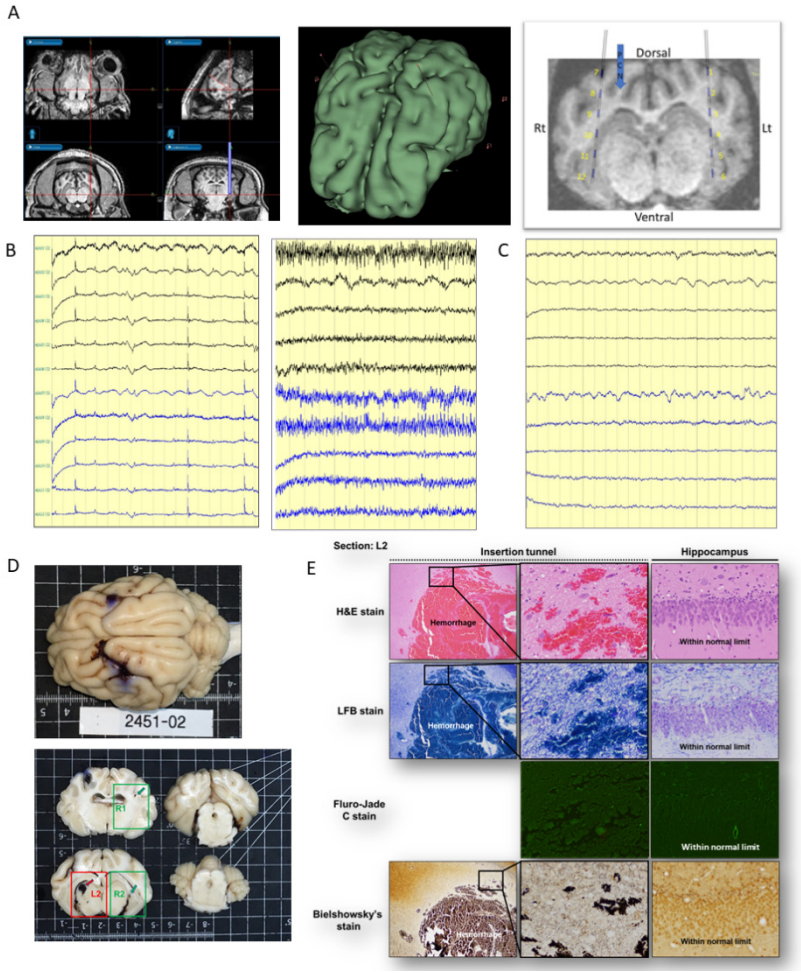

Figure 2. (**A**) The neuro-navigation plan for the depth electrodes implanted at bilateral hippocampi and the subcortical injection of the PCN. (**B**) Epileptic form discharge induced by the PCN injection. The paroxysmal sharp waves (**left**) progressed to the generalized polyspike (**right**) 20 min after the 5 µL PCN injection in one pig. (**C**) The polyspike was attenuated after stimulation with 5 mV. (**D**) Gross and slice view of the pig brain to confirm the depth electrode trajectories. (**E**) Histopathology exam revealed no neuronal or axonal injury after the stimulation.

4. Discussion

In this pilot study, we developed a novel tES device and verified it using a living porcine acute epilepsy model. Using depth electrodes, we detected the local stimulation frequency by envelope modulation. The assessment of the intracranial electrical field during tACS may provide novel insights that could extend beyond tES. We also performed this investigation to establish the viability of using pigs as an animal model to intraoperatively monitor investigational device implantation and to validate the use of depth electrodes to define and then modify the transcranial electric field. No acute neuronal or somitic injuries were noted within and beyond the hippocampi. This indicates the biosafety of this device as well as the parameters of electric stimulation.

We applied all electrodes directly onto the skull bone and demonstrated that it was possible to manipulate and steer the field arising from three-electrode AC stimulation in a noninvasive way. Measurements from human cadavers have revealed that a significant fraction of the current applied to the scalp is lost due to shunting effects from the skin and soft tissue, and serial resistance of the skull [1]. To minimize the shunting effect, we applied all electrodes directly onto the skull bone. Using this methodology, the electrical field remained at maximum at the surface and declined in the deeper regions, such as the hippocampi. The results are concordant with previous literature in which electrical

field strength was strongest in the superficial brain regions with maximum values of about 0.5 mV/mm with 1 mA stimulation [19]. From a neurosurgeon's point of view, subgaleal implantation of the device may minimize both rates of infection and shunting effects.

Nevertheless, it is reasonable to determine that the electrical field delivered to the hippocampus is enough to induce neural firing. When a neuron is about to emit a spike, even a weak electric field can bias the spike threshold. In vitro experiments have shown that coupling an oscillatory field to intracellularly generate oscillation can be effective at gradients as small as 0.2 mV/mm [22], while 1 mV/mm can induce measurable subthreshold effects and affect the timing of action potentials [23]. Several experiments performed on rodents have demonstrated an average electrical field of up to 6.8 ± 3.8 mV/mm, which was induced using a higher current compared to that used in human studies [24,25]. During transcranial stimulation in rats, the lowest electrical field sufficient to affect the timing of spiking activity in widespread cortical and hippocampal areas has been reported to be around 1 mV/mm [1]. In our swine model, we showed that 1 mA tACS with a specific frequency was sufficient to cause an effective electric field to influence epileptic spikes. We did not examine the distribution of the electric field during 5 mA stimulation; however, it would theoretically be proportional to the current value. Whether such acutely applied weak stimuli can have beneficial or deleterious effects on brain function can only be determined using targeted recordings and additional behavioral measures. With our porcine seizure model, we showed that the epileptic form discharge was effectively neutralized after interference tACS delivery, which could be a potential treatment for epilepsy.

Our interference t-ACS was targeted in the hippocampus, which is an embedded archicortex and neocortex in advanced mammals, including swine and primates, due to the brain-folded nature that develops during neuroembryology. It is difficult to deliver electrical stimulation to such a deep-seated region through noninvasive methods. Fields perpendicular to the soma-dendritic axis may have little influence on the apical dendrites of pyramidal neurons. Therefore, the electrical field induced by tES is not only related to the current amplitude, but is also related to the three-dimensional construction of neural tissue [26]. Hippocampal DBS has been shown to clinically improve seizure outcomes [27]. In the present study, we show that noninvasive tES could potentially achieve a similar goal. The planned envelope stimulation at the local region from the two high-frequency currents was 140 Hz because this frequency when applied to the anterior nucleus of the thalamus has been shown to be therapeutic [4] for epilepsy patients. We studied the hippocampus as it is another crucial point on the circuit of Papez for neurostimulation epilepsy treatment [28]. Further studies are needed to investigate whether high- or low-frequency stimulation is better to stop epileptic activity. Rashid et al. demonstrated that low-frequency stimulation applied at a frequency of 1 Hz significantly reduced both the excitability of the neural tissue as well as the seizure frequency in a rat model of human temporal lobe epilepsy [29]. A previous study revealed that seizure-triggered, feedback tES can dramatically reduce spike-and-wave episodes in a rodent model of generalized epilepsy [30]. Despite the successful demonstration that weak, periodic, electric fields diminished epileptic seizure, the mechanism of action remains unclear. Previous studies indicated tACS frequencies of 1–5 Hz are excitatory similar to anodal transcranial direct current stimulation (tDCS), while those higher than 10 Hz, such as cathodal tDCS, are inhibitory [31,32]. Furthermore, optic low-frequency stimulation could effectively reduce epileptiform activity in the hippocampus through a γ-aminobutyric acid (GABA)-A-mediated mechanism, which is the activation of glutamate decarboxylase (GAD)-expressing hippocampal interneurons [33]. In the future, we will track changes in the concentrations of neuropeptides and cytokines in the extracellular fluid of the hippocampus using chronically implanted microdialysis probes.

To the best of our knowledge, previously not many large mammal models were used for studying acute seizure provocation in Taiwan. Almost all relative experiments were performed on rodents, and the kindling medication was kinetic acid. We modified the swine model from a previous study [21] and successfully replicated the acute epileptic scenario.

Although the cost of experimentation is much higher for pigs compared to rats, the porcine brain is a larger volume and has a much more complex gyretic brain construction with a deep-folded hippocampus, which is more similar to human brains. It is reasonable to assume that data that are more applicable to primates will be obtained when using intracranial depth electrodes. Furthermore, since simultaneous video and EEG recordings are useful to confirm seizures in clinical as well as experimental conditions [34,35], we would apply video EEG in a future setting. To setup a reliable animal model is important for further translational research on intracranial electrophysiological monitoring. The development of electrodes with both sensing and stimulation activity is ongoing, and we hope that our model will provide further evidence for their biocompatibility.

Limitation

We did not quantitively demonstrate the number of epileptic discharges per second before and after tACS due to the small sample number. In addition, we could not rule out the collateral influence from electric shunting outside the targeted area because the local electrical field could not be captured at the region far from depth electrode implantation. Further studies are needed to investigate whether this may be resolved by increasing the resolution of sampling. Another notable limitation of the current study was the accumulation effect of the repeated stimulation on neural excitation since one pig received multiple stimulation cycles within a single day of experimentation. Lateralized stimulation with different stimulation parameters on each hemisphere is worthy of consideration since stimulation on one side may cause less influence on a contralateral brain. This methodology would need to be validated in the future.

5. Conclusions

In this epileptic swine model, we verified the characteristics of the frequency, electrical field, and biosafety of our novel TES device. We also made direct recordings within living porcine brains and analyzed the local field electrical potential. The results demonstrate the advantages of using depth electrodes, which are both a feasible and safe probing modality, for investigating intracranial electrical distribution. These findings will allow us to develop techniques that can eventually be translated to patient treatment.

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Data Availability Statement: Not applicable.

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