A Testbed to Explore the Optimal Electrical Stimulation Parameters for Suppressing Inter-Ictal Spikes in Human Hippocampal Slices

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***Abstract*— New interventions using neuromodulatory devices such as vagus nerve stimulation, deep brain stimulation and responsive neurostimulation are available or under study for the treatment of refractory epilepsy. Since the actual mechanisms of the onset and termination of the seizure are still unclear, most researchers or clinicians determine the optimal stimulation parameters through trial-and-error procedures. It is necessary to further explore what types of electrical stimulation parameters (these may include stimulation frequency, amplitude, duration, interval pattern, and location) constitute a set of optimal stimulation paradigms to suppress seizures. In a previous study, we developed an *in vitro* epilepsy model using hippocampal slices from patients suffering from mesial temporal lobe epilepsy. Using a planar multi-electrode array system, inter-ictal activity from human hippocampal slices was consistently recorded. In this study, we have further transferred this *in vitro* seizure model to a testbed for exploring the possible neurostimulation paradigms to inhibit inter-ictal spikes. The methodology used to collect the electrophysiological data, the approach to apply different electrical stimulation parameters to the slices are provided in this paper. The results show that this experimental testbed will provide a platform for testing the optimal stimulation parameters of seizure cessation. We expect this testbed will expedite the process for identifying the most effective parameters, and may ultimately be used to guide programming of new stimulating paradigms for neuromodulatory devices.**

I. Introduction

Epilepsy is the condition of recurrent unprovoked seizures. It affects an estimated 2.2 million Americans and is the fourth most common neurologic disorder nationwide. With approximately 150,000 new cases diagnosed annually, it is estimated that 1 in 26 Americans will develop epilepsy in their lifetime, with children and older adults being the largest affected segments of the population. A common type of epilepsy is partial epilepsy arising from the temporal lobe, in particular the hippocampus. Although it is common, when treated surgically, it is the most successfully treated form of epilepsy. About 30% of patients with epilepsy have treatment resistant epilepsy, despite numerous trials of anti-seizure medications.

The concept of neuromodulation and neurostimulation has received increasing attention over the past decade[1]. This strategy entails the stimulation of elements of the central nervous system, either directly or via cranial nerves. The most widely utilized treatment is that of vagus nerve stimulation (VNS). This approach achieves bipolar stimulation of the cervical trunk of the vagus nerve by a fully implanted battery-generator [2]. This approach has been demonstrated to have a significant palliative effect on medically intractable epilepsy patients who are not amenable to surgical therapy. More recently, deep brain stimulation (DBS) has been shown to have a positive effect of the reduction of seizures in medically intractable patients [3]. In contrast to VNS, DBS involves the direct stimulation of the brain using a stereotactically placed electrode, targeting anterior nucleus of the thalamus. The effect of DBS is not dissimilar to that of VNS and represents another alternative for medically intractable patients. A new neurostimulation strategy has emerged that is fundamentally different from both VNS and DBS. With VNS and DBS, no attempt is made to stimulate the actual seizure focus. Responsive neurostimulation (RNS), in contrast, attempts to directly stimulate the seizure focus in response to seizure activity detected by electrocorticography (Ecog) [4]. While the initial data on RNS is very promising, the approach as it currently exists has some features that clearly need to be refined and optimized. For mesial temporal lobe epilepsy (MTLE) patients, the stimulating electrode is implanted along the long axis of the hippocampus with no ability to stimulate specific subpopulations of neurons or structures within the architecture of the hippocampus. In addition, there is little strategy for the programming of the stimulation paradigm. Based on clinical criteria as well as Ecog recordings, exploring the optimal stimulation parameters requires time-consuming, trial-and-error efforts on the part of the clinician.

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Direct stimulation for the treatment of seizures has been employed in different seizure models, with different parameters, both on human clinical trials and animal studies [5], [6]. From clinical studies of patients with MTLE, Yamamoto et al. showed 0.9 Hz and 50 Hz of electrical stimulation to the epileptic focus has inhibitory effects [7]. Kinoshita et al. also showed the inter-ictal spikes were reduced after low-frequency electrical stimulation applied in the epileptic area [8], [9]. Some other groups demonstrated reduction in inter-ictal spiking by open-loop stimulation [10]–[12]. Tellez-Zenteno et al. reported subthreshold electrical stimulation with 190 Hz reduced seizures [13], and Boon et al. applied a wide range of high frequency (130 Hz to 200 Hz) in twelve patients and most of them had a seizure reduction rate over 50% [10]. Lesser et al. compared the termination effect on afterdischarges of different patterns of brief burst stimulation [14]. In the rat model, Wyckhuys concluded Poisson distributed inter-impulse interval stimulation reduced seizure rates with less (25%) stimulation intensity than fixed inter-impulse interval stimulation, which implied the increase of battery life [15]. The latter two studies brought out the idea that different patterns of stimulation may contribute to improve the therapy.

We have previously reported an *in vitro* model of epilepsy using human hippocampal slices [16]. We applied a planar multi-electrode array (MEA) system, in which the spatio-temporal inter-ictal activity from 500 micrometer-thick human hippocampal slices can be consistently recorded in high-potassium (8 mM), low-magnesium (0.25 mM) aCSF with additional 100 μM 4-aminopyridine (HiK-LowMg-4AP). Using this *in vitro* seizure model, we have developed an experimental paradigm that allowed us to trial varied stimulation parameters, and to observe the influence of these variations on the inter-ictal spikes. Once the origin of inter-ictal spikes was identified, the stimulation electrode was placed close to the spiking region, and the pre-programmed stimulation impulses were then applied to the slice. Our hypothesis is, the number of inter-ictal spikes should decrease with electrical stimulation. We have tested with different stimulation durations, frequencies, and interval patterns. The use of the MEA system, combined with the microscopy imaging system helped us to identify the seizure focus easily, and locate the stimulation site accurately. We also tried different stimulation paths, i.e., orthodromic stimulation and antidromic stimulation. The results show that this *in vitro* seizure model provided an ideal paradigm to test the efficacy of different stimulation parameters on spike suppression.

II. Materials and Methods

A. Human Hippocampal Slice Preparation

The hippocampal tissue is obtained from patients suffering from intractable MTLE during curative epilepsy surgery. Candidates for temporal lobectomy were consented for this university IRB approved study (#HS-10-00162) that uses their tissue for our study. The surgeries were performed in the standard fashion with no alterations in technique to accommodate the study. A vibratome (Leica VT1200) was setup close to the operative suite prior to tissue resection. The neurosurgeon removes about 1.5 cm of the hippocampal head and body *en bloc*, immediately placing it into a petri dish filled with 4°C sucrose. The tissue was then quickly sliced to 500 micrometer-thick slices with the vibratome. In each case, approximately 15 slices were prepared, and then transported from the hospitals to our lab with a self-made mobile oxygen-temperature sustaining system. More details can be found in [16].

B. Electrophysiological Recording Setup

Electrophysiology data were collected through an MEA60 system (Multi Channel Systems, Germany). The data were sampled at a frequency of 10 kHz per channel and were recorded using MC_Rack. The MEA60 system was assembled over an inverted microscope (Leica DM-IRB, Germany). Using the transparent glass-based planar multi-electrode array (MEA, 500/30iR-Ti), the slice image and its corresponding position to the electrodes was clearly observed. This feature enabled us to record the neural activity in a broad region and from different subregions simultaneously. Once the slice was transferred onto the array, a metallic ring with nylon mesh attached to it was gently placed on the top of the slice. A small brush was used to move the slice to the corresponding recording site. The temperature was maintained at 32-34°C. The inter-ictal spike activity was induced by perfusing HiK-LowMg-4AP aCSF to the slice.

C. Stimulation Configuration

The electrical stimulation pattern was programmed using MC_Stimulus and pre-loaded into the stimulator (STG1008). The impulses were delivered to the slice through a bipolar stimulation electrode made out of twisted Nichrome wires. The stimulation electrode was controlled by a micromanipulator. The position of the electrode tip could be monitored and confirmed through the microscopic image system (Leica DFC450C Digital camera and LAS imaging suite), which enabled us to have more precise positioning capability for our study. Once the origin of inter-ictal spikes was identified, the stimulation electrode was placed close to the spiking focus. If two stimulation electrodes were applied, one was placed on the afferent path (orthodromic stimulation) and the other on the efferent path (antidromic stimulation). In this study, the intensity of 500 μA, biphasic current, with a 100 μs duration in each phase was applied in all stimuli. The detail of the formulation of each different stimulation scheme, such as stimulation frequencies, durations, and inter-impulse intervals will be described in the Results Section. There was a 5 minute resting time between each stimulus train.

D. Seizure Focus Identification

The seizure focus was first identified by the channel demonstrating the largest inter-ictal spike amplitude. It was also verified by the current source density (CSD) analysis. In this experiment, the 2D CSD analysis was performed using toolbox provided from INCF (http://software.incf.org/) [17]. The raw data was first filtered (0.1-1KHz), then averaged

(from consecutive 100 inter-ictal spikes). A segment of 300 ms of the averaged inter-ictal spike waveform was exported to reconstruct current distribution map.

III. Results

A. The in vitro Seizure Model in Human Hippocampal Slices

The inter-ictal activity was consistently recorded in HiK-LoMg-4AP aCSF, and the slice viability was maintained for a long period of time using the preparation procedures described. As a rule, each slice was monitored for 3 to 5 hours. However, successful recordings could be obtained for longer periods of time. Figure 1(A) shows the inter-ictal activity recorded for three hours in the same slice. Figure 1(B) demonstrates recording for more than 11 hours after the slice was resected from the brain. The data shown in Figure 1(A) and (B) are from different surgical cases.

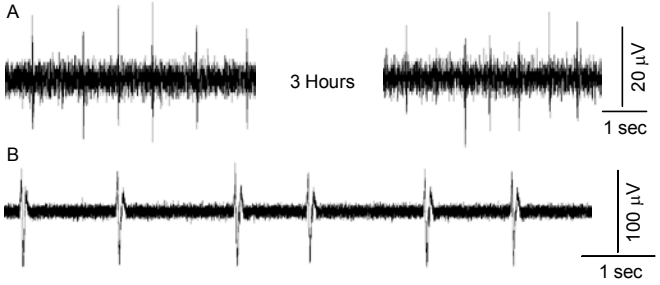

Figure 1. Tissue viability and stability were well preserved in the slice. (A) The inter-ictal activity was sustained for 3 hours. (B) The inter-ictal activity at 11 hours after the tissue was resected from the patient.

B. 2D CSD analysis of the inter-ictal activity

2D CSD analysis was applied and an example of the result is presented in Figure 2. The accurate location of the inter-ictal zone can be identified through the CSD analysis. The distribution of current source and sink of the inter-ictal spike was used to confirmed the placement of the stimulation electrode. In this Figure, two stimulation electrodes were placed at the seizure focus, the upper one is the afferent, and the bottom one is efferent.

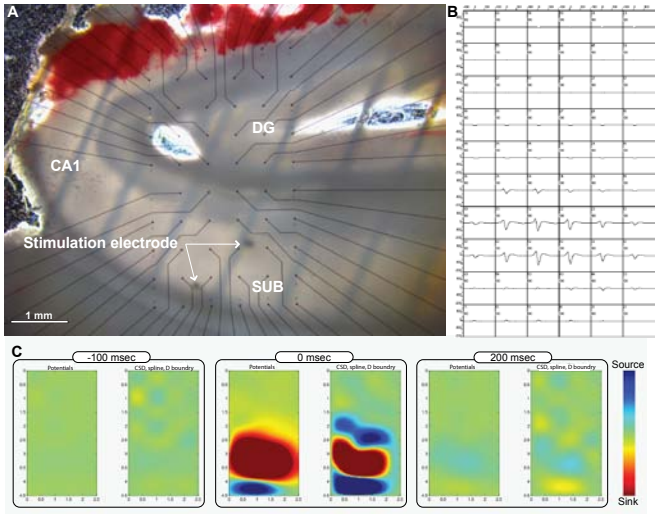

Fig. 2. A demonstration of the 2D CSD analysis. (A) A photo of a human hippocampal slice with the MEA, (B) 60 channel data recorded from the array. Only a segment of 300 ms of the spike was exported (from -100 ms to 200ms, where the minimum point of the spike is set as 0 ms). (C) Snapshots of the potential (left), and current source (right) distribution at different time periods.

C. Electrical Stimulation to Suppress Inter-Ictal Spikes

In order to observe the suppressive effects caused by the electrical stimulation, we applied varying stimulation frequencies (1, 5, 60, 100, 130, 200 Hz) and durations (5, 10, 30 sec, 1 min, and 2 min) in a pilot study. Some examples are showed in Figure 3. The results showed that the longer stimulation durations suppressed the inter-ictal activity for longer periods of time. The inhibition was not obvious for stimulation durations of 10 and 30 sec, regardless of frequency. In the 1 minute stimulation paradigm, both of the amplitude and the number of inter-ictal spikes were reduced.

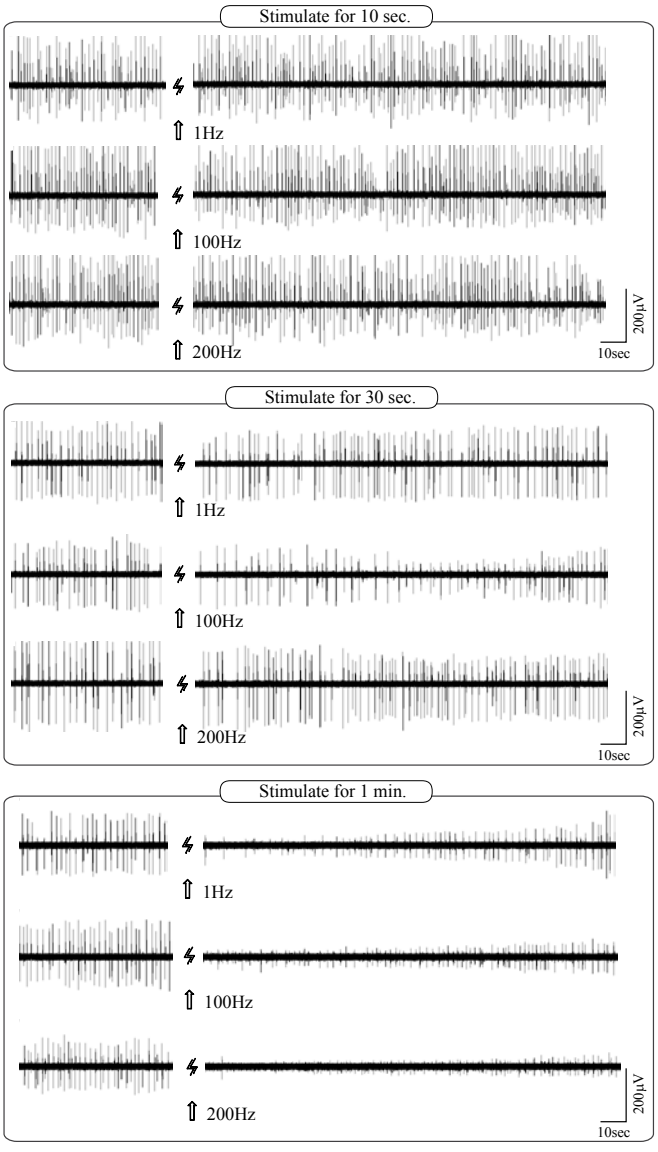

Fig. 3. Examples of suppression effects by electrical stimulation. Varying stimulation frequencies (1, 100, 200 Hz) and durations (10, 30 sec., and 1 min.) were applied to suppress inter-ictal spikes. 1 min stimulation duration had a noticeable effect independent of frequency.

We also applied longer durations of stimulation (2.5 min) of varying patterns, and through varying pathways to suppress the inter-ictal spikes. In this set of experiments, random interval trains (RITs) and constant interval trains (CITs) were

delivered to the slice both orthodromically and antidromically. RIT stimulation is composed of 300 pulses of Poisson distributed random intervals with a mean frequency of 2 Hz (i.e., a mean interval of 500 ms). CIT stimulation has the same 2 Hz mean frequency with fixed intervals (500 ms), and equal numbers of stimulation pulses (300 pulses). The inter-ictal spikes were also suppressed by these stimulation patterns. As shown in Fig. 4, inter-ictal spikes were immediately reduced after RIT stimulation was applied both antidromically as well as orthodromically, and then slowly returned to the baseline, demonstrating the continued viability of the tissue.

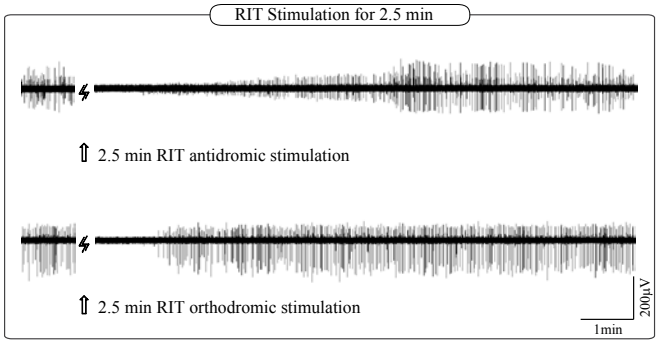

Fig. 4. Both antidromic and orthodromic RIT stimulation inhibit inter-ictal spikes.

The effectiveness of inter-ictal spike suppression between different stimulation parameters was compared by calculating the average number of inter-ictal spikes before and after the stimulation was applied. In average, the number of inter-ictal spikes was decreased by 80% immediately after the stimulation, then slowly returned to the baseline. The suppression period (the time for the mean inter-ictal spike rate returned to the baseline) caused by the RIT stimulation was 150 sec., and was 100 sec. by the CIT stimulation. The difference between orthodromic and antidromic stimulations is not significant.

IV. Discussions

Epilepsy surgical specimens from human epileptic hippocampal tissue provided an opportunity to study the response of hippocampal neural networks to neurostimulatory modalities. Here we have demonstrated an *in vitro* testbed for examining the efficacy of varied electrical stimulation parameters to suppress inter-ictal neuronal responses. In our model, stimulation duration needed to be at least 1 minute to observe significant inter-ictal spike inhibition. Similar inhibitory effect could also be seen in RIT or CIT stimulations, and in orthodromic or antidromic stimulations.

More experiments on varying combinations of parameters, and more comprehensive analysis (such as spike amplitude reduction or spike interval increment) are in progress. Since the inter-ictal focus from different patients could be observed from different regions including dentate gyrus, CA1, and Subiculum, further comparison between stimulation parameters for specific seizure zones will also be considered. Finally, an optimal paradigm for suppressing seizures in patients with epilepsy will be explored to assist the clinical team managing neuromodulatory devices.

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