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Detailed spectral profile analysis of electrocorticograms during freezing against penicillin-induced epileptiform discharges in the anesthetized rat

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ABSTRACT

Objectives: Cryosurgery is an alternative technique for minimally invasive treatment of lesions. We have recently examined cryosurgery for epilepsy in animal models, and found that penicillin G (PG)-induced epileptiform discharges (EDs) mostly vanished after freezing. However, EDs were provoked again after insufficient freezing. Inadequate freezing is not visually detectable during and just after freezing and is not predictable beforehand. To manage this problem, we examined whether intraoperative monitoring of electrocorticograms (ECoGs) can predict recurrence of EDs after cryosurgery.

Methods: A palm-sized cryoprobe system was applied to focal seizures in a Wistar rat model in which EDs were induced in advance by intracerebral injection of PG. During stable induction of EDs, the cryoprobe was carefully inserted into the epileptic region and this region was immediately frozen. After the series of prefreezing, freezing, and postfreezing, rats in which PG-induced EDs relapsed within 3 h were defined as the ED-relapsed (EDR) group, and other rats were defined as the ED-vanished (EDV) group. Time-frequency analysis was conducted on the ECoGs in each group through each freezing series.

Results: Relapse of PG-induced EDs on ECoG after the freezing series was associated with the remaining power of the delta band in the freezing period more strongly in the EDR group than in the EDV group.

Conclusions: Success or failure of the freezing procedure can be predicted by the specificity of the delta band of the ECoG obtained intraoperatively.

1. Introduction

Cryosurgery is an alternative technique for minimally invasive resection of lesions (Gage et al., 2009; Korpan, 2001) that is a simple procedure to perform, and has hemostatic (Ganz, 1974) and anesthetic (Schneider et al., 1985) effects. Cryosurgery has been primarily applied to the skin and breast (Korpan, 2001; Yiu et al., 2007), but rarely to the brain (Cooper, 1962, 1964; Hass and Taylor, 1953; Rand and Markham, 1964; Rowbotham et al., 1959; Tytus, 1961) because of the adverse effects of pathological deterioration. To verify the effectiveness of cryosurgery for neurosurgical operation, especially surgical treatment for intractable epilepsy, we recently proposed a newly developed palm-sized cryoprobe system (Tokiwa et al., 2015). The system was applied to a Wistar rat model of focal seizure, in which epileptiform discharges (EDs) were induced in advance by cortical application of the potent epileptogenic substance penicillin G (PG). In a series of experiments, we showed that PG-induced EDs could be eliminated at the onset of the freezing procedure. However, the EDs were occasionally provoked again after the freezing period due to inadequate freezing protocols (e.g., freezing speed, area, and temperature). These EDs were unexpected and were not visually detectable during freezing or immediately after freezing. In this study, we conducted time-frequency analysis of ECoGs in a freezing procedure series to define an approach to therapy using freezing.

Abbreviations: ECoG, electrocorticogram; ED, epileptiform discharge; EDR, epileptiform discharge-relapsed; EDV, epileptiform discharge-vanished; FFT, fast Fourier transform; GABA, gamma-aminobutyric acid; IRDS, interictal regional delta slowing

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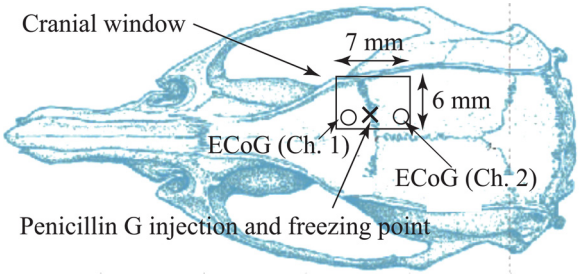

Fig. 1. Dorsal view of the Wistar rat skull. The figure shows the craniotomy location. Two silver ball electrodes (Ch. 1 and Ch. 2) are marked by open circles and are located over the dura. The insertion point of the cannula and cryoprobe is the same and marked by an "x". The insertion depth of the cryoprobe is deeper than that of the cannula. This figure is based on a schematic reported by Watson (2007).

2. Materials and methods

2.1. Wistar rat model

Wistar rats (n = 12; weight, 377 ± 56 g) were anesthetized with isoflurane (1%–2%) via a nosepiece to maintain anesthesia. The rectal temperature was maintained at 37 °C ± 0.5 °C using a heating pad (ATC-402; Unique Medical, Tokyo, Japan). The skull was fixed using a stereotactic apparatus (SR-6N; Narishige, Tokyo, Japan) and the skin on the skull was cut after subcutaneous injection of lidocaine (2.0%). The right parietal area of the skull was exposed and a craniotomy was performed using a dental drill. The craniotomy area was located between 3 mm rostrally and 4 mm caudally from the bregma and between 1 mm and 7 mm laterally from the sagittal suture, as shown in Fig. 1.

To record electrocorticograms (ECoGs), two silver ball electrodes (UL2-2020; Unique Medical Co.) were placed anteroposteriorly so as to not block blood flow in the craniotomy area and over the dura mater. All experiments were performed under appropriate conditions in accordance with the Declaration of the Bioethical Standards of Animal Experiments at the Kyushu Institute of Technology (Kitakyushu, Japan).

2.2. Epilepsy model

There are many animal models of epilepsy (Fisher, 1989). For this study, we chose the PG-induced epileptic model for two reasons: (1) the model is a common method for induction of simple epileptic focal seizures and EDs can be induced within dozens of minutes after injection of PG (Fisher, 1989); and (2) the epileptic focus is localized within a few millimeters (Noebels and Pedley, 1977). PG alters the excitation-inhibition balance in cortical tissues by blocking gamma-aminobutyric acid (GABA) receptor-associated chloride channels and thus induces local rhythmic EDs, as in the interictal stage of epilepsy (Canan et al., 2008; Curtis et al., 1972; Fisher, 1989; Purpura and Penry, 1972; Wong and Prince, 1979).

To inject PG into the brain, a part of the dura was carefully incised and a cannula was inserted to a 2.5-mm depth without damaging blood vessels on the brain surface. The injection point was in the right primary somatosensory and motor cortex. Penicillin G potassium (400 IU for injection, 400 IU/μL 0.9% sodium chloride solution; Meiji Co., Tokyo, Japan) was injected for 5 min at a rate of 0.2 μL/min (SP100i Syringe Pump; WPI Co.) using an injection cannula (φ 0.3 mm; model EIM-40; EiCOM Co., Tokyo, Japan).

2.3. Cortical freezing using the cryoprobe

We used a cryoprobe system that has previously been shown to be effective for freezing in Wistar rat brain tissue (Tokiwa et al., 2015). Fig. 2 shows a component drawing of the system, which comprises a tank unit and a probe unit that are connected using a nonconducting, flexible, and fatigue-resistant high-pressure tube. The size of the probe tip is 2 mm long and 0.7 mm in diameter. The important characteristic of the cryoprobe, which is a tricoaxial tube, is that it has a high contrast in temperature, which freezes tissues deep inside the brain without damaging superficial surrounding healthy tissue. In principle, the steady-state freezing temperature at the probe tip is conceptually the same as the boiling temperature of the refrigerant.

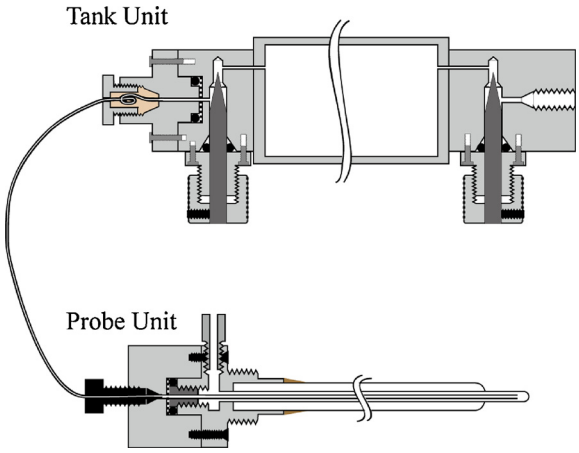

Fig. 2. Component drawing of the palm-sized cryoprobe system (Tokiwa et al., 2015). The system comprises a tank unit and a probe unit, which are connected by a nonconducting, flexible, and fatigue-resistant high-pressure tube. The probe needle is 70 mm long and the diameter of the probe tip is 0.7 mm. The weight of the entire system is 336 g.

During stable induction of epileptiform activities by injection of PG (around 10 min after the PG injection), the cannula was removed and replaced by the probe unit. The probe tip was inserted 3.0-mm deep from the cortical surface, which was 0.5-mm deeper than the injection cannula. The inserted position was adjusted so that the egg-like frozen region covered the PG-induced epileptogenic region. Taking into account the size of epileptogenic lesions induced by PG, the refrigerant R-410a (boiling point, −51.4 °C) was used to create a 4.5-mm diameter egg-like freezing area. The freezing period was set at approximately 20 min, as previously reported (Tokiwa et al., 2015).

2.4. Data acquisition and analysis

Silver ball electrodes were connected to a digital data acquisition system (Digidata 1322A; Axon Instruments, Union City, CA, USA) via a differential alternating current amplifier (DPA-2016; Dia-Medical System, Tokyo, Japan). ECoGs were recorded from after the dura incision until 3 h after freezing with a sampling rate of 10 kHz (low-pass filter 300 Hz, 0.3-s time constant), and were stored on a computer. Platinum wires were placed beneath the scalp and in the left hind leg as the reference and ground, respectively. The positions of the two silver ball electrodes are shown in Fig. 1.

The recorded ECoGs were analyzed using MATLAB (Mathworks, Natick, MA, USA). First, to conduct fast Fourier transform (FFT) analysis with a Hamming window, these data were visually assessed offline and divided into three distinct periods, as follows: 1. The prefreezing period, with mature PG-induced EDs and behavioral seizures provoked every 1 or 2 s. 2. The freezing period, in which the cryoprobe system is used for approximately 20 min. 3. The postfreezing period, about 3 h after the freezing period. In the EDR group, the ECoG contained PG-induced EDs. Two-minute continuous and noise-free segments from each episode were extracted for FFT analysis. Each sample was then divided into a 1-s half-overlapping window and the mean FFT values of each window were transferred for further analysis. Only the 0.5–40 Hz range of all spectra was used for further analysis and other faster