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An *in vitro* seizure model from human hippocampal slices using multi-electrode arrays☆

Min-Chi Hsiao[a,*], Pen-Ning Yu[a], Dong Song[a], Charles Y. Liu[b], Christianne N. Heck[c], David Millett[c,d], Theodore W. Berger[a]

[a] Department of Biomedical Engineering, Center for Neural Engineering, University of Southern California, Los Angeles, CA 90089, USA
[b] Department of Neurological Surgery and Neurology, USC Keck School of Medicine, USC Center for Neurorestoration, Department of Biomedical Engineering, and Rancho Los Amigos National Rehabilitation Center, USA
[c] Department of Neurology, USC Keck School of Medicine, USC Center for Neurorestoration, Los Angeles, CA 90033, USA
[d] Rancho Los Amigos National Rehabilitation Center, Downey, CA 90242, USA

HIGHLIGHTS

- We report methods to prepare hippocampal slices from temporal lobectomy subjects.
- This is the first application of using planar MEA to study human hippocampal tissue.
- A portable system was designed and fabricated to transport human brain tissue.
- A novel approach to induce epileptiform in human epileptogenic hippocampal slices is described.
- Inter-ictal like activity was consistently observed in DG, CA1, and SUB subregions.

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ABSTRACT

Temporal lobe epilepsy is a neurological condition marked by seizures, typically accompanied by large amplitude synchronous electrophysiological discharges, affecting a variety of mental and physical functions. The neurobiological mechanisms responsible for the onset and termination of seizures are still unclear. While pharmacological therapies can suppress the symptoms of seizures, typically 30% of patients do not respond well to drug control. Unilateral temporal lobectomy, a procedure in which a substantial part of the hippocampal formation and surrounding tissue is removed, is a common surgical treatment for medically refractory epilepsy. In this study, we have developed an *in vitro* model of epilepsy using human hippocampal slices resected from patients suffering from intractable mesial temporal lobe epilepsy. We show that using a planar multi-electrode array system, spatio-temporal inter-ictal like activity can be consistently recorded in high-potassium (8 mM), low-magnesium (0.25 mM) artificial cerebral spinal fluid with 4-aminopyridine (100 μM) added. The induced epileptiform discharges can be recorded in different subregions of the hippocampus, including dentate, CA1 and subiculum. This new paradigm will allow the study of seizure generation in different subregions of hippocampus simultaneously, as well as propagation of seizure activity throughout the intrinsic circuitry of hippocampus. This experimental model also should provide insights into seizure control and prevention, while providing a platform to develop novel, anti-seizure therapeutics.

1. Introduction

Epilepsy is the condition of recurrent unprovoked seizures. Epilepsy 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. About 30% of patients with epilepsy have treatment resistant epilepsy (TRE) – epilepsy that persists despite

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* Corresponding author at: 3641 Watts Way, Los Angeles, CA 90089-2520, USA. Tel.: +1 213 740 8061; fax: +1 213 740 5687.
E-mail addresses: mhsiao@usc.edu, minchi.hsiao@gmail.com (M.-C. Hsiao), penning@usc.edu (P.-N. Yu), dsong@usc.edu (D. Song), cliu@med.usc.edu (C.Y. Liu), check@med.usc.edu (C.N. Heck), millett@med.usc.edu (D. Millett), berger@usc.edu (T.W. Berger).

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numerous trials of anti-seizure medications. New interventions using neuromodulatory devices such as vagus nerve stimulation, deep brain stimulation, and responsive neurostimulation are available or are under study for the treatment of TRE (Nagel and Najm, 2009; Morrell, 2006; Fisher, 2011; Skarpaas and Morrell, 2009; Nahas et al., 2005; Milby et al., 2008; Stacey and Litt, 2008). A common type of epilepsy is "partial epilepsy", arising from the temporal lobe, and in particular the hippocampus. Although hippocampal partial epilepsy is common, when treated surgically, it is the most successfully treated form of epilepsy. Surgical removal of the seizure focus, namely the hippocampus and anterior temporal lobe structures, may provide complete control of seizures in 60–90% of patients.

Epilepsy surgical specimens from human epileptic hippocampal tissue may provide an ideal substrate for studying the neural networks within epileptic zones. Electrophysiological study of sclerotic human hippocampal tissue, has yielded important findings, particularly following the development of methods to section and preserve thin hippocampal slices. Spontaneous inter-ictal like activity was first recorded from the subiculum (SUB) in hippocampal slices using a combination of intracellular and extracellular tungsten electrodes (Cohen et al., 2002), but could not be recorded from the sparse CA3 or CA1 regions or from the well-preserved dentate gyrus (DG). Paired hilar stimulation in combination with a high extracellular potassium concentration was subsequently shown to promote a variety of epileptiform phenomena recorded from the DG in hippocampal slices, including intermittent spike-waves, periodic 1 Hz spiking, and seizure-like events (Gabriel et al., 2004). Finally, spontaneous inter-ictal like activity from the CA2 region of sclerotic hippocampi was recorded under physiological conditions, distinct from independent and more frequent activity in the SUB (Wittner et al., 2009). Whether each of these structures is capable of generating epileptiform activity in mesial temporal lobe epilepsy, which aberrant signals are critical for ictogenesis, and whether the mechanism of ictogenesis is similar among heterogeneous groups of patients with mesial temporal lobe epilepsy remain central unanswered questions.

The development of miniaturized electrodes for recording extracellular, intracellular and membrane activity has been central to modern neuroscience. The innovation of multi-electrode arrays (MEAs) has permitted high-resolution electrical recordings and stimulation in studies of epilepsy, motor control, hippocampal prosthesis, and other brain-computer interface applications (Novak and Wheeler, 1986; Taketani and Baurdy, 2006; Gonzalez-sulser et al., 2011; Wagenaar et al., 2005; Kowal et al., 1998; Hsiao et al., 2013; Berger et al., 2001, 2005). The recent use of MEAs in the study of rat hippocampal slices under high K^+ conditions has led to identification of a hippocampal network for the initiation and propagation of epileptiform discharges (Liu et al., 2013).

We provide here methods for isolating human hippocampal tissue from subjects undergoing epilepsy surgery, specifically temporal lobectomy, preserving viability of the human tissue, and the first application of the MEA recording technique to study the electrophysiological characteristics of human epileptogenic hippocampal tissue.

2. Materials and methods

2.1. Human hippocampal tissue preparation

The *in vitro* seizure model was developed using the surgical byproduct hippocampal tissue from patients suffering from TRE. Patients first underwent a standard pre-surgical workup. Those identified as candidates for temporal lobectomy were consented for surgery in the standard manner. Following consent for surgery as the primary treatment for their epilepsy, they were then consented for this study (University of Southern California Institutional Review Board approval number: HS-10–00162). The surgery was performed in the standard manner with no alterations in technique to accommodate the study. During the surgery, the head and body of the hippocampus were dissected *en bloc* by the neurosurgeon. The tissue was immediately placed into a petri dish filled with cold sucrose solution (4 °C), as seen in Fig. 1, because this low temperature slows down the metabolic rate of cells and reduces the energy consumption. To avoid resistance during the slicing procedure, the pia in the choroidal fissure was carefully dissected away. The final size of the dissected hippocampal tissue varied in each case, but generally, the ranges of cross-section dimensions were approximately 15–20 mm in length, and 10–15 mm in width.

2.2. Hippocampal tissue slicing procedure

Once the corresponding orientations and major landmarks of the tissue were confirmed with the neurosurgeon, either the posterior or the anterior face of the specimen was quickly glued on the specimen plate with Cyanoacrylate glue. A gel block made of 3% agarose, prepared one day before the experiment and cut into ~3 cm × 1 cm × 1 cm cubes, was glued next to the specimen to avoid leaning back due to the advancing blade, as described in (Wang and Kass, 1997). The specimen plate then was put into the buffer tray, which was filled with icy sucrose solution and kept oxygenated. The tissue then was sliced using a vibratome (Leica VT1200, Germany), as shown in Fig. 2.

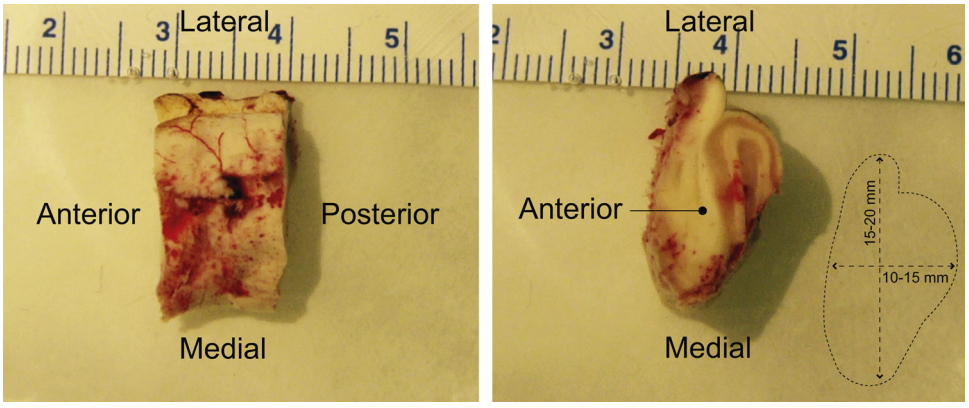

Fig. 1. Resected hippocampal tissue from the right hemisphere of a patient with epilepsy.

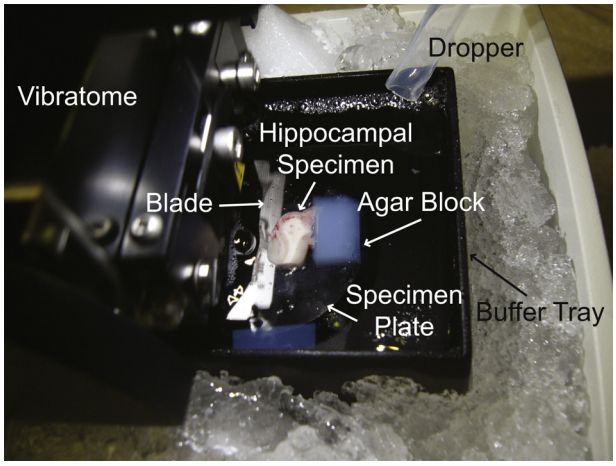

Fig. 2. The preparation of human hippocampal tissue for vibratome slicing.

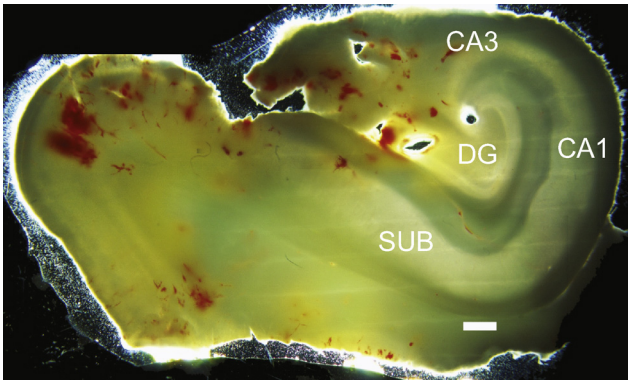

Fig. 3. Photomicrograph of a 500 μm-thick coronal section of a human hippocampal slice from a patient with epilepsy (scale bar: 1 mm).

The optimal vibratome parameters for harvesting high-quality slices are 0.20 mm/s for the advancing speed and 1.25 mm for the vibrating amplitude. Slower advancing speeds (*e.g.*, between 0.05 and 0.10 mm/s) and higher vibrating amplitudes (up to 1.50 mm) were used sometimes when distortion of the tissue specimen was observed during the slicing session. The slices included in this study were 500 μm thick coronal slices. A digital photomicrograph shown in Fig. 3 illustrates the unmistakable, identifying cytoarchitecture of the human hippocampus visible in a representative slice from the present study.

The slicing procedures were carried out at a substerile site immediately adjacent to the operating theater. Slices were prepared one at a time and quickly transferred to compartment wells using a dropper. In all cases, special care was taken to slice the block of resected tissue as quickly as possible. After excision, it took on average 20–30 min to finish the slicing process; approximately 15 slices could be prepared during this time from one block of tissue.

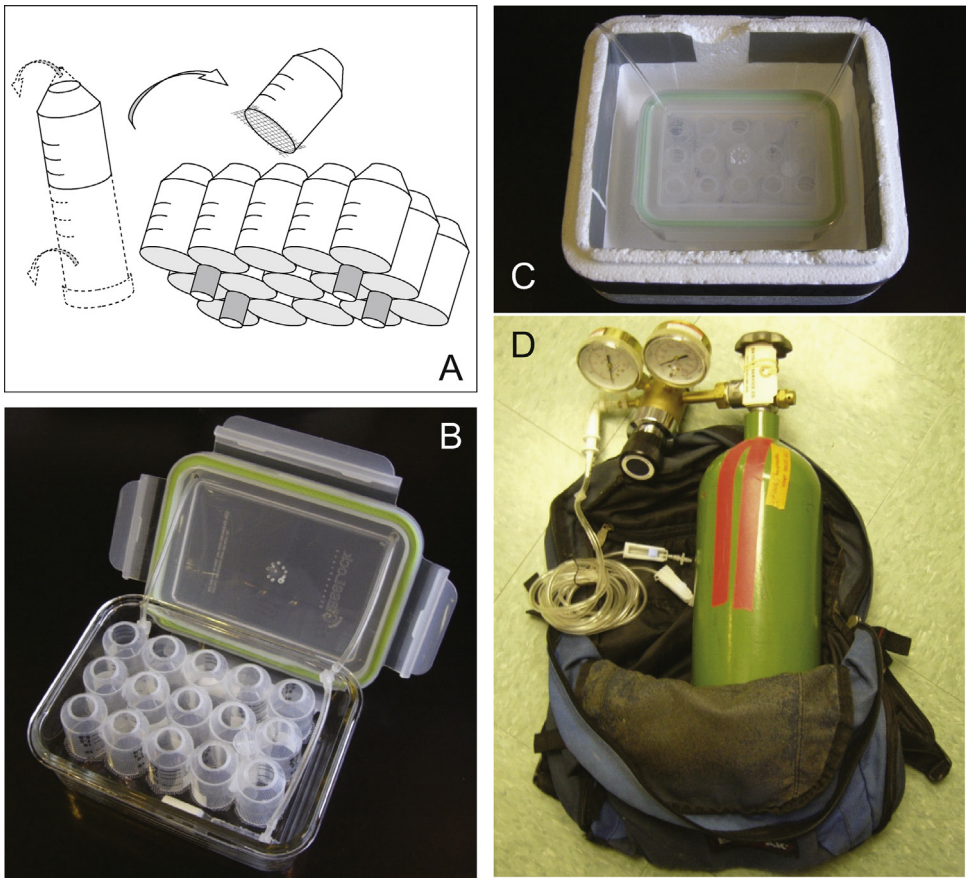

Fig. 4. The MOTS system. (A) 15 compartment wells made by centrifuge tubes were glued together. The tip and the lower half of the tubes were cut off. Nylon mesh was glued at the bottom of the trimmed tube. The slanted top protected the slices when swaying occurred during carrying and transportation phases. (B) Photo of the compartment wells in the glass container. Two gas dispensers were installed on the sides. The sealable lid was tightened after all slices were collected to avoid spill of the sucrose solution. (C) The covered glass container was put into the styrofoam container to be carried during the transportation session. Crushed ice was put inside the styrofoam container to maintain an appropriately cold environment for the slices. (D) The portable tank was carried by a backpack. The oxygenation gas was delivered to all solutions.

2.3. MOTS system for transporting brain slices

In this study, the temporal lobectomy surgery was performed in an operation theater of the Keck Hospital of USC, on the Health Sciences Campus. As in many universities, the medical school and non-medical schools of USC are located on different campuses (both within Los Angeles, CA). Thus, electrophysiology testing of the tissue was conducted in a laboratory operating as part of the Neuroscience Program on the University Park Campus of USC, located 7 mi from the Hospital (travel time, 25–40 min). In order to accommodate this practical constraint, a customized Mobile Oxygen-Temperature Sustaining (MOTS) system was designed and fabricated to sustain the viability of the human hippocampal slices during transportation. The MOTS system was composed of:

(i) Slice compartment wells: 15 tubes (VWR centrifuge tubes, 50 mL, #89004) were cut to half height and glued to each other side-by-side by Silicone (All-Glass Aquarium Co., Inc.). A nylon mesh was glued at the bottom of each tube to hold the slices in each compartment. The internal diameter is 25.4 mm, allowing slices to unfold completely. Usually, one slice (no more than two) was contained in each compartment well.
(ii) Glass and Styrofoam Containers: a glass container with sealable lid (Glasslock RP-518, 177 mm × 131 mm × 68 mm) was used. Two gas dispersion tubes (Chemglass, CG-203) were cropped and then installed in the container, and were connected to the oxygenation gas tank through Tygon tubes (R-3603). Each dispersion tube is attached to a regulator to make fine flow adjustments. The glass container was put in a Styrofoam container (220 mm × 190 mm × 115 mm) surrounded with crushed ice to maintain at 4 °C throughout the whole session, including slicing and transportation.
(iii) Oxygenation gas tank: in order to make the whole system transportable, the gas tank selected was a 24 cubic feet gas cylinder (18 in. tall and 5 in. in diameter). The tank contains 95% Oxygen and 5% Carbon Dioxide. A CGA-500 regulator was used to adjust the output pressure and flow. During slicing, transportation, and experimental testing, all solutions were bubbled with this mixed gas, and the pH should be kept in 7.4 (Gabriel et al., 2004; Wang and Kass, 1997).

In summary, the slice compartment well is placed inside the glass container filled with sucrose and put into the styrofoam container that preserves the temperature at 4 °C until the slices have been carried to the laboratory (Fig. 4).

2.4. Solution preparation and time of usage

The sucrose solution in which slices were maintained contained: Sucrose 248 mM; KCl 1 mM; $NaHCO_3$ 26 mM; Glucose 10 mM; $CaCl_2$ 1 mM; and $MgCl_2$ 10 mM (Huberfeld et al., 2011). It was used during in the slicing and transportation sessions. NaCl was removed to decrease the neurotoxic effects of passive Na^+ influx followed by cell swelling and lysis. In addition, to preserve the ice-cold temperature throughout the whole session of tissue preparation, frozen sucrose cubes could be prepared and subject to use. In our case, the frozen sucrose were made with 50 ml centrifuge tubes (in total 6–8 tubes), and placed inside the Sucrose container and buffer tray.

Normal artificial cerebral spinal fluid (aCSF) is made of NaCl 124 mM; KCl 4 mM; $NaHCO_3$ 26 mM; Glucose 10 mM; $CaCl_2$ 2 mM; and $MgCl_2$ 2 mM (Huberfeld et al., 2011). It was prepared and kept at room temperature (24–26 °C). After slices arrived at the laboratory, the sucrose solution in the glass container was changed to normal aCSF and the glass container then was switched into a water bath (Model 182, Precision Scientific, Inc.) and maintained at 30–31 °C. Before conducting electrophysiological recordings, slices were immersed in normal aCSF for at least 1 h to permit maximal recovery from surgery, slicing, and transportation.

2.5. Electrophysiology experiment setup

Electrophysiology data were collected through an MEA1060-Inv extracellular, multi-electrode array recording system with 60 input channels (Multi Channel Systems, Germany). This system consisted of 60 pre-amplifiers (1200× gain), a data acquisition device (MC_Card), and an 8-channel stimulus generator (STG1008), all operated using software provided by Multi Channel Systems (MC_Rack v3.9.1 and MC_Stimulus v2.0.6). Fig. 5 shows an example of the MEA recording array (A) and (B) relative to a human hippocampal slice, as well as representative data recorded from all 60 channels. Using the transparent glass-based planar MEA, the slice image and its corresponding position to the electrode pads can be observed clearly. The multi-electrode array used in this study was the MEA 500/30iR-Ti (Fig. 5A). Fig. 5B shows the 10 × 6 layout of the electrode array (electrode diameter: 30 μm, inter-electrode spacing 500 μm, electrode impedance: 30–50 kΩ). The overall distributed area covered by the electrode array was 11.25 mm^2. This feature enabled recording neural activity from two to three hippocampal subregions simultaneously.

In this study, electrical stimulation was applied to confirm that the slice was viable. An external bipolar electrode of twisted Nichrome wires (diameter: 50 μm, A-M system #762000) was used; biphasic currents with a 100 μs duration in each phase were applied to all stimulation schemes. The schemes included paired-pulse, quadruplet-pulse, and input/output (*I*/*O*) curve stimulations. The *I*/*O* curve stimulation protocol included current intensities ranging from 100 to 1000 μA, with 100 μA increments. The stimulation schemes were programmed using MC_Stimulus and pre-loaded into the stimulus generator.

Data were sampled at a frequency of 10 kHz per channel and were recorded using MC_Rack. The MEA1060-Inv system was situated over an inverted microscope (Leica DM-IRB, Germany). In each experiment, the position of the slice on the MEA was recorded by a digital image capture system (Diagnostic Instruments, Spot RT Digital Camera) with SPOT (v4.6.4.3) and Adobe Photoshop (Adobe v7.0) software. The experimental setup is shown in Fig. 6. Two circulation pumps (Fisher Scientific model 13-876-4, circulation rate: 6 mL/min) were used to perfuse aCSF solution into the bath surrounding the slice. The solution temperature was controlled by a circulated heating tank (HAAKE fisons-DC3) and was maintained at 32–34 °C (repeatedly measured with the thermocouple thermometer, Cole-Parmer EX-91210-31).

3. Results

3.1. MOTS system maintained tissue viability

The key factors for slice viability are time, temperature, and oxygen supply. For this translational project, the human hippocampal tissue was resected in an operating room physically located a considerable distance from the electrophysiology laboratory. Slicing the human tissue occurred at the hospital immediately after the tissue was resected followed by rapid transport of the slices to the laboratory. The MOTS system was designed to preserve the viability of the slice through maintenance of the oxygenation supply and bath temperature in a highly portable and low-cost manner.

The viability of the slice was verified by several physiological indices. First, the input/output (*I*/*O*) relationship of electrophysiological responses to electrical stimulation of major excitatory afferents was measured for each slice. Fig. 7 shows an example of a subthreshold field potential DG granule cell response to perforant

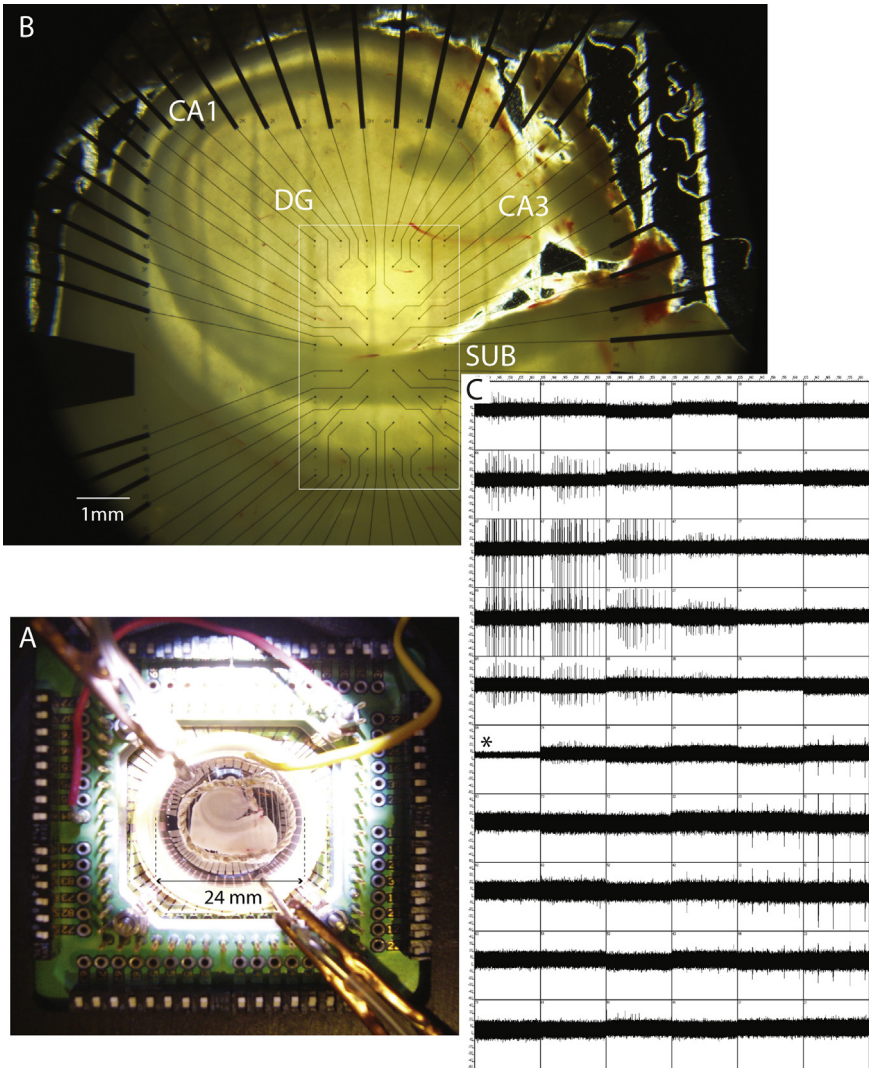

Fig. 5. A schematic of the MEA1060-Inv system data presentation diagram. (A) A photo of an epileptic human hippocampal slice submerged in the MEA recording chamber. (B) The image of the slice on the 10 × 6 MEA captured by a digital image system through an inverted microscope. The superimposed white rectangular frame demonstrates the region where the neural activities could be recorded. (C) Actual 60 channel data recorded by the 10 × 6 array. Each channel (box) displays the instant data recorded from the corresponding electrode (*X* axis: 30 s and *Y* axis: 100 μV in this case). The channel demarcated with an asterisk was assigned as the internal reference, or ground.

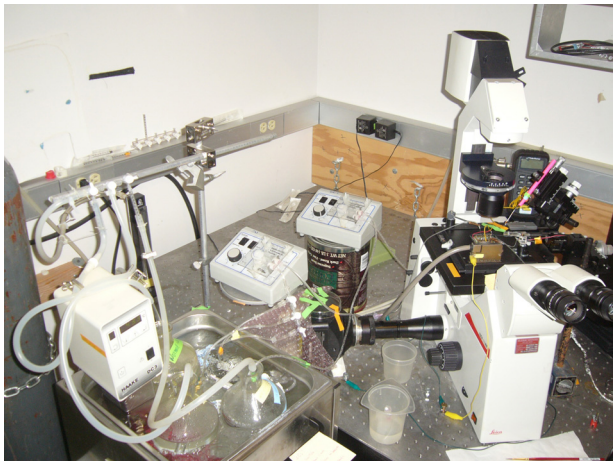

Fig. 6. The experimental setup including the MEA1060-Inv system embedded with the inverted microscope. A circulation heating tank for maintaining the perfusion solutions at 32–34 °C. Two pumps (in-flow and out-flow) circulate the solutions to the slice in the MEA.

path (PP) stimulation. In this experiment, two adjacent recording electrodes close to the granule cell body layer were selected from the MEA, as sketched in Fig. 7A. Examples of the evoked responses recorded from each electrode are shown in Fig. 7B. The inversion of the polarity of the waveforms demonstrates that the chosen electrode sites of the MEA captured the synaptic generator of perforant path dendritic activation, *i.e.*, the current sink (negative-going wave); the positive-going wave indicates the current source caused by the return of current (Andersen et al., 1971, 2006). To quantify the responses, field potential amplitudes were defined as the peak value (either positive or negative) from the baseline. Fig. 7C shows the *I*/*O* curves from the same recording electrodes selected in 7B; the amplitudes increase in correlation with increases in stimulation intensity, reaching a plateau when the response is maximum (to stimulus intensities greater than approximately 600 μA).

Paired-pulse or quadruplet-pulse stimulation also was applied to verify that nonlinearities of synaptic transmission were preserved in slices of human hippocampal tissue. Nonlinearities in responses to synaptic inputs reflect feedforward and feedback mechanisms due to local circuitry and/or intrinsic biochemical events that alter the response to subsequent inputs. For example, Fig. 8A shows commonly occurring paired-pulse facilitation

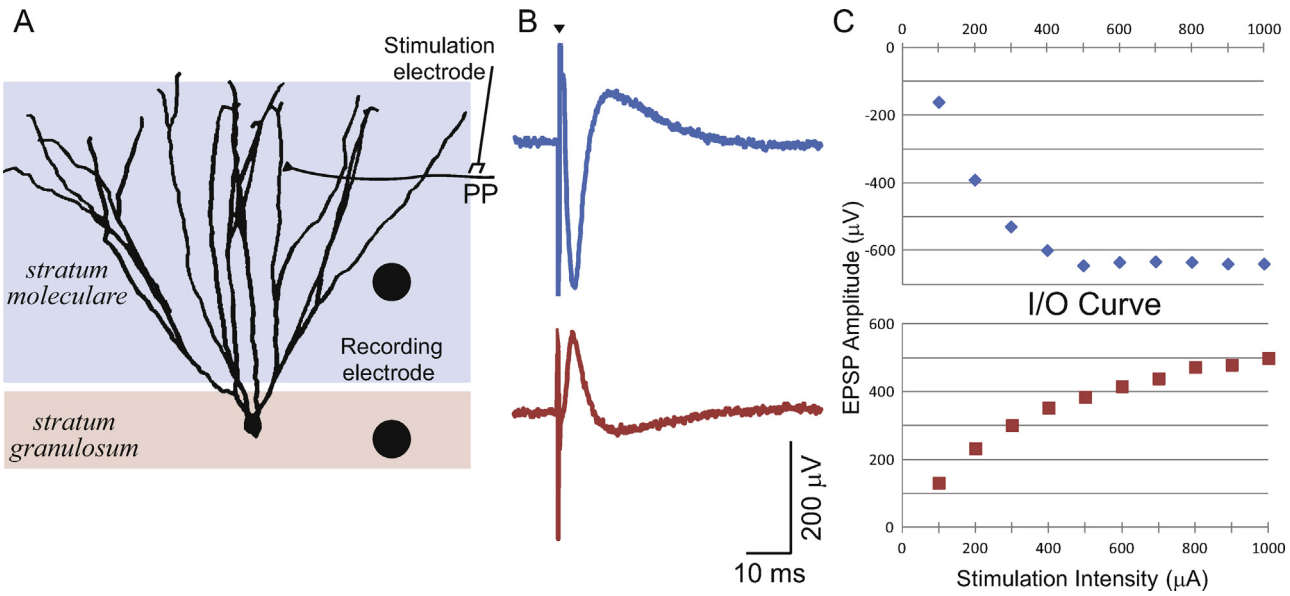

Fig. 7. Electrical stimulation was applied to verify the viability of the slice. An *I/O* relationship of the DG response to the perforant path (PP) stimulation is shown here. (A) A sketch of the stimulation and recording electrode, and their corresponding position to the slice. (B) An evoked field potential recorded from the electrode located at *stratum granulosum* and *stratum moleculare* electrodes, respectively. (C) The *I/O* curve from the same stimulation and recording electrode.

recorded from the perforant path-DG pathway. Population spike amplitude is measured by taking the distance between the minimum point of the negative peak and the intersection of its upward projection to the line linking two positive peaks, as shown in Fig. 8B, measure a. Paired-pulse facilitation is believed to reflect a temporary presynaptic accumulation of calcium; a higher concentration of calcium facilitates the amount of neurotransmitter released, thus increasing the number of neurons activated postsynaptically as reflected in the amplitude of the population spike. Because the accumulation is temporary, it is observed only in response to intervals of approximately 60 ms and less.

The time course of paired-pulse facilitation reflects the time course of mechanisms responsible for removing free Ca^{2+} from the presynaptic space. Given that multiple mechanisms affect membrane excitability, and that different mechanisms have different time courses, then it is reasonable to assume that different interspike intervals will activate different mechanisms. Thus, a train of varying inter-impulse intervals will lead to a profile of facilitations and suppressions determined by the specific mechanisms and combinations of mechanisms activated by the particular interimpulse intervals in the train. In Fig. 8C, partial results are shown for electrically stimulating the perforant path with a segment of a train of impulses having a random series of inter-impulse intervals. Although at present nothing can be said about the specific mechanisms activated by the train, it is obvious that, as predicted, amplitude of the population spike varies substantially as a function of the stimulation inter-impulse interval. This result is consistent with the hypothesis that the multiple mechanisms underlying synaptic transmission in large part have been preserved during the slicing and transport procedures. Subsequent studies will investigate this hypothesis further.

The entire set of procedures, including human hippocampal tissue resecting, slicing, transportation, and recording, has been tested successfully in our experiments. These results demonstrate that the MOTS system is adequate for ongoing tissue study.

3.2. Epileptiform discharges was induced by HiK^{+}-$LoMg^{2+}$-4AP aCSF

With the aim of developing an *in vitro* seizure model using an MEA system, we followed the approaches developed by other groups using several different seizure induction protocols. These included lowering the extracellular Mg^{2+} concentration to increase the glutamatergic neurotransmission; applying bicuculline and picrotoxin to decrease the GABAergic inhibition (Bernard, 2005; Köhling et al., 1998, 2005; Straub et al., 1996). We also attempted antidromic stimulation of the hilar region with a higher K^{+} concentration (10–12 mM) environment as described in (Gabriel et al., 2004; Kivi et al., 2000; Wahab et al., 2010). However, these methods did not consistently induce epileptiform discharges in our setup. In our preliminary experiments, the slice activity was continuously monitored for at least 4 h, using the approaches

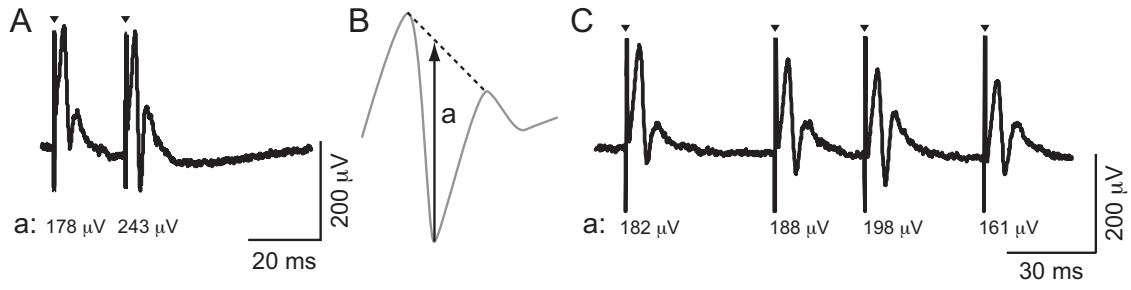

Fig. 8. Dentate gyrus population spikes evoked by (A) a paired-pulse train and (C) quadruplet-pulse train stimulation of the perforant path. The varying amplitudes show that the internal synaptic transformation was still well preserved in the slice. (B) Illustration of the amplitude measurement for population spike.

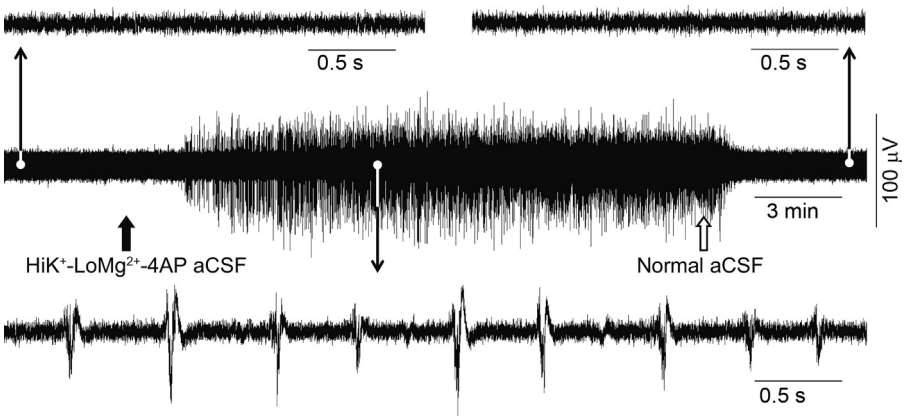

Fig. 9. The epileptiform discharges induced by HiK$^+$-LoMg^{2+}-4AP aCSF. The data on the top panel shows no inter-ictal like activity recorded when the slice was perfused with normal aCSF. In the middle panel, HiK$^+$-LoMg^{2+}-4AP aCSF was perfused to the slice for 20 min. The inter-ictal like activity appeared within 3 min in the presence of HiK$^+$-LoMg^{2+}-4AP aCSF and ceased with normal aCSF wash-out within 2 min. The lower panel shows the spike profile at a higher time resolution.

above, there were either no spikes recorded or only 5–10 spikes appeared during the whole session. Attempts to increase the K^+ (from 4 to 8 mM) and decrease the Mg^{2+} concentration (from 2 to 0.25 mM) in the aCSF, as described by Huberfeld et al. (2011) also were unsuccessful in producing inter-ictal discharges. It is known that 4-aminopyridine (4AP) interferes with potassium channels and induces different kind of epileptiforms (Brückner and Heinemann, 2000). 4AP has been applied to produce epileptiform discharges in rat hippocampal or entorhinal cortex-hippocampal slices for the development of anti-epileptic drugs (Gonzalez-sulser et al., 2011; Perreault and Avoli, 1992; Brückner et al., 1999). In our setup, adding 4AP(100 μM) into the high potassium (8 mM) low magnesium (0.25 mM) aCSF, successfully resulted in consistent, reproducible inter-ictal like activity recorded by MEAs. Fig. 9 demonstrates the inter-ictal like activity produced in the presence of HiK$^+$-LoMg^{2+}-4AP aCSF, and discontinuation of this epileptiform activity once normal aCSF was perfused into the slice.

3.3. Induced inter-ictal like activity was observed in multiple regions of human hippocampal slices

The MEA system enabled simultaneous extracellular recordings from varied sites of each slice. The actual area of inter-ictal foci can be clearly identified through the microscope, and the image then can be captured by the digital camera. During each experiment, if all 60 channels did not record any neuronal activity, the slice would be moved gently by a small brush to another spot. Hence different areas of the slice could easily be explored. If no activity was found in the entire slice, it would be discarded as non-viable. In this study, we have obtained data from 27 surgical cases, 8 cases did not demonstrate inter-ictal like activity. In the remaining 19 cases, a total of 28 slices were tested. HiK$^+$-LoMg^{2+}-4AP aCSF evoked epileptiform discharges were identified from different hippocampal regions including DG (16 slices), CA1 (5 slices), and SUB (9 slices). The spiking discharges were not observed in CA3 probably because in some cases, the CA3 subregion was not preserved intact due to surgical difficulties. Fig. 10 shows some examples of the data recorded in various regions of human hippocampus.

3.4. Stability of the in vitro seizure model

Our data have shown that slice viability can be maintained very well for a long period of time using the experimental paradigm described. Fig. 11(A) demonstrates recording from a slice that has been resected from the patient for 11 h. Once it has been placed into the recording chamber, the inter-ictal like activity can still be evoked. Typically, each slice was monitored for at least 3 h. However, successful recordings could be obtained for longer periods of time. An example of the inter-ictal like activity that has been evoked for 3 h from the same slice is shown in Fig. 11(B).

4. Discussions

Optimizing experimental procedures to obtain excellent, physiologically viable slices is always the first critical step in developing an *in vitro* slice preparation. This is particularly true in the case of developing a human slice preparation for the study of hippocampal seizures. The reasons are that: (1) the hippocampi of study candidates consist mainly of cases with sclerotic tissue; (2) often the slicing blade cannot smoothly cut through the tissue due to intra-hippocampal blood vessels and physical distortions in the extracted specimen; (3) the tissue endured surgical procedures in the context of preparing for the hippocampectomy that required a considerable number of physical manipulations. We found that there are two main parameters of vibratome operation that could be adjusted to improve the process of slicing; these included advancing speed and vibration amplitude. Changing the advancing speed directly influenced the time to finish slicing, the slower speed took longer period of time to completion. The adjustable vibration amplitude was particularly helpful when severe sclerotic tissue or blood vessel concentration is high. We found that slicing with a wider amplitude had a higher probability of generating slices that were intact and unbroken. Our aim was to complete slicing within 20 min. The initial advancing speed was set to 0.20 mm/s, and the initial vibrating amplitude was set to 1.25 mm. Once we observed that these parameters produced distortions in the hippocampal specimen, the amplitude was increased to as much as 1.50 mm, sometimes accompanied with a lowered advancing speed (down to 0.05 mm/s). If blood vessels were observed, they would be removed manually using a small surgical scissors before slicing, whenever possible.

Another key point for achieving good slice viability was use of the MOTS system. The MOTS system was developed mainly with respect to portability. The size and weight of each component was taken into consideration before final selection. The oxygen gas cylinder was 6.6 kg and was able to fit into a regularly sized backpack for physical transport. The styrofoam container was lightweight, able to be hand-carried, and capable of being maintained internally at 4 °C for at least 4 h with crushed ice. The inclined top of each slice compartment well (see Fig. 4A) could also avoid spilling from any accident that might occur during transportation. For the

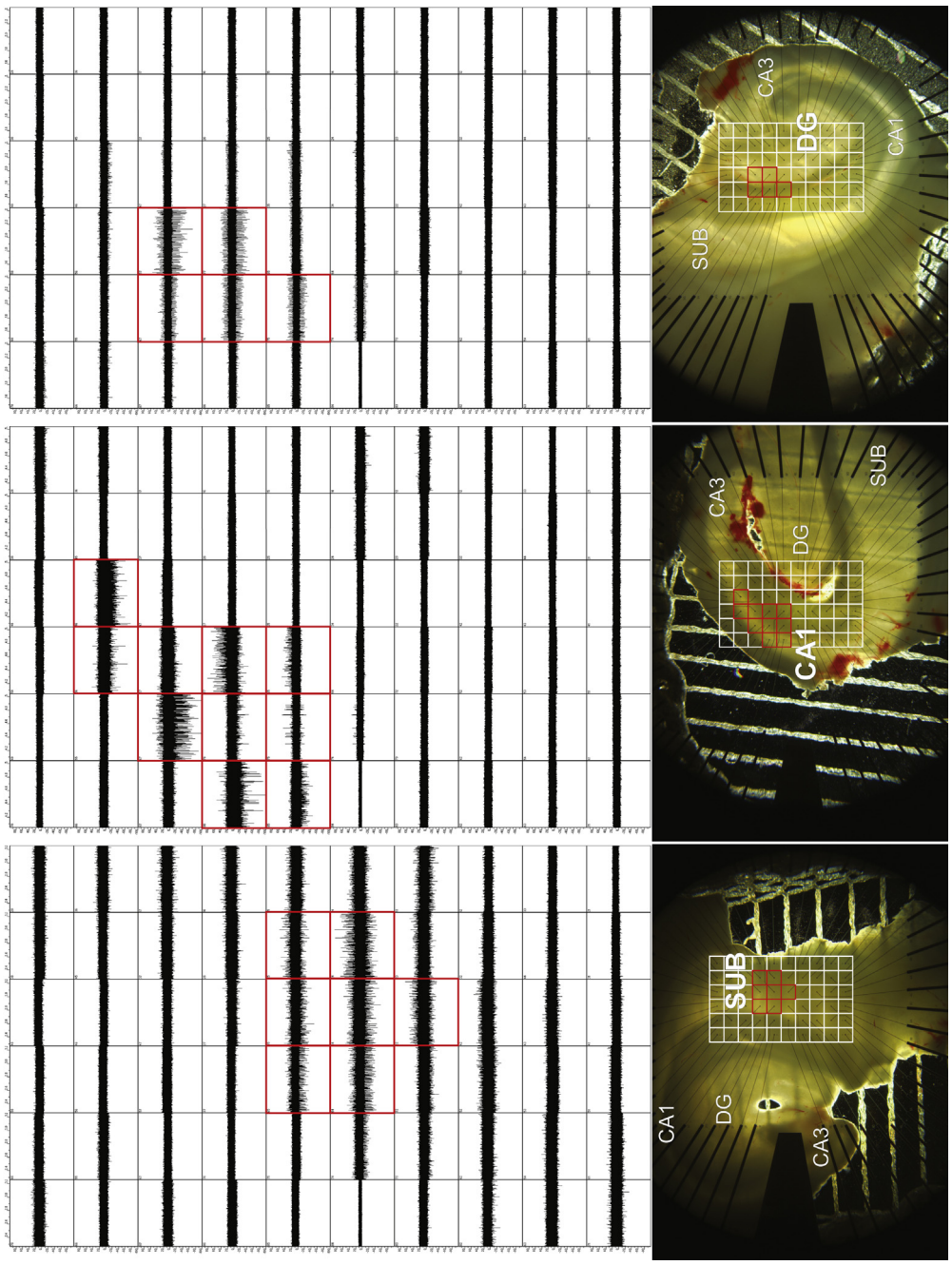

Fig. 10. HiK^{+}-LoMg^{2+}-4AP aCSF induced inter-ictal like activity recorded in SUB, CA1 and DG from human hippocampal slices (*X* axis: 1 min and *Y* axis: 200 μV).

environmental conditions critical to our study (transportation time, traffic, distance, and weather), we found it was not necessary to have a circuit board and power source to control the temperature (Knowles, 1985; Köhling et al., 1996). Our results showed that the specially designed MOTS tissue transport system successfully preserves the viability and stability of human hippocampal slices. Investigation of human brain tissue is going to be much more important in the future. It is often the case that the medical schools or the hospital facilities have a considerable physical distance from experimental laboratories. A portable system for surviving brain slices is very crucial to this type of research. We believe that the MOTS system could be very useful for many other groups who want to explore human tissue.

The size of the resected hippocampal tissue varied significantly between patients. In general, the range of the cross-section dimension was approximately 15–20 mm in length, and 10–15 mm in width, as shown in Fig. 1. In the early stages of this study, several different profiles of MEAs were tested as the recording interface with human hippocampal slices. The 20 × 3 array (Fig. 12A, electrode diameter: 30 μm, inter-electrode spacing: 50 μm, covering area: 0.19 mm^2) had a higher spatial resolution but could only record from a small portion of the total tissue region. The 8 × 8 array could cover a larger area (Fig. 12B, electrode diameter: 30 μm, inter-electrode spacing: 200 μm, covering area: 1.96 mm^2). Because cross-section of the hippocampus in the human is quite large, it could only cover a small part of a single region of the slice. Lastly, we tried the 10 × 6 array (Fig. 2C, electrode diameter: 30 μm, inter-electrode spacing: 500 μm, covering area: 11.25 μm^2), which was well-adapted to this particular study of human hippocampal tissue. The 10 × 6 array enabled us to record neural activity from

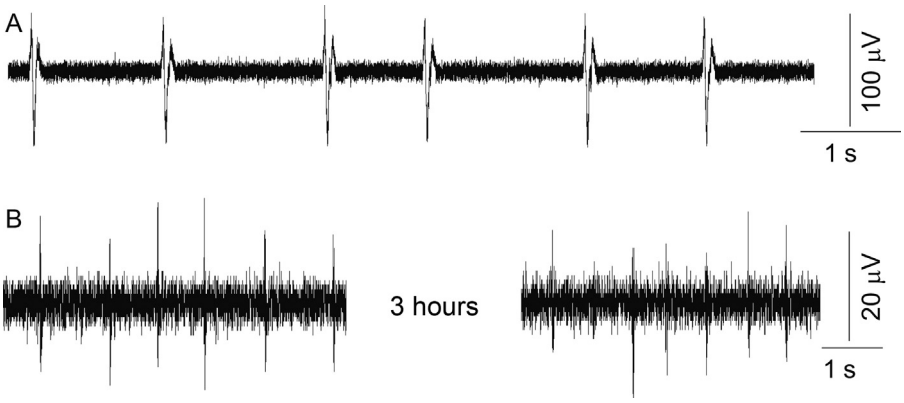

Fig. 11. Tissue viability and the stability were preserved in the slice that was prepared and transported by our custom-designed tissue transport system. (A) Inter-ictal like activity still could be recorded for 11 h after the tissue was resected from the patient. (B) Inter-ictal like activity sustained for 3 h in one slice. The data shown in (A) and (B) are from different surgical cases.

broad cross-section of hippocampal tissue, as well as from different hippocampal subregions simultaneously.

We had previously designed conformal arrays according to the cytoarchitecture in the rat hippocampal slices, in order to develop neuroprosthesis for hippocampus (Gholmieh et al., 2006; Hsiao et al., 2006). In the future, it will also be very beneficial to have a conformal array specially designed for human hippocampal slices.

In the preliminary experiments of this study, we attempted to record spontaneous ictal-like (seizure) activity from human hippocampal slices. The slices were perfused with normal aCSF and their viability was verified by the electrical stimulation procedures described in Section 3.1, and was then monitored for at least 4 h. In our recordings, no spontaneous ictal discharges were observed, which is consistent with the findings of a previous study (Köhling et al., 2005).

We had tried different approaches to induce ictal or inter-ictal activity as described in Section 3.2, including decreasing the concentration of extracellular Mg^{2+}, increasing the concentration of K^+, or applying bicuculline and picrotoxin. However, we were not able to record either ictal or inter-ictal activity by the methods introduced previously. It should be noted that all of the previous protocols were conducted using an interface recording chamber, where the bottom surface of the slice is maintained wet by the perfusing solution while the top surface is kept moist by warmed and humidified gas mixture. The experiment paradigm described here was conducted utilizing a submersion chamber. This difference in

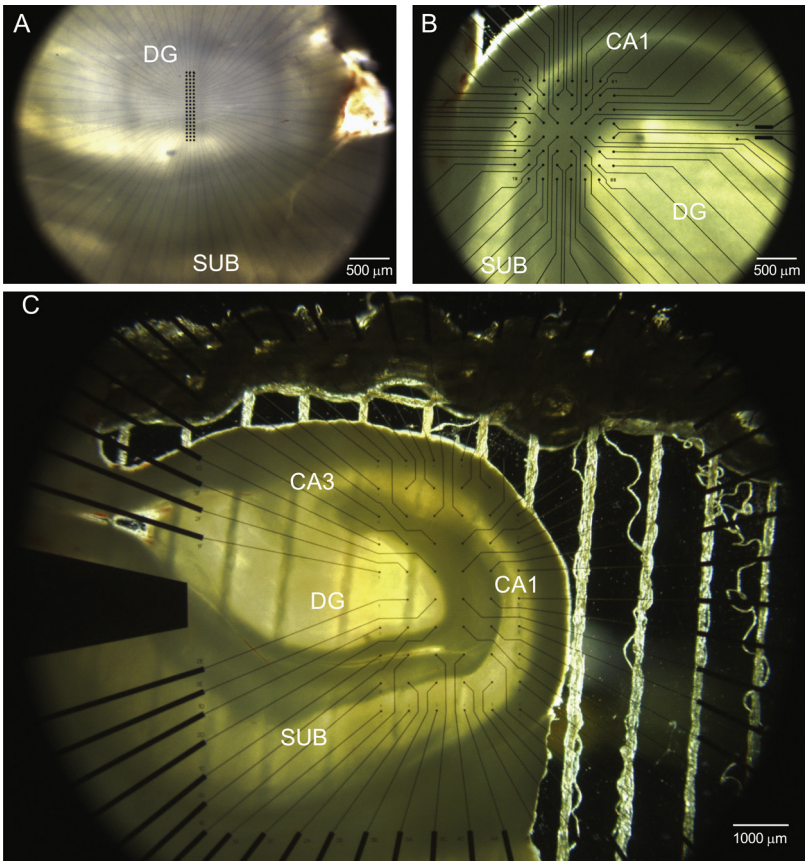

Fig. 12. Different layout of MEAs were tried in the experiments. (A) A 20 × 3 array, (B) An 8 × 8 array, and (C) A 10 × 6 array (see details in text).

slice recording chambers may be the reason why epileptiform could not be observed. After we discovered that epileptiform discharges could be induced in high-potassium (8 mM), and low-magnesium (0.25 mM) aCSF with the addition of 100 μM 4AP, we also tried combinations of different concentrations, including ([K^+]: 4 and 8 mM; [Mg^{2+}]: 0.25 and 2 mM; [4AP]: 0 and 100 μM). Only 8 mM K^+, 0.25 mM Mg^{2+} aCSF plus 100 μM 4AP consistently produced inter-ictal like activity. Among all the approaches we tried, however, none led to ictal activity (where the epileptiform last for at least 20 s, as described in Gabriel et al. (2004) and Huberfeld et al. (2011)).

In conclusion, the techniques and procedures described here establish a new *in vitro* paradigm for the study of partial epilepsy in human hippocampal slices. The MEA technology was adapted to human tissue enabling simultaneous recording in a broad area and from multiple subregions. Inter-ictal like activity was consistently observed in presence of HiK$^+$-LoMg^{2+}-4AP aCSF, and was recorded in DG, CA1, and SUB fields, and often observed in a small region of each field. This provided a consistent environment for studying the spatio-temporal electrical characteristics of human epileptic hippocampal tissue. We foresee that this *in vitro* model can be used to improve closed-loop neural stimulation techniques to treat the intractable epilepsy. It can also be applied to explore the electrophysiological characteristics of human epileptic brain tissue, and to discover the pharmacological effects of different anti-epileptic drugs.

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