EPILEPTIFORM DISCHARGE INHIBITION USING A MULTIFUNCTIONAL CRYOGENIC MICROPROBE FOR MINIMALLY INVASIVE BRAIN SURGERY

TOSHITAKA YAMAKAWA[1], TAKESHI YAMAKAWA[2],
SATORU ISHIZUKA[2], ZIMIN LEV GRIGORIEVICH[2],
MICHIYASU SUZUKI[3] and MASAMI FUJII[4]

A microprobing system which has the functions of measuring the intracranial EEG (electroencephalogram)/temperature, providing the brain stimulation current, and freezing brain tissue is proposed for the minimally invasive brain cryosurgery of intractable epilepsy treatment. The experimental results using brain samples of a rat shows that the focal cooling/freezing using the cryogenic microprobe suppresses the epileptiform discharge.

Key words: epilepsy, cryogenic surgery, minimally invasive, microelectrodes, *in vitro*.

1. INTRODUCTION

About 50 million people worldwide have epilepsy at any one time. The lifetime prevalence of epilepsy (*i.e.* the number of people presently in the world who have epilepsy now or have had it in the past or will experience it in the future) is approximately 100 million people. The mean prevalence of active epilepsy which causes continuing seizures and needs treatments is 0.82% of the general population around the world [9]. Seizures in about 70% of the epilepsy patients can be controlled with medications, even though it cannot be cured. However, up to 30% of the patients do not respond to the medications even with the best (strongest) available medicines. In that case, surgeries are applied to remove the brain tissue of the epileptogenic focus. Here, the epileptogenic focus is the roots of abnormal EEG (electroencephalogram) which causes seizures.

In the most cases of epilepsy surgeries, two times of craniotomy are necessary as follows: one is for detection of the epileptogenic focus, and the other is for resection of the brain tissue around the detected focus. However, the detection accuracy of the focus is up to several centimeters. Additionally, the tissue of the focus is resected with the margin of few centimeters. Thus the risk of the side and after effects cannot be avoided.

As a new surgical approach to the treatment for intractable epilepsy, vagus nerve stimulation (VNS) was developed [1]. In this method, a pacemaker-like

instrument is implanted to the patient in order to block the transmission of the ictal spikes on vagus nerve. However, the ratio of 50% seizure reduction (one of the characteristics for the treatment effectiveness) is still lower than 40% [3], [5].

RF thermocoagulation is being developed as another candidate surgical treatment for intractable epilepsy [4]. However, since heat generated by the radio-frequency AC current is used to necrotize the focus, the possibility of cytopathic effect (mutation of the cells which can develop cancer, sometimes) cannot be avoided.

On the other hand, cryosurgery was developed as a low invasive surgical method and has been applied to treat liver cancer, renal cancer, breast cancer, skin cancer, uterine myoma, etc. Mostly, the necrotized tissue is absorbed by the surrounding normal cells within several months since cryo-injury do not cause cell mutation – this can easily be observed with the difference between the burned meat and the frozen meat. However, cryosurgery for brain is still under development, and its effectiveness for epilepsy has not been confirmed.

In this paper, a multifunctional cryogenic microprobe is proposed to be compatible with the minimally invasive brain microsurgery. The proposed microprobe system has the functions of measuring the intracranial EEG (IC-EEG)/temperature, providing brain stimulation current, and freezing the brain tissue around the microprobe's tip for few millimeters.

Section 2 describes system configuration and the detail of the proposed multifunctional cryogenic microprobe. Here, the measurement/control system, the constitution of the probe, the surgery procedure in brief, and the manufacturing process are explained.

The experimental results of the animal tests using a rat are mentioned in Section 3. The functions of the electrodes and the effect of refrigeration performed by the probe are confirmed with the experiments using sliced hippocampus samples.

Section 4 summarizes the conclusions.

2. CRYOGENIC MICROPROBE SYSTEM

Figure 1 shows the block diagram of the proposed minimally invasive multifunctional microprobe system. This system mainly consists of 4 blocks: the multi-functional cryogenic microprobe, the EEG instrumentation amplifier, the brain stimulation current source, and the thermocouple amplifier.

The system operation is briefly explained below.

1. The multifunctional cryogenic microprobe is inserted to the pre-detected epileptogenic focus which is the break-out area of the epileptiform discharges: the abnormal neuronal bursts which cause the epileptic seizures.

2. In order to confirm that the probe is inserted to the correct location (*i.e.* the epileptogenic focus), IC-EEG is measured by the EEG amplifier with SW1 and

SW2 opened. If the epileptiform discharge is observed, the probe inserted position is determined to be an epileptogenic focus. As a confirming option, it is stimulated by the current (enough for activating epileptogenic focus and less than activating normal neurons) through the microelectrodes with SW1 and SW2 closed when the epileptiform discharge is not observed.

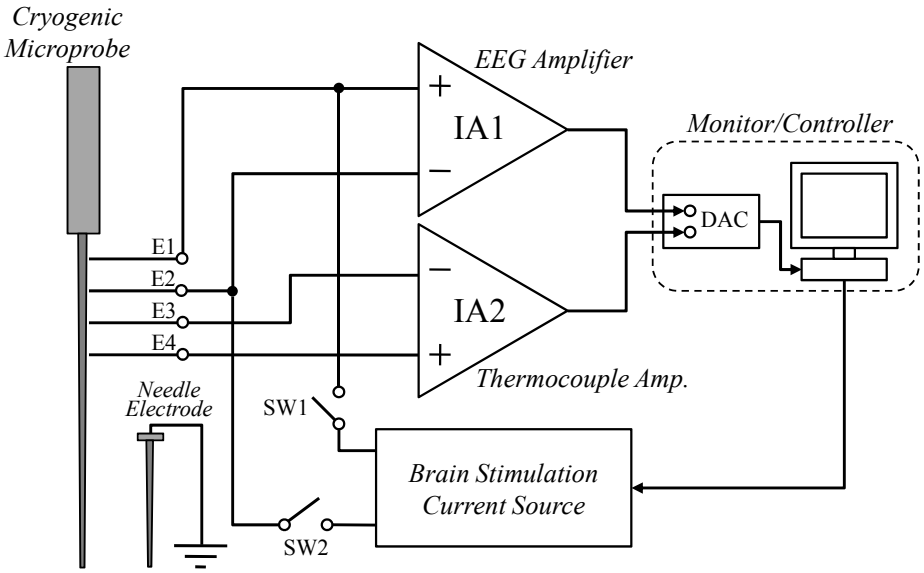

Fig. 1. Proposed minimally invasive multifunctional cryogenic microprobe system.

3. The refrigerant gas flows into the probe through the inner pipe, then the temperature of the probe's tip falls. Hence the brain tissue around the tip is frozen.

4. The brain stimulation current is applied in order to confirm that the brain tissue of the epileptogenic focus is adequately neutralized. When the epileptiform discharge does not occur after the stimulation, the epileptogenic focus no longer exists.

The details of the each block are explained in the following subsections.

MULTIFUNCTIONAL CRYOGENIC MICROPROBE

The multifunctional microprobe and its cross sectional view are shown in Figure 2.

The needle pipe consists of the microelectrodes and coaxial pipes. The outer pipe is made of stainless steel SUS304 and acts as a negative thermocouple electrode (ELECTRODE 3). The inner pipe is made of Kovar (Fe54%-Ni29%-Co17%) and acts as a positive thermocouple electrode (ELECTRODE 4) and a refrigerant guide to evaporate it. The refrigerant gas flows through a small hole made on the inner pipe, and temperature around the hole is reduced due to Joule-Thompson effect. Thus the

tip of the needle pipe acts as the cooling/freezing probe and also acts as the temperature measuring probe. The diameter of the tip is 0.8 mm.

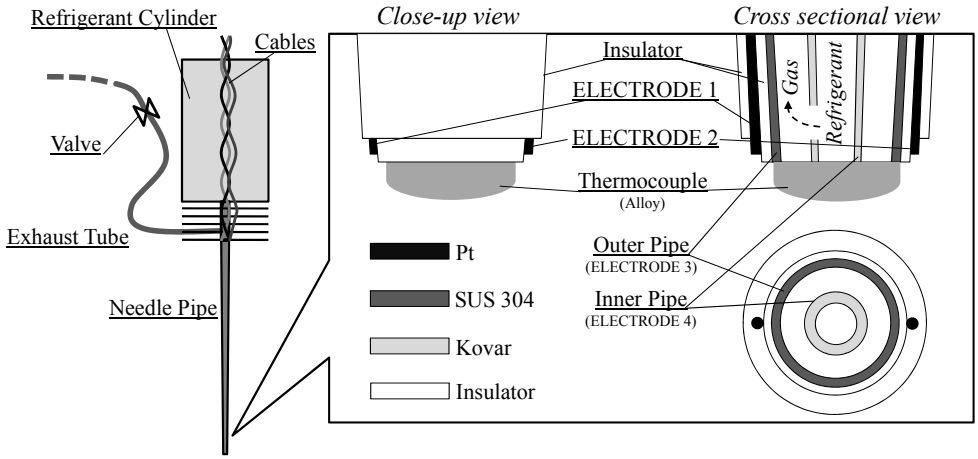

Fig. 2. The proposed cryogenic microprobe and its cross sectional view.

The refrigerant cylinder is filled with the refrigerant gas, and is connected to the inner pipe of the needle pipe. Here, HFC-152a (hidrofluoro-carbon) is used as the refrigerant gas, since it has relatively high boiling point (–24°C). The freezing ability of the microprobe and the accuracy of temperature measurement using the thermocouple element (welded tip) have already been confirmed [8].

In order to obtain electrical isolation between the outer pipe and electrodes, an insulator is formed on the outer pipe by using a fluoropolymer heat-shrinkable tube.

The microelectrodes (ELECTRODE 1, ELECTRODE 2) which are made of ϕ76μm platinum wires are fixed on the surface of the insulator by using a bio-compatible bond, and after that, the insulator is again formed to clip the electrodes. The tip of the electrodes including the insulators are polished in order to treat the contacting surface and to reduce the mismatch of the contacting impedance. The photograph of the fabricated multifunctional cryogenic microprobe and its microscopic photograph of the probe's tip are shown in Figure 3 and Figure 4, respectively.

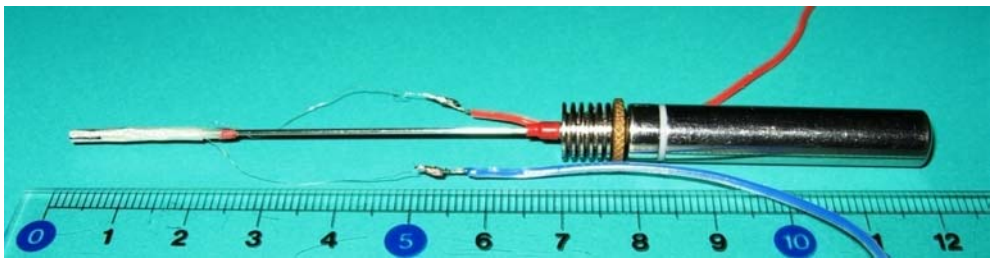

Fig. 3. Photograph of the multifunctional cryogenic microprobe.

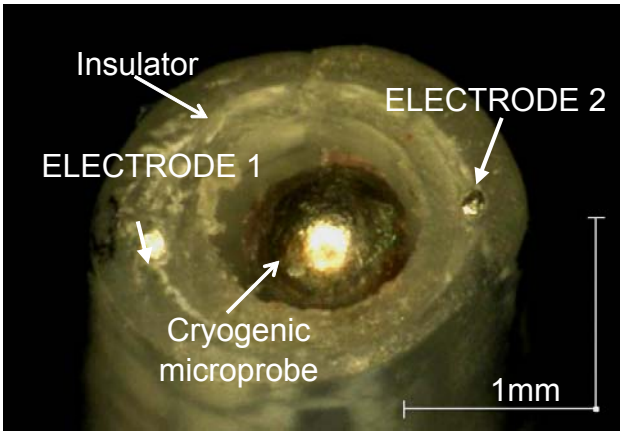

Fig. 4. Microscopic photograph of the probe's tip.

3. EXPERIMENTAL RESULTS

3.1. MEASUREMENT OF EPILEPTIFORM DISCHARGE USING THE MICROELECTRODES

Generally, the sliced hippocampus samples of a rat (see Figure 5) are used for imitating human epilepsy in animal experimentation [6]. In this experiment, hippocampus of a male Wister rat (40 weeks old, 240 g) was sliced with the thickness of 400 μm, and the sliced hippocampus samples were put on the test bench under 35ºC Ringer solution reflux. Here, since epilepsy can easily be developed in the CA3 area (as well as CA1) of the rat's hippocampus samples, epilepsy was formed on the CA3 area by intermittent pulse current stimulation through ELECTRODE 1. The stimulation technique and measurement protocol used in the experiments are known as theta burst stimulation and are well used in the animal tests of epilepsy [2], [7].

In the experiments, the self-produced biomedical signal amplifier and the data acquisition system of Digidata 1322A of Axon Instruments were used for measurement. The voltage signal was sampled with the sampling rate of 10 kS/sec., and the sampled data was treated by the pCLAMP software ver. 10 of Axon Instruments. The multifunctional cryogenic microprobe including the microelectrodes was set up on a micromanipulator, in order to align the contact between the tip and the sliced sample with 0.01 mm precision in tri-axial direction. The tip of the probe was contacted the sliced hippocampus vertically. The voltage difference between ELECTRODE 1 (+) and ELECTRODE 2 (–) was observed, and an Ag/AgCl needle electrode was put in Ringer solution to be used as the reference of the measurement system. The current stimulation was performed with an Electric Stimulator System of Nihon Kohden which includes a stimulation signal generator and an isolator. The stimulation current parameters were adequately controlled

according to the protocol mentioned in [7] with the amplitude limitation of 40 μA. The all procedures using the probe were operated under a stereo-microscope Nikon SMZ which has the variable magnification of ×8~×50.

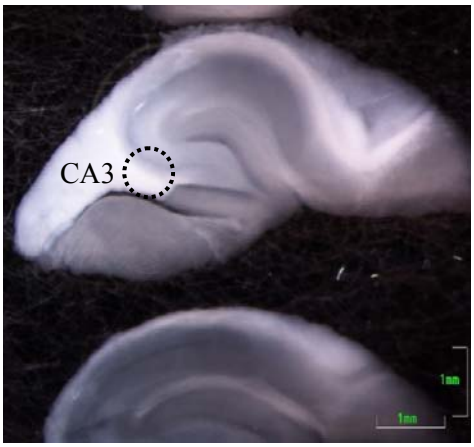

Fig. 5. Microscopic photograph of the sliced hippocampus sample.

The epileptiform discharge was observed by using the electrodes as shown in Figure 6. Figure 7 shows the continuous epileptiform discharges which often appear in the epileptogenic focus. From the results, the proposed microelectrodes on the cryogenic microprobe successfully performed the functions of spike measurement and stimulation.

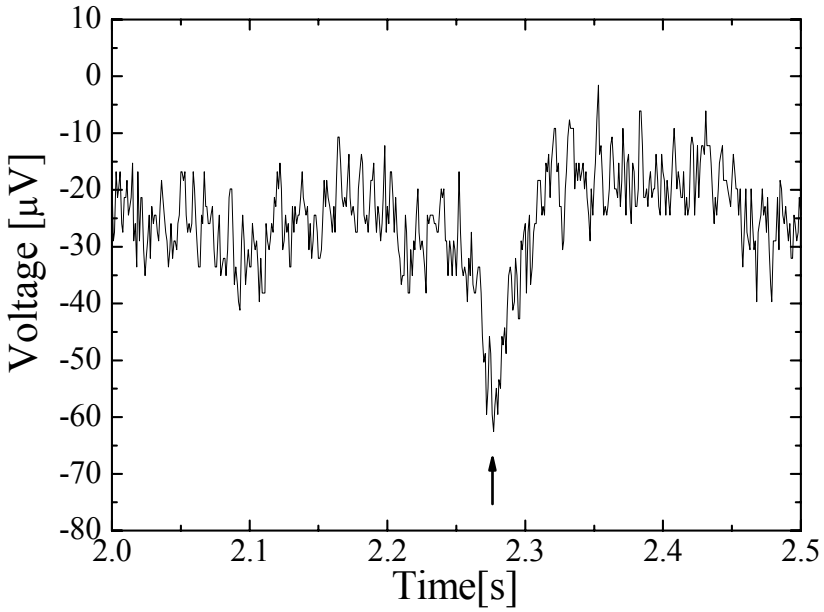

Fig. 6. Epileptiform discharge on a sliced hippocampus measured by the microelectrodes.

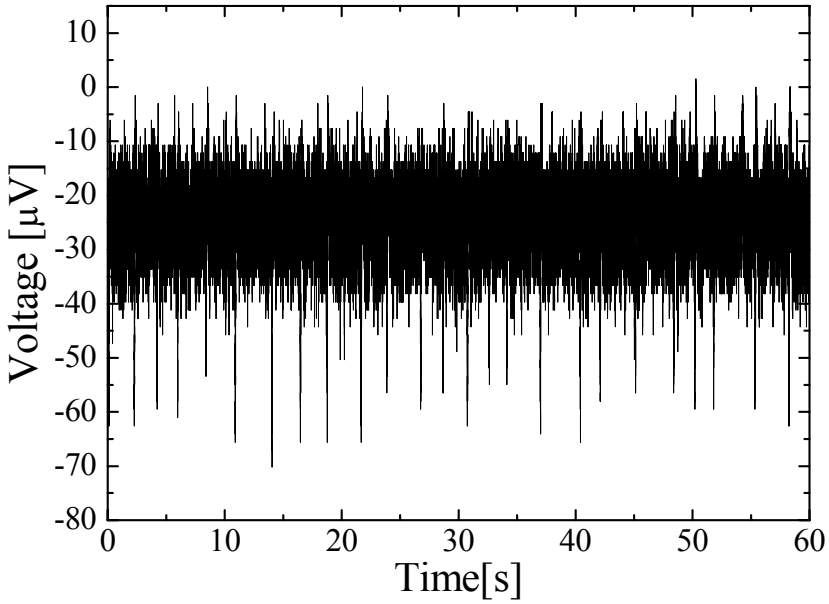

Fig. 7. Epileptiform discharges measured by the microelectrodes under a long period.

3.2. EPILEPTIFORM DISCHARGE INHIBITION UNDER REFRIGERATION

The epileptogenic focus evoked on CA3 area was refrigerated by the proposed probe with continuous flowing of HFC-152a.

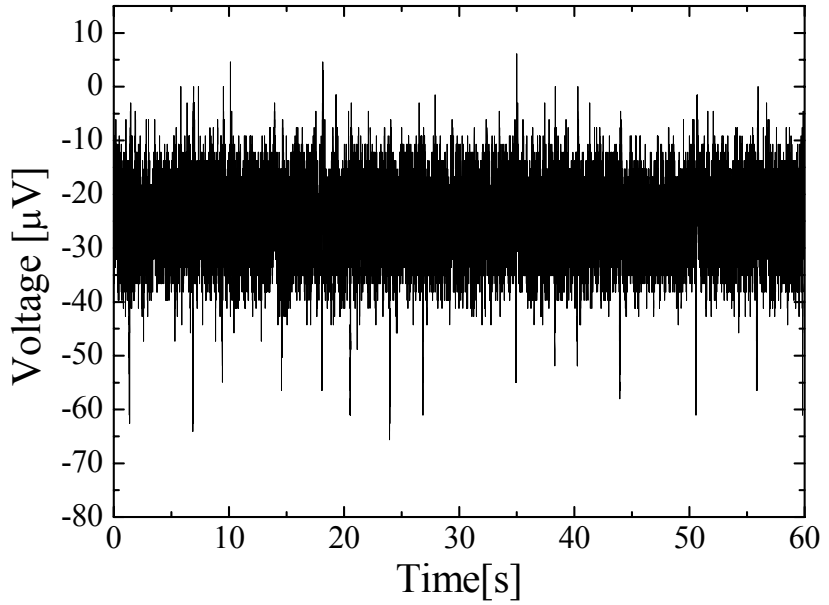

Fig. 8. Epileptic spikes measured after cooling of 50 seconds.

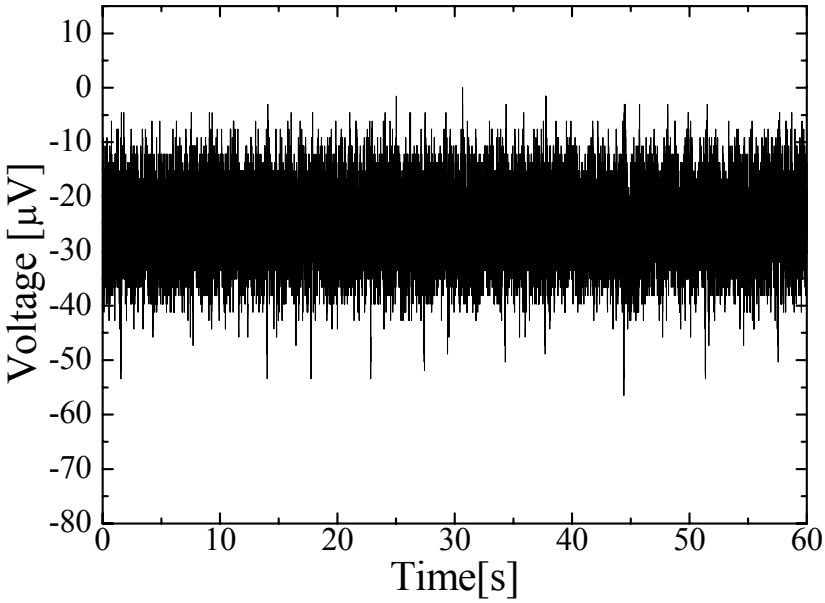

Fig.9. Epileptic spikes measured after cooling of 150 seconds.

Figures 8 and 9 show the epileptiform discharges after cooling of 50 seconds and 150 seconds, respectively. As shown in Figure 7, the epileptic spikes appeared 25 times in a minute. After cooling of 50 seconds and 150 seconds, the numbers of the spikes were reduced to 15 times and 12 times in a minute, respectively.

The spikes finally disappeared after cooling of 4 minutes, and the spikes were never appeared after 1 hour, 6 hours, and 24 hours from cooling. The results suggest that refrigeration using the proposed cryogenic microprobe caused irreversible inhibition of the epileptiform discharges, i.e. the epileptogenic focus on the sliced hippocampus was necrotized.

4. CONCLUSIONS

A multifunctional cryogenic microprobe was proposed for minimally invasive brain surgery of the treatment for intractable epilepsy. The proposed probe was manufactured with non toxic materials, and the shape was designed to be low invasive for human brain surgery. Experimental results showed that the microelectrodes equipped on the probe were able to apply the stimulation current to the brain tissue and measure the epileptiform discharge. In addition, the epileptiform discharges were irreversibly suppressed and inhibited by the focal refrigeration performed by the cryogenic microprobe. The epileptogenic focus on sliced hippocampus of a rat was successfully necrotized on *in-vitro* animal tests.

These results suggest that the multifunctional cryogenic microprobe can be used in epilepsy surgery, and it makes the surgery less invasive since the probe can be inserted from a small hole perforated on the patient's skull.

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[1]*Shizuoka University, Faculty of Engineering, Japan*
[2]*Kyushu Institute of Technology,*
Graduate School of Life Science and Systems Engineering, Japan
[3]*Yamaguchi University, Graduate School of Medicine, Japan*
[4]*Yamaguchi University Hospital, Department of Neurosurgery*
3–5–1 Jyohoku, Naka-ku, Hamamatsu-shi, 432-8561 Japan
E-mail: ttyamak@ipc.shizuoka.ac.jp.