distribution from slow to fast frequency bands (Canan et al., 2008). In this study, we investigated whether focal brain cooling at different temperatures can attenuate EDs of any frequency.

2. Methods

Male Sprague–Dawley rats (body weight 500–600 g, Chiyoda. Kaihatsu, Japan) were housed in individual plastic cages (*L* 40 cm, *W* 25 cm, *H* 25 cm) at a constant temperature (22 °C) under a 12-h light/dark cycle with access to water and food ad libitum. The animals were 11–12 weeks old at the start of the study. All experiments were performed according to the Guidelines for Animal Experimentation of Yamaguchi University School of Medicine.

2.1. Preparation

The animals were anesthetized with urethane (1.25 g/kg, i.p.). Lidocaine, a local anesthetic, was applied at pressure points and around the area of surgery. After the initial surgery, the animals were fixed in a stereotaxic apparatus (SR-6, Narishige Co., Tokyo, Japan). Body temperature was maintained at 37 ± 1 °C with a heating pad (BWT-100, Bio Research Center Co., Japan). The depth of anesthesia was monitored throughout the experiment by testing for reflexes and monitoring changes in heart rate in response to tail pinching.

2.2. Epilepsy model

Penicillin G potassium (Sigma, Japan) was dissolved in 0.9% saline at a concentration of 400 IU/µl. A rectangular opening (4 × 10 mm) in the cranium was made above the left sensorimotor cortex to allow insertion of a guide cannula and placement of the cooling device (a Peltier chip with a heat sink) (Imoto et al., 2006) on the dura-arachnoid membrane. A thin thermocouple (IT-24, Physitemp, Japan) was placed between the Peltier chip and the brain surface. A small slit in the dura was made and the injection cannula (0.4 × 19 mm, NN-2719S, Terumo, Japan) was inserted at a depth within 1 mm from the brain surface. Penicillin G was administered into the left sensorimotor cortex for 5 min at a rate of 5 µl/min (total 2000 IU). Administration was performed via a 10-µl Hamilton syringe (MS-10 type, Ito Corp. Fuji, Japan) attached to a microinfusion pump (ESP-64, Eicom, Japan), after the dura-arachnoid membrane had been carefully incised at the point of entry of the needle. The stereotactic coordinates relative to the bregma were 1 mm (posterior) and 3 mm (lateral).

2.3. Electrophysiological recording

Continuous EEG recordings were made during each experiment, as previously described (Imoto et al., 2006). An Ag/AgCl electrode for recording EEGs (Unique Medical Co., Fukuoka, Japan) was positioned stereotactically on the cortical surface at the left
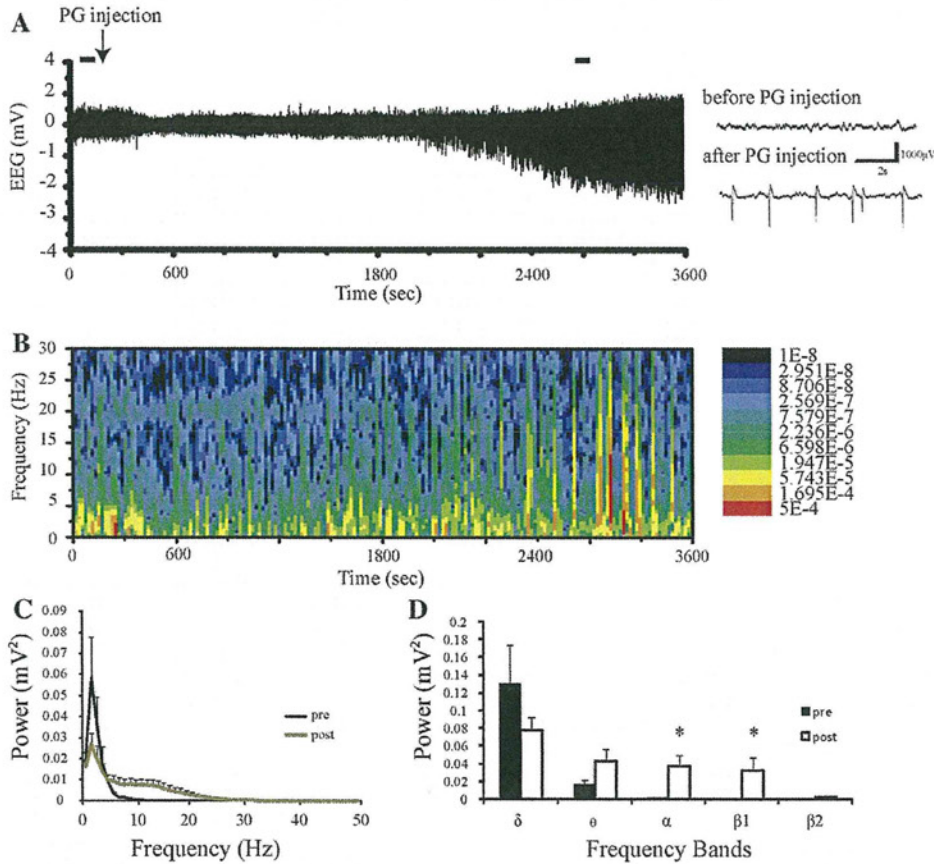

Fig. 1. (A) An example of epileptic discharges produced by intracortical administration of penicillin G. Bars indicate durations for EEG spectral analysis pre- and post-penicillin injection (arrow), respectively. EEG traces (right). (B) FFT analysis. (C) Spectral profiles of epileptic activity (*n* = 5, mean ± SE) pre- (black) and post- (gray) penicillin injection. (D) Average EEG power (*n* = 5, mean ± SE) pre- (black) and post- (white) penicillin injection for each frequency band. *$P < 0.05$ (paired *t*-test).

sensorimotor cortex, just beneath the cooling device. A reference electrode was inserted in the neck muscle and EEGs were recorded continuously by a digital electroencephalograph (GE Healthcare, Japan). An ED was determined as a sharp wave with a duration <70 ms (Chatrian et al., 1974). The conditions for recording EEGs were time constant: 0.3 s; high-frequency filter: 10 kHz; notch filter: on.

2.4. Cooling conditions

We previously reported that cooling the cortical surface to 15 °C increased the latency for withdrawal in limbs contralateral to the cooling cortex in thermal withdrawal tests (Fujioka et al., 2010), suggesting that cooling below 15 °C induces sensorimotor dysfunction. Therefore, the temperatures targeted by the focal brain cooling device were 25 °C, 20 °C, and 15 °C.

2.5. Data analysis

Before induction of EDs, EEGs were obtained for 1 min within the first 3 min as basal activity. EEGs during EDs were recorded for 1 min when the amplitude reached a supramaximal level. In cooling, fast Fourier transform (FFT) analysis was performed for spontaneous discharges for the first 1 min after the cortical temperature reached the target value. The sampling rate of the EEG recording was 2000 Hz. Frequency analyses were performed using FFT (one epoch of 100 s) with a Hamming window. After FFT, the absolute band power was calculated for prominent EEG spectral bands (Delta: 1–4 Hz, Theta: 4–9 Hz, Alpha: 9–14 Hz, Beta 1: 14–24 Hz and Beta 2: 24–30 Hz).

2.6. Statistical analysis

All results are expressed as means ± standard error of the mean. For spectral analysis, a paired t-test was used for comparison of pre- and post-drug injection or non-cooling and cooling data. The level of significance for all analyses was set at $P < 0.05$.

3. Results

First, we investigated the changes in the power spectrum after penicillin G injection. A typical example of EDs induced by penicillin G is shown in Fig. 1. No EDs were observed prior to injection. At 30 min after drug injection, the amplitude of EDs began to increase
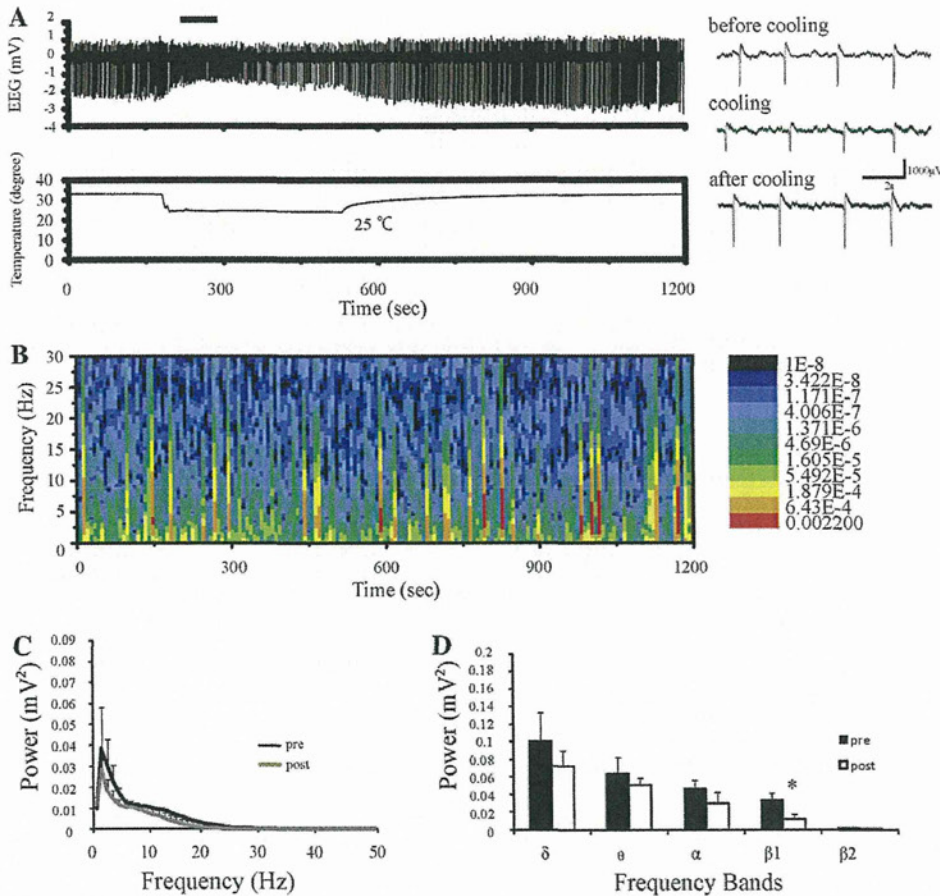

Fig. 2. (A) An example of a change in epileptic discharge (top) with the temperature of the cortical surface (bottom) controlled at 25 °C. The bar indicates the duration of EEG spectral analysis after focal cooling. EEG traces (right). Note that the decreased spectral power recovered to the level before focal cooling. (B) FFT analysis. (C) Spectral profiles of pre- (black) and post- (gray) cooling treatment for epileptic activity (n = 5, mean ± SE). (D) Average EEG power (n = 5, mean ± SE) pre- (black) and post- (white) cooling for each frequency band. *$P < 0.05$ (paired t-test).

Fig. 3. (A) An example of a change in epileptic discharge (*top*) with the temperature of the cortical surface (*bottom*) controlled at 20 °C. The bar indicates the duration of EEG spectral analysis after focal cooling. EEG traces (right). (B) FFT analysis. (C) Spectral profiles of epileptic activity (*n* = 5, mean ± SE). (D) Average EEG power (*n* = 5, mean ± SE) pre- (black) and post- (white) cooling for each frequency band.*P < 0.05 (paired t-test). Other notations are the same as those in Fig. 2.
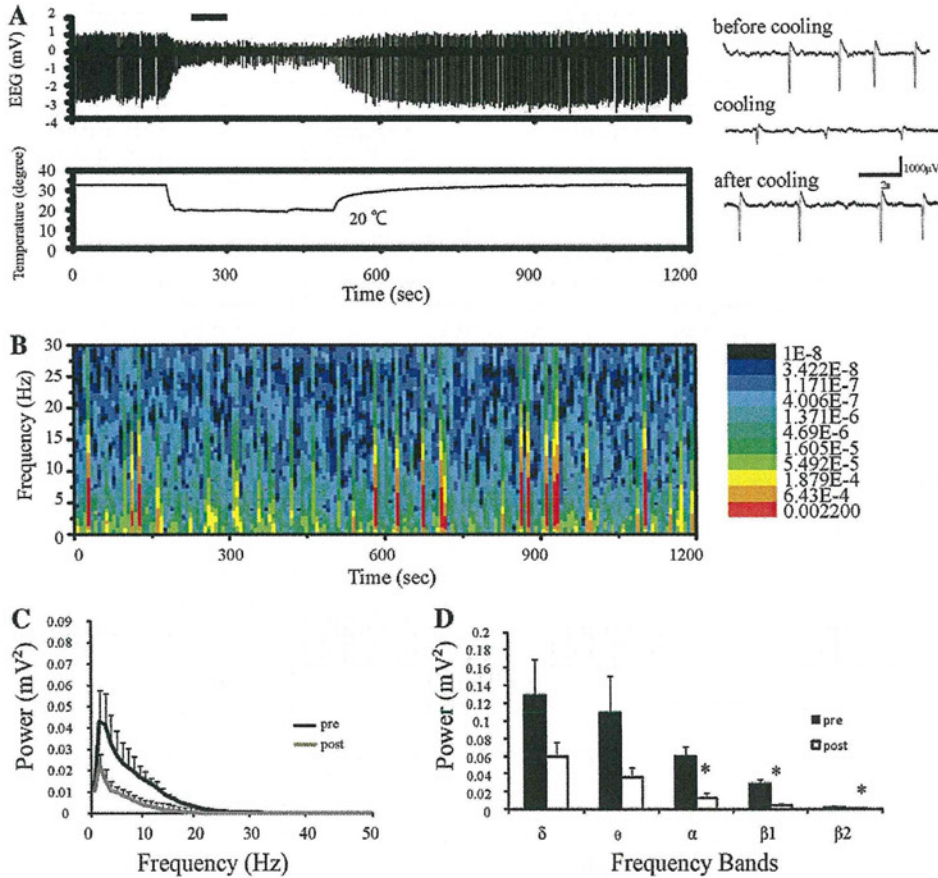
and lasted at least 1 h (Fig. 1A). The mean ED rate was 0.64 ± 0.07 Hz. There was a significant increase in the number of EDs per min between pre and post-drug infusion (P < 0.01). To quantify the frequency profiles, the power spectrum was calculated by FFT analysis for all animals (*n* = 5). A power distribution within 10 Hz was predominant during pre-treatment, while the distribution shifted toward faster frequency bands after injection of penicillin G (Fig. 1B and C). There was a significant increase in spectral power for the Alpha wave (pre 0.002 ± 0.0002 vs. post 0.037 ± 0.012 mV^2) and Beta1 wave (0.001 ± 0.0002 vs. 0.032 ± 0.015 mV^2) bands (Fig. 1D, P < 0.05). To investigate the frequency components in the EDs, FFT analysis was performed for each ED. Wave bands other than the Delta wave exhibited significant increases (Theta wave, 5.7 times; Alpha wave, 22.6 times; Beta1 wave, 12.2 times; Beta2 wave, 5.2 times).

Focal brain cooling was performed after adequate EDs were observed. Compared with pre-cooling, EDs were weakly and insignificantly suppressed by cooling to 25 °C (pre 0.68 ± 0.07 vs. post 0.63 ± 0.09 Hz, P = 0.44; Fig. 2A); moderately but significantly suppressed at 20 °C (0.70 ± 0.01 vs. 0.59 ± 0.06 Hz, P < 0.05; Fig. 3A); and completely attenuated at 15 °C (0.62 ± 0.01 vs. 0.47 ± 0.06 Hz, P < 0.05; Fig. 4A). The temperature of the cortical surface was maintained at 34 °C before cooling and decreased to the target

temperature within 3 min in all cooling protocols (Figs. 2A, 3A, and 4A).

To examine changes of EEG frequency during cooling, FFT analyses were performed, as shown in Fig. 1B. We verified that the changes in spectra were reversible (Figs. 2B, 3B and 4B). At 25 °C, only the Beta1 band was significantly suppressed (pre 0.033 ± 0.009 vs. post 0.011 ± 0.006 mV^2, P < 0.05; Fig. 2C and D). At 20 °C, the Alpha band (0.059 ± 0.001 vs. 0.013 ± 0.006 mV^2) and Beta1 band (0.028 ± 0.004 vs. 0.004 ± 0.002 mV^2) were significantly suppressed (P < 0.05, Fig. 3C and D). At 15 °C, every spectral band was significantly suppressed (P < 0.05, Fig. 4C and D).

4. Discussion

This is the first study to use frequency analysis to investigate the effects of focal cooling in rats with EDs. The results are summarized as follows: (1) penicillin G increased faster frequency components (Alpha range: 9–14 Hz; Beta range: 14–30 Hz), (2) cooling the brain to 25 °C suppressed the Beta EEG bands, (3) cooling the brain to 20 °C suppressed not only the Beta EEG bands but also the Alpha EEG bands, and (4) cooling the brain to 15 °C suppressed both the fast and slow frequency EEG bands.
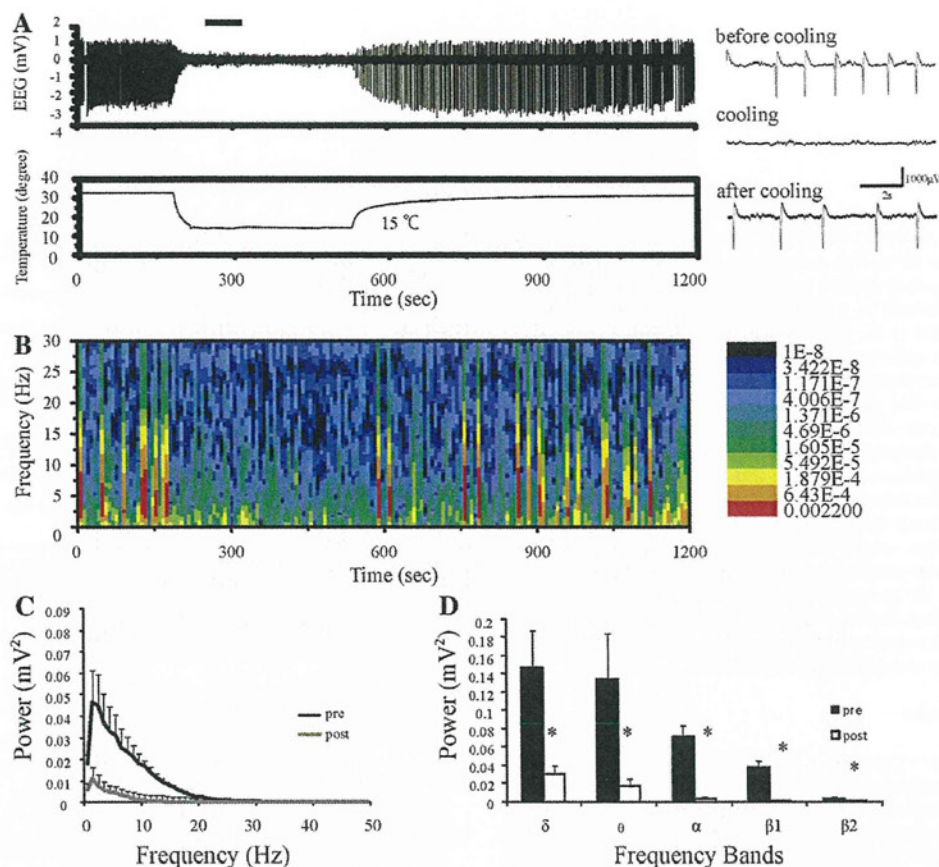
Fig. 4. (A) An example of a change in epileptic discharges (*top*) with the temperature of the cortical surface (*bottom*) controlled at 15 °C. The bar indicates the duration of EEG spectral analysis after focal cooling. EEG traces (right). (B) FFT analysis. (C) Spectral profiles of epileptic activity (*n* = 5, mean ± SE). (D) Average EEG power (*n* = 5, mean ± SE) pre- (black) and post- (white) cooling for each frequency band.*P < 0.05 (paired *t*-test). Other notations are the same as those in Fig. 2.

The spectral shift from slow to fast frequency induced by penicillin G was responsible for disinhibition of synchronous firing activity in the cortex, consistent with previous studies (Canan et al., 2008). Penicillin G was infused into the superficial layers through a small slit in the dura, after which the drug gradually diffuses into deep layers within 15 min (Ludvig et al., 2008). However, the origin of faster frequency components was not considered to be due to hyperactivity in deep layers. This is because i.c.v. infusion in a previous study (Canan et al., 2008) and intracortical administration in this study induced faster frequency components in Alpha and Beta wave bands, while GABA A receptor disinhibition with bicuculline in neocortical layer V generates discharges of at most 1 Hz (Castro-Alamancos, 2000). Therefore, induction of faster frequency components may be organized in superficial layers.

In this study, the cooling device predominantly reduces the temperature in superficial layers, since a previous study showed that there is a temperature difference of approximately 5° between the cortical surface just under the cooling device and deep layers (Oku et al., 2009). Therefore, mild cooling at 20 °C could terminate Alpha waves in the cortex, while 25 °C cooling suppresses only Beta wave bands. Because 15 °C cooling suppressed neuronal activity in the subcortex, there may be a faster frequency of neuronal networks in the cortex than in the subcortex. The exact mechanism of the antiepileptic effects of focal brain cooling has not been

elucidated. However, we have obtained preliminary results (Fujii et al., 2011) that suggest that reductions of metabolism, cerebral blood flow and glutamate release are involved in termination of seizures, in addition to suppression of synaptic transmission (Eilers and Bickler, 1996) and/or the difference of activity in response to cooling between pyramidal neurons and inhibitory interneurons (Motamedi et al., 2012).

In the rat sensorimotor cortex, including the barrel cortex, there are widespread horizontal excitatory connections among functional columns in layers II/III and V/VI (Feldmeyer et al., 2002; Petersen et al., 2003). Our previous study demonstrates even cooling to 15 °C above brain surface cannot decrease the temperature below 20 °C in layers V/VI (Fujii et al., 2012), suggesting that the effect of focal cooling on termination of ED is within layers II/III. It is reasonable to assume that even cooling at 25 °C can terminate the conductance of faster frequency EEGs along layers II/III, although we did not record an EEG from each layer directly. Accordingly, axons in layers II/III may be involved in routes for propagating EDs. In addition, maintenance of the temperature of the cooling site at 20 °C caused significant suppression of the amplitude of the EDs. Our results are also consistent with reports showing that the optimum temperature of the cortical surface for terminating seizures is approximately 15–25 °C (Yang et al., 2002, 2003; Fujioka et al., 2010). A recent study demonstrated that focal brain cooling from 20 to 15 °C did not suppress normal

function (Fujii et al., 2012). Therefore, we conclude that focal brain cooling to at least 20 °C does not affect normal EEG activity.

High EEG frequency spectra (>30 Hz) did not appear in the current study, in contrast to experiments performed under arousal conditions (Buzsaki et al., 1988; Bragin et al., 1999). Additionally, epileptic seizures were not observed, in contrast to a recent report using a similar method (Rothman, 2009). Our method differs from those previous studies in that we selected a stable and prolonged urethane anesthesia. Nevertheless, we considered that the observed epileptic discharges in our study were interictal (Holmes et al., 1987; de Guzman et al., 2010), although induction of seizure may depend on the degree of anesthesia. These disagreements are due to the limitation of performing experiments in anesthetized animals, and further work is required to test the effects of cooling in free moving rats established in our laboratory (Fujioka et al., 2010; Fujii et al., 2012). A recent study demonstrated that high frequency oscillation is linked to seizure onset (Bragin et al., 2010), and therefore it is possible that focal cooling at a suspected epileptogenic focus can terminate EDs and seizures.

Acknowledgements

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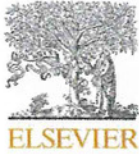
Intra-operative monitoring of lower extremity motor-evoked potentials by direct cortical stimulation

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ARTICLE INFO

HIGHLIGHTS

- We advocate a new method for lower-limb motor evoked potential (MEP) monitoring.
- This method is available in the supratentorial surgery.
- Lower-limb MEPs were consistently recorded by direct cortical stimulation.
- Optimal stimulation site was 2 cm lateral from the midline on the motor cortex.
- This monitoring technique can be applied to standard pterional craniotomy.

ABSTRACT

Objective: Motor-evoked potentials (MEPs) are commonly recorded from upper-extremity muscles, whereas lower-extremity MEP (LE-MEP) monitoring has not been adequately established. The goal of the study was to develop a MEP monitoring method using direct cortical stimulation (DCS) for predicting motor deficits of lower extremities.

Methods: Intra-operative LE-MEP monitoring was performed in 22 patients. After craniotomy, a subdural electrode was placed on the cortex so that the optimal contact was positioned 2 cm lateral from the midline on the motor cortex. The electrodes for stimulation consisted of a cathode at Fpz and an anode at the optimal contact site on the motor cortex. After stimulation was performed with short trains of five stimuli, LE-MEPs were recorded from the lower-limb muscles.

Results: LE-MEPs were consistently recorded in all patients. Disappearance or amplitude reduction of MEP waveforms was observed in five patients, but the MEP waveforms had recovered and remained at the control level by dural closure, and no permanent motor deficit was observed in any patient.

Conclusions: We accomplished LE-MEP recording during supratentorial surgery using monopolar DCS with a subdural electrode placed on the convex side of the motor cortex.

Significance: A useful method of intra-operative LE-MEP recording was described.

1. Introduction

Intra-operative monitoring of the motor evoked potential (MEP) has become common during neurosurgical procedures to avoid the occurrence of motor deficit, especially following the manipulation of the peri-rolandic area (Burke and Hicks, 1998; Kombos et al., 1999, 2001, 2009; Sala et al., 2007; Sloan et al., 2008; Suzuki et al., 2003; Szelényi et al., 2005; Tanaka et al., 2007; Taniguchi et al., 1993). The MEPs are commonly recorded from upper-extremity muscles or the spinal cord at the cervical epidural space (the so called D-wave) after transcranial electrical stimulation or direct stimulation of the motor cortex (Cedzich et al., 1996; Chen et al., 2007; Fujiki et al., 2006; Journée et al., 2007; Kaneko et al., 1988; Krammer et al., 2009; Nagle et al., 1996; Rothwell et al., 1994; Suzuki et al., 2003; Szelényi et al., 2005, 2007a,b; Szelényi et al., 2010; Taniguchi et al., 1993; Yeon et al., 2010). Recently, MEP monitoring using direct cortical stimulation (DCS) has been recommended for supratentorial surgery, because transcranial stimulation may stimulate the motor tracts deep to the motor cortex and therefore miss ischaemia in the cortical region (Rothwell et al., 1994).

The hand area of the motor cortex is usually selected as a DCS site when recording MEPs with DCS (Chen et al., 2007; Neuloh and Schramm, 2004a; Neuloh et al., 2007). However, this monitor-

ing method is difficult for detecting blood-flow insufficiency in the anterior cerebral artery (ACA) during aneurysm surgery or motor deficit of the lower extremities during procedures for peri-rolandic tumour resection. Furthermore, the few studies of MEP monitoring of the lower extremities have indicated a low success rate of monitoring (Neuloh and Schramm, 2009; Szelényi et al., 2005). Therefore, we herein describe a useful monitoring method using DCS for predicting motor deficits of the lower extremities. The methodology and results of intra-operative monitoring of the lower-extremity MEP (LE-MEP) are described in this technical study.

2. Clinical material and methods

All protocols were approved by the Yamaguchi University Ethical Review Committee. Written informed consent to intra-operative monitoring and surgical procedures was obtained from each patient or their legal guardian.

2.1. Patient population

Intra-operative LE-MEP monitoring was applied to 22 consecutive patients (nine males and 13 females) ranging in age from 40 to 85 years (mean 63.0 ± 10.8 years). These patients were undergoing surgical procedures for aneurysms of the ACA territory and for brain tumours that were adjacent to the primary motor cortex for the lower extremities. Ten patients had an anterior communi-

cating artery (AcomA) aneurysm, four had an ACA aneurysm, three had an internal carotid artery (ICA) aneurysm, one had a middle cerebral artery (MCA) aneurysm, three had meningiomas and one patient had a glioblastoma. The backgrounds and symptoms of the patients are summarised in Table 1.

2.2. Neurophysiological monitoring methods

2.2.1. Placement of subdural electrodes for cortical stimulation

We applied LE-MEP monitoring for three types of craniotomy: a standard pterional craniotomy, a bifrontal craniotomy and a craniotomy in the range of which the motor cortex was exposed. Depending on the location of craniotomy, the placement of subdural electrodes for DCS was arranged as follows. In a standard pterional craniotomy for ICA, MCA and AcomA aneurysms, 'Corkscrew' electrodes (CS electrodes; Nicolet Biomedical, WI, USA) were placed at the Cz' of the International 10–20 System on the scalp (2 cm posterior from the midpoint of the nasion–inion line) and C3/C4 of the International 10–20 System (approximately 7–7.5 cm lateral from the midline on the central sulcus line) as landmarks for the insertion of a subdural grid and were also used as stimulation sites before craniotomy for recording of MEPs. After the craniotomy, a 3×4 grid electrode (12 contacts, each 5 mm in diameter with a 10-mm centre-to-centre inter-electrode distance; Unique Medical Co. Ltd., Tokyo, Japan) was subdurally inserted toward C1/C2 of the International 10–20 System and between the two

Table 1
Background, stimulus parameters, MEP changes during surgery and postoperative outcome in 22 patients.

Case no.	Age/ sex	Diagnosis	Symptoms/signs	Stimulation		MEP change during surgery	Postoperative de novo motor deficit (duration)
				Intensity (mA)	Duration (ms)		
1	40/M	ICA thrombosed giant aneurysm	No	23.0–26.6	1	Disappeared for 30 min	Recovered (35 days)
2	44/M	SAH, AcomA aneurysm	Headache	14.5–16.5	05	No change	No
3	52/F	SAH, ICA–AchA aneurysm	Headache/vomiting	18.0	05	No change	No
4	52/F	SAH, AcomA aneurysm	Headache/vomiting/ consciousness disturbance	18.0–22.0	0.5	No change	No
5	53/F	Parietal glioblastoma	Left clumsy hand/left sensory disturbance	19.0	0.5	No change	No
6	56/M	Frontal parasagittal meningioma	Partial seizures of right fingers	19.0	0.5	No change	No
7	56/M	Frontal falx meningioma	No	21.0	0.5	No change	Transient (8 days)
8	59/F	SAH, AcomA aneurysm	Headache/vomiting	12.5	0.5	Disappeared for 8 min	No
9	62/F	Unruptured AcomA aneurysm	No	22.5	05	No change	No
10	62/M	SAH, ApomA aneurysm	Headache/vomiting/ consciousness disturbance	155	05	No change	No
11	65/F	SAH, AcomA aneurysm	Headache/consciousness disturbance	16.0	05	No change	No
12	66/F	SAH, AcomA aneurysm	Consciousness disturbance	16.0–17.0	1	No change	No
13	67/M	SAH . AcomA aneurysm	Headache	20.0	0.5	No change	No
14	68/F	Unruptured AcomA aneurysm	No	20.3	0.5	No change	No
15	69/M	SAH, distal ACA aneurysm	Headache/vomiting	23.5	0.5	20–50% reduction for 15 min	No
16	70/F	Frontal parasaggital meningioma	Right hemiparesis	20	0.5	No change	No
17	70/F	Unruptured ICA–PcomA aneurysm	No	20.5	05	No change	No
18	72/M	ACA thrombosed giant aneurysm	Disorientation/left hemiparesis	20.0–22.0	1	Disappeared for 20 min	Recovered (26 days)
19	72/F	SAH, distal ACA aneurysm	Headache	30.0	05	80% reduction for 90 s	No
20	72/M	SAH, AcomA aneurysm	Headache/vomiting	20.0	0.5	No change	No
21	76/F	SAH, distal ACA aneurysm	Headache	16.0	05	No change	No
22	85/F	SAH, MCA aneurysm	Headache	24.5	0.5	No change	No

ICA; internal carotid artery, SAH; subarachnoid haemorrhage, AcomA; anterior communicating artery, ACA; anterior cerebral artery, AchA; anterior choroidal artery, PcomA; posterior communicating artery.
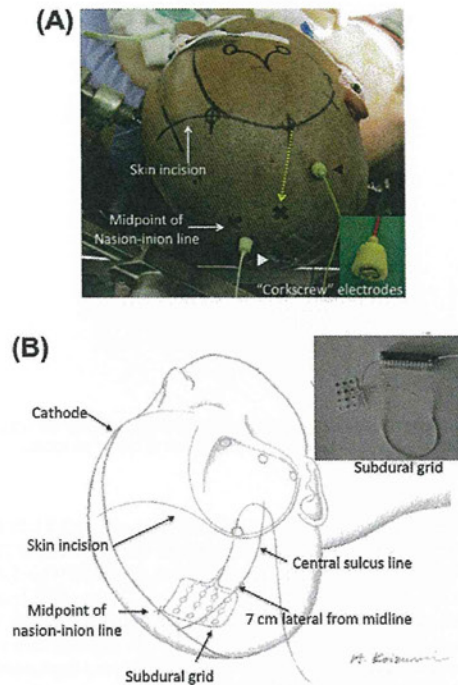
Fig. 1. Neurophysiological MEP monitoring methods. Intraoperative photograph (A) and schematic drawing (B) showing the placement of the subdural grid for electrical stimulation during pterional craniotomy. A: "Corkscrew" electrodes placed at Cz′ (white arrowhead) and C3 of the international 10–20 system (black arrowhead). A yellow dotted line and a bold "×" mark (C2 of the international 10–20 system) indicate the direction of grid insertion. B: the ideal position of the subdural grid is indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

landmarks ('Corkscrew' electrodes) on the scalp (Fig. 1A). Ideally, the optimal contact was located 2–2.5 cm lateral from the midline on the motor cortex for recording constant MEP responses (Fig. 1B). A strip electrode was also available for use when damage of well-developed bridging veins was likely from angiograms, but we generally used a grid electrode because an optimal contact was easily selected by a multi-channel somatosensory evoked potential (SEP) recording with a single insertion trial. In cases of bifrontal craniotomy for ACA distal aneurysms or frontal lobe tumours, a subdural strip (six contacts) was placed bilaterally and parallel to the sagittal sinus 2 cm lateral from the midline as the grid crossed the surface of the motor cortex. In cases where the motor cortex was exposed in the range of craniotomy, a strip or grid electrode was placed directly over the motor cortex on the convex side.

2.2.2. Stimulation and recording conditions

The contact in the subdural grid or strip providing the highest MEP amplitude was chosen for the optimal site of the anode. The subdermal needle electrode was placed at the Fpz (International 10–20 System) for the cathode. Anodal-electrical monopolar stimulation was performed for eliciting LE-MEPs with DCS, with short trains of five stimuli consisting of rectangular pulses with an individual pulse width of 0.5 or 1.0 milliseconds (ms) and an interstimulus time interval of 2 ms. Compound muscle action potentials were recorded using sterilised needle electrodes inserted into the soleus or anterior tibial muscles or abductor hallucis muscles (the abductor hallucis muscle was commonly used as a recording site). A bandpass filter was set from 30 to 3000 Hz.

Intra-operatively, the stimulus intensities of DCS were maintained at 2 mA above the threshold level of the MEP without exceeding 30 mA (Neuloh et al., 2004). An amplitude decrease of 50% or more in three or more consecutive MEP recordings was taken as a warning criterion, even when increasing the stimulus intensities by 20% or more (Fujiki et al., 2006; Neuloh et al., 2004; Neuloh and Schramm, 2004b). These MEPs were recorded using Viking Select and Endeavor monitoring systems (Nicolet Instruments, Biomedical Division, Madison, WI, USA).

2.3. Anaesthesia

Anaesthesia was induced with a bolus of propofol (1 mg kg^{-1}) and remifentanil (0.5 μg kg^{-1}), and maintained with propofol (6 mg kg^{-1} h^{-1}) and remifentanil (0.1–0.2 μg kg^{-1} min^{-1}). A short-acting muscle relaxant was administered as a bolus for intubation purposes only.

3. Results

The stimulus parameters and results of LE-MEP monitoring are listed in Table 1. MEP monitoring was consistently performed in all patients. The applied stimulus intensities ranged from 12.5 to 30.0 mA. A pulse width of 1.0 ms was selected in the first three cases for obtaining constant LE-MEP waveforms. Thereafter, the pulse width was reduced to 0.5 ms to avoid excitotoxicity because in our experience, LE-MEPs could be constantly recorded with a shorter pulse width.

Significant MEP changes were observed in five patients (23%) during the surgical procedures. These changes occurred after the temporary occlusion of an artery supplying blood flow to the motor cortex of the lower extremity and the MEPs had recovered almost to the control level by dural closure. Postoperatively, although de novo motor weakness was found in the first postoperative examination in two patients (cases #1 and #18), permanent motor deficit did not occur in these patients. The initial Medical Research Council (MRC) Scale scores for muscle strength and deficit duration to full recovery of these patients (cases #1 and #18) were as follows: case #1, left extremity: 2/5, for 42 days; case #18, right extremity: 3/5, left extremity: 2+/5, for 26 days. Of the five patients who had MEP changes, one (case #1) developed lacunar infarction in the right basal ganglia. The other four patients did not develop new lesions based on postoperative neuroimaging with magnetic resonance imaging (MRI) or computed tomography (CT). There were two true positives, three false positives, 16 true negatives and one false negative for transient early postoperative motor deficits (Table 1). These data gave a sensitivity of 66.7% (2/3 cases), a specificity of 84.2% (16/19 cases), a positive predictive value (PPV) of 40.0% (2/5 cases) and a negative predictive value (NPV) of 94.1% (16/17 cases).

No significant MEP changes were observed during surgery, but a transient motor weakness was observed postoperatively in a patient with falx meningioma (case #7). Motor weakness was found in the first postoperative examination and the initial MRC Scale score was 3+/5 in the right extremity, with a deficit duration to full recovery of 8 days. Body movements, which could interfere with microsurgical dissection, were not induced by DCS. Minor and controllable bleeding from the bridging vein occurred in one patient during placement of a grid electrode. No other adverse events were observed in the manipulation of LE-MEP monitoring.

4. Illustrative cases

A 59-year-old female patient (case #8) was admitted with symptoms of a severe headache and vomiting. Neuroimaging
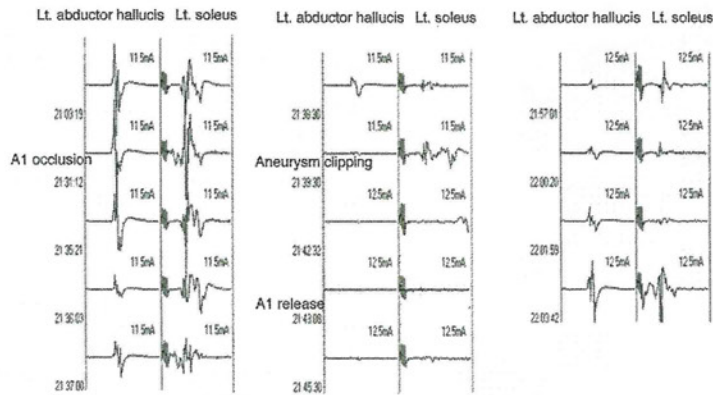
Fig. 2. The waveforms of MEPs recorded from the left soleus and the adductor hallucis are indicated. The MEP decreased in amplitude and disappeared after the proximal right-ACA (A1) was occluded. Although the MEPs disappeared for 8 min, they recovered after permanent clipping of the aneurysm and release of the A1 occlusion.

revealed subarachnoid haemorrhage (SAH) and an AcomA aneurysm. Right pterional craniotomy and neck clipping of the aneurysm were immediately performed. Preparation for MEP monitoring was performed as described above and MEPs were recorded during the microscopic procedure. The amplitudes of the MEPs recorded from the left soleus and abductor hallucis muscle gradually decreased and then the MEPs disappeared after temporary occlusion of the proximal ACA. After permanent clipping was applied, the ACA was reperfused. Although the MEP waveforms disappeared for 8 min, they had recovered to the control level by the time of dural closure (Fig. 2). Postoperatively, no motor deficits were identified.

A 72-year-old male patient (case #18) was admitted with a symptom of impaired orientation. Neuroimaging revealed an azygos ACA and a huge ACA aneurysm that was partially thrombosed (Fig. 3A–D). The patient underwent bifrontal craniotomy and neck clipping of the aneurysm. Subdural strips (1 × 6 contacts) were placed through the craniotomy bilaterally and parallel to the sagit-

Fig. 3. Axial (A) and Gd-enhanced sagittal (B) T1-weighted MR images of a patient (case 18) with a thrombosed aneurysm of the ACA. Anterior (C) and lateral (D) views of digital subtraction angiography showing an azygos ACA and a thrombosed aneurysm.

tal sinus from 2 cm lateral to the midline (Fig. 4A and B). Before applying permanent clips for the aneurysm, the proximal portion of the ACA (azygos ACA) was temporarily occluded. MEP waveforms disappeared 20 min after occlusion. However, 22 min after releasing the temporary clip, the MEP waveforms had recovered to 75% of the previous amplitude (Fig. 4C). Although the patient temporarily had motor weakness in both lower extremities, the motor deficit had fully resolved at 26 days postoperatively.

A 56-year-old male patient (case #6) presented with focal seizures of the right fingers. The patient was diagnosed with a parasagittal meningioma of the left peri-rolandic area (Fig. 5A and B) and underwent surgery. Because the motor cortex was exposed in craniotomy, a subdural grid was placed directly on the motor cortex to cover the entire precentral gyrus on the convex side (Fig. 5C). The central sulcus was identified using a neuronavigation system (Stealth Station, Medtronics Inc., Minneapolis, MN, USA) and SEP recording. MEP waveforms were consistently recorded from the muscles of the upper or lower extremity by stimulating a contact on the hand area or on the motor cortex closest to the midline, respectively. The meningioma was safely removed while confirming no reduction in the MEP amplitudes of the upper and lower extremities during the excision (Fig. 5D).
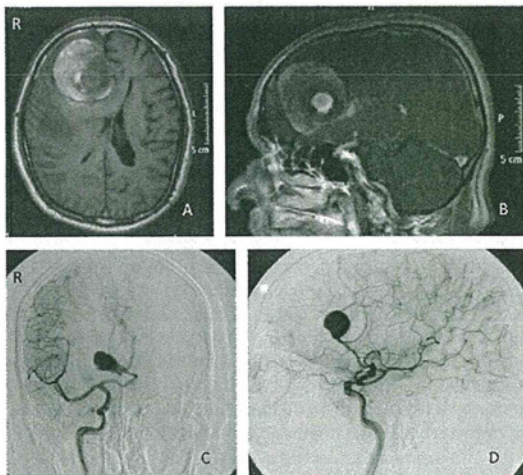
5. Discussion

In the present study, we demonstrated consistent recording of LE-MEPs by applying DCS using a subdural electrode placed over the motor cortex on the convex side close to the midline. Szelényi et al. found that MEP waveforms from the lower-limb muscles could be recorded in only 55% of patients undergoing surgery for aneurysms in the ACA territory (Szelényi et al., 2005). Furthermore, there have been no detailed descriptions of methodology for LE-MEP monitoring using DCS for accurate monitoring. It is commonly believed that because the leg area of the motor cortex is hidden in the sagittal plane, it is difficult to monitor LE-MEPs. However, LE-MEPs were consistently recorded in the present series by identifying the optimal stimulation site on the primary motor cortex on the convex side, even in a pterional approach. Our study also indicates that LE-MEPs can be consistently recorded without placing an electrode for stimulation in the sagittal plane after dissecting the interhemispheric fissure.

It is necessary to explain why LE-MEPs could be recorded by stimulation of the convex side of the motor cortex close to the midline. As the leg area of the motor cortex is located in the interhemispheric
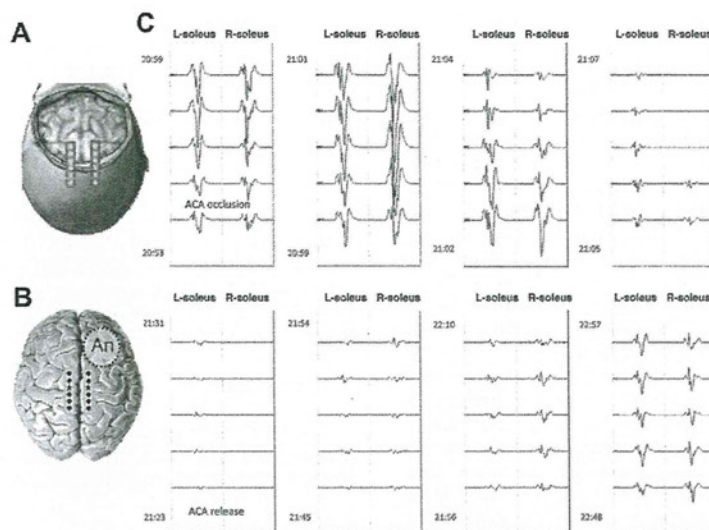
Fig. 4. Location of stimulus electrodes and MEP waveforms during surgery. Location of subdural strips with six contacts inserted from a cranial window (A). The stimulation sites are indicated by asterisks (B). The location of the aneurysm is indicated by An and a dashed circle-line. MEP waveforms disappeared 20 min after application of the temporary clip to the azygos ACA (C). However, the waveforms recovered to the control level 22 min after releasing the temporary occlusion.
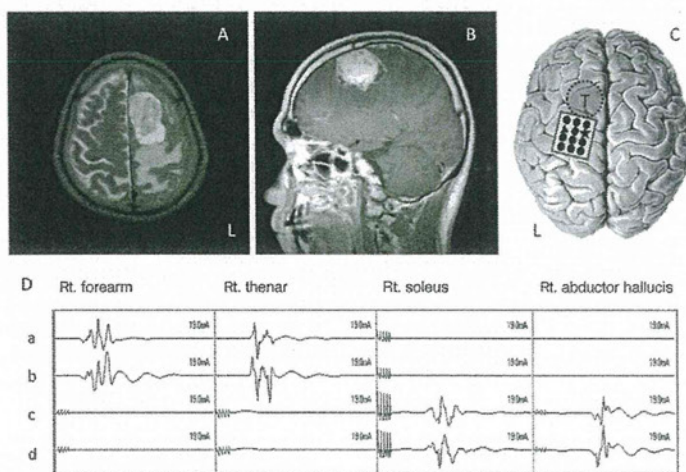

Fig. 5. Axial T2-weighted MR image (A) and sagittal Gd-enhanced T1-weighted MR image (B) of a patient (case 6) with a parasagittal meningioma. A 4 × 3 grid was placed directly on the motor cortex to cover the entire precentral gyrus (C). The stimulation sites for the lower and upper limb MEPs are indicated by an asterisk and a triangle, respectively. The location of the tumor is indicated by a T and a dashed circle. MEPs (D) were successfully recorded from the muscles of the upper (a and b) or lower (c and d) extremities, respectively.

fissure, we believe that the LE-MEP responses are elicited by stimulation of subcortical fibres from the leg cortex, rather than the pure leg motor cortex. In our experience, it was difficult to record LE-MEPs by bipolar stimulation between the two contacts over the motor cortex on the convex side. The most consistent recording was obtained by anodal–monopolar stimulation of the motor cortex with a frontal cathode at Fpz. Kombos et al. pointed out that because the current spreads transversely to the surface of the cortex with bipolar stimulation and longitudinally with monopolar stimulation, the effect of bipolar stimulation is on the level of the cortex, but monopolar stimulation spreads to the subcortical area and pyramidal tract (Kombos et al., 2001). Furthermore, for these reasons,

bipolar cortical stimulation is recommended for intra-operative mapping of cortical functions such as speech (Kombos et al., 1999), and monopolar stimulation is recommended for monitoring of subcortical motor pathways (Kombos et al., 2009). According to previous studies and our own experience, the direction of the current spread significantly influences the elicitation of LE-MEPs, and the validity of our methodology for LE-MEP monitoring can be proved. However, when using monopolar DCS, a strong current may spread in the deep subcortical layer. Therefore, it is important to recognise that 'false-negative' results may occur due to direct cortical injury in the interhemispheric fissure and leave the efferent subcortical pathway intact, although direct damage of the leg motor

cortex only is unlikely in standard pterional and bifrontal craniotomy.

Based on the results that significant MEP changes were observed in five patients when the proximal arteries were temporarily occluded during aneurysmal clipping, we showed that the MEP responded sensitively to the insufficiency of arterial blood flow in the motor pathway of the lower extremity. Postoperatively, although two of these patients had transient weaknesses of the lower extremity, permanent motor deficit was not observed in any of the patients. Transient and mild motor weakness was observed postoperatively in a patient with falx meningioma (case #7), although significant MEP changes were not observed. We believe that damage to the supplementary motor area due to surgical manipulation may have induced transient motor weakness without significant MEP changes because the tumour was located over the interhemispheric premotor area. These findings indicated that the preservation of the MEP waveforms sensitively reflects the avoidance of permanent motor deficits of the lower extremity.

Transcranial electric stimulation (TES) has also been applied for LE-MEP monitoring in other studies, especially in the field of spine surgery (Chen et al., 2007; Deletis and Sala, 2008; Deletis et al., 2009; Kelleher et al., 2008; Langeloo et al., 2007; Rothwell et al., 1994; Szelényi et al., 2005). Among these studies, LE-MEP monitoring during supratentorial surgery was found to have a success rate of only 66% (Chen et al., 2007). Furthermore, TES has several disadvantages in comparison to the DCS method, including a lack of reproducibility of the MEP waveforms, which can lead to false-positive judgements. This uncertainty of waveforms is caused by an increase in the distance between the scalp stimulation site and the motor cortex because of the gradual dilatation of the space between the brain and the dura mater during supratentorial surgery. Therefore, higher-intensity stimulation is applied towards the end of surgery. However, care must be taken in interpreting MEP waveforms elicited by high-intensity TES, as this stimulation can activate the corticospinal tract as deeply as the pyramidal decussation (Rothwell et al., 1994), and this may cause false-negative results in supratentorial surgery. By contrast, in DCS, when the subdural space is dilated and electrical conduction into the brain is reduced, close contact of the electrode and the cortex can be obtained by inserting compressing materials such as neurosurgical sponges in the subdural space. Another disadvantage of TES is possible patient movement induced by excessive muscle contractions caused by high-intensity stimulation. Therefore, DCS using a subdural electrode is more favourable than TES in LE-MEP monitoring during supratentorial surgery.

SEP monitoring was previously considered to be a surrogate technique to avoid postoperative motor dysfunction of the lower limbs (Holland, 1998; Min et al., 2001; Neuloh and Schramm, 2004a; Sako et al., 1998). However, there is a serious problem with recording, which is referred to as a 'false negative' in SEP monitoring (Sako et al., 1998). Holland et al. reported the occurrence of stroke despite normal SEP recordings during aneurysm surgery (Holland, 1998). Min et al. described a false-negative rate of 11% for SEP in aneurysm surgery (Min et al., 2001). Therefore, SEP monitoring alone may be insufficient for intra-operative monitoring of the motor function of the lower limbs due to a lack of accuracy.

Exposure or dissection of the interhemispheric fissure for placing the subdural electrode on the leg motor cortex is not possible in a pterional or bifrontal approach. Therefore, we performed LE-MEP monitoring by placing the subdural electrode on the motor cortex of the convex side close to the midline. This is a unique and safer method. Adverse events associated with MEP monitoring are rare (MacDonald, 2002; Szelényi et al., 2005), with incidences of seizures of 0.85% for TES and 1% for DCS (Szelényi et al., 2005). Subdural bleeding from bridging veins occurred in 2% of patients during placement of a subdural electrode (Szelényi et al., 2005).

Although no significant adverse events were experienced in the current series, careful and gentle insertion is required to place the electrodes and avoid vein injury. Attention must also be paid to the stimulus intensity and frequency during surgery to avoid body movements and seizures.

6. Conclusion

We herein describe a useful method for monitoring LE-MEPs during supratentorial surgery using anodal–monopolar DCS with a frontal cathode. LE-MEPs were consistently recorded by DCS using a subdural electrode placed over the convex side of the motor cortex close to the midline. This monitoring technique can also be applied to standard pterional craniotomy.

Acknowledgements

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FULL-LENGTH ORIGINAL RESEARCH

Cooling of the epileptic focus suppresses seizures with minimal influence on neurologic functions

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SUMMARY

Purpose: Focal brain cooling is effective for suppression of epileptic seizures, but it is unclear if seizures can be suppressed without a substantial influence on normal neurologic function. To address the issue, a thermoelectrically driven cooling system was developed and applied in free-moving rat models of focal seizure and epilepsy.

Methods: Focal seizures limited to the unilateral forelimb were induced by local application of a penicillin G solution or cobalt powder to the unilateral sensorimotor cortex. A proportional integration and differentiation (PID)–controlled, thermoelectrically driven cooling device (weight of 11 g) and bipolar electrodes were chronically implanted on the eloquent area (on the epileptic focus) and the effects of cooling (20, 15, and 10°C) on electrocorticography, seizure frequency, and neurologic changes were investigated.

Key Findings: Cooling was associated with a distinct reduction of the epileptic discharges. In both models, cooling of epileptic foci significantly improved both seizure frequency and neurologic functions from 20°C down to 15°C. Cooling to 10°C also suppressed seizures, but with no further improvement in neurologic function. Subsequent investigation of sensorimotor function revealed significant deterioration in foot-fault tests and the receptive field size at 15°C.

Significance: Despite the beneficial effects in ictal rats, sensorimotor functions deteriorated at 15°C, thereby suggesting a lower limit for the therapeutic temperature. These results provide important evidence of a therapeutic effect of temperatures from 20 to 15°C using an implantable, hypothermal device for focal epilepsy.

KEY WORDS: Epilepsy, Implantable device, Focal brain cooling, Therapeutic temperatures.

Focal or selective brain cooling is a candidate treatment for epilepsy (Stacey & Litt, 2008; Rothman, 2009). The first clinical application was performed almost 50 years ago and demonstrated clear suppression of epileptic seizures in patients with intractable epilepsy (Ommaya & Baldwin, 1963). A number of subsequent studies have confirmed the strong suppressive effect of cooling on epileptic discharges (Vastola et al., 1969; Reynolds et al., 1975; Sartorius & Berger, 1998; Hill et al., 2000; Yang & Rothman, 2001; Karkar et al., 2002; Imoto et al., 2006; Yang et al., 2006; Tanaka et al., 2008) and epileptic seizures (Sourek & Travnicek, 1970; Burton et al., 2005).

Despite the long history of investigation, the clinical feasibility of focal brain cooling remains unclear. One of the crucial but unresolved issues is to clarify whether "therapeutic temperatures" really exist, since focal brain cooling can suppress epileptic seizures, but is also associated with suppression of synaptic transmission. Indeed, a series of in vivo experiments have shown cooling-induced deterioration of various neurologic functions, including visual function in cats (Lomber et al., 1996; Lomber & Payne, 2004), auditory function in cats (Malhotra et al., 2004), and motor function in monkeys (Sasaki & Gemba, 1984; Brinkman et al., 1985). The cooling temperatures of the cortical surface in these experiments were not explicitly described, but neurologic deterioration was presumably induced by excessive suppression of synaptic transmission. Therefore, to address the issue of clinical feasibility, it is necessary to clarify whether seizure suppression can be achieved with a minimal influence on neurologic function.

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This issue was preliminarily investigated by Karkar et al. (2002) wherein bath application of 4°C saline on the cortex in a patient with epilepsy did not influence the amplitudes of motor-evoked potentials. Another study of cooling showed that network synchronization in hippocampal slices was terminated without blocking normal synaptic transmission (Javedan et al., 2002). Although these results are promising, they do not provide direct evidence. Therefore, we addressed the effect of cooling on seizure and neurologic functions in awake, free-moving rats using an implantable cooling system, with a focus on the eloquent cortex.

METHODS

Cooling system

A cooling device originally developed in our laboratory (Imoto et al., 2006; Oku et al., 2009; Fujioka et al., 2010) was used in the study. This device includes a cooling component and a heat-processing component. The cooling component (about 11 g in weight) consists of a proportional integration and differentiation (PID)–controlled thermoelectric chip (6.0 × 6.0 mm; maximum current (I max) 1.8A, maxmum voltage (V max) 2.5V, maximum power (Q max) 2.4W; Ferrotec Corp., Tokyo, Japan). The cooling side of the thermoelectric chip is attached to a pure silver plate (thickness of 1 mm) for direct cooling of the cortex. To avoid contact injuries with the brain, a fine thermocouple (Physitemp 23T, Clifton, NJ, U.S.A.) is embedded in the silver plate (Fig. 1A). The thermoelectric chip is controlled by a PID controller (Yamatake Corp., Tokyo, Japan). Each PID value was selected by automatic tuning of the controller to minimize overshooting or undershooting of a target temperature. The temperatures of the brain surface were cooled to 20, 15, and 10°C. Heat from the thermoelectric chip was transferred via a copper-made heat sink (6 × 6 mm with a thickness of 4 mm; see Imoto et al., 2006; Tanaka et al., 2008; Fujioka et al., 2010). The heat sink, with two water channels inside, was connected to the heat processing component via medical catheters (TYGON, R-3603, Saint-Gobain Performance Plastics, Akron, OH, U.S.A.) filled with Ringer's lactate. The
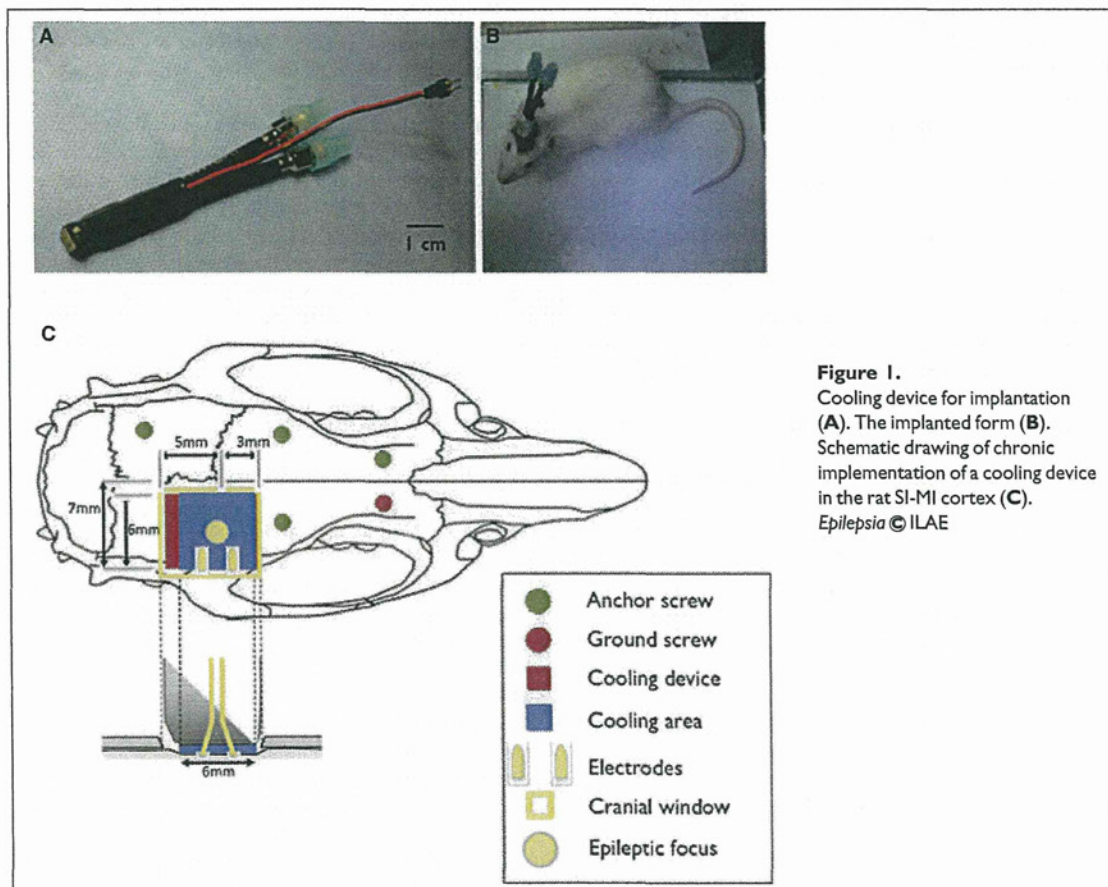

Figure 1.
Cooling device for implantation (**A**). The implanted form (**B**). Schematic drawing of chronic implementation of a cooling device in the rat SI-MI cortex (**C**).
Epilepsia © ILAE

heat-processing component includes a helium-gas cooler (TwinBird Corp., Tsubame, Japan) and a direct current (DC)-driven pump (flow rate of 200 ml/min), which circulates Ringer's solution at a controlled temperature of 20°C. Cooling was started manually in the current study.

Focal seizure and epilepsy models

Animal experiments were performed using protocols approved by the Yamaguchi University School of Medicine Institutional Animal Care Committee. Male Wistar rats (Chiyoda Kaihatsu Co. Ltd., Tokyo, Japan) (450 ± 50 g) housed in a temperature-controlled room (23.0 ± 2.0°C) were used in the study (n = 29 in total). Following induction of anesthesia by 4% sevoflurane, atropine (0.01 mg/kg) was injected subcutaneously and a mixture of ketamine (40 mg/kg, i.m.) and xylazine (4 mg/kg, i.m.) was administered for maintenance of anesthesia. The rectal temperature was maintained at 37 ± 0.2°C using a heating pad. The skull of the rat was fixed using a stereotactic apparatus (Narishige, Tokyo, Japan) and the skin on the skull was cut following injection of lidocaine (2%). A craniotomy was made with a dental drill over the ipsilateral sensorimotor (SI-MI) area (1.5–7.5 mm lateral, 3.0 mm anterior, and 4.0 mm posterior to the bregma). The cooling device was implanted and fixed in place with medical resin (Unifast II; GC Corp., Tokyo, Japan). The cooling component (6.0 × 6.0 mm) cools the entire somatotopic representation center, except for the tongue and lips, in rats (Fig. 1B,C) (Hall & Lindholm, 1974).

We used a focal seizure model (n = 12) and a focal epilepsy model (n = 6) for induction of focal seizures limited to the unilateral forepaw area. Rats with seizures outside the forepaw area were excluded from the study. Focal seizures were induced by intracortical infusion of a 4% NaCl solution of penicillin G (PG) using a syringe pump (0.3 μl/min at a concentration of 200 IU/μl, up to 1,200 IU) until continuous seizures were stably but minimally induced. Intracortical infusion was performed using a fine needle (28 gauge) with a tip length of 0.8 mm, which was attached to the center of the cooling component. The needle was stereotactically implanted on the eloquent area (i.e., the forepaw area of the sensorimotor [SI-MI] cortex at a depth of 0.8, 1.0 mm anterior, and 3.6 mm lateral to the bregma) using medical resin. Once seizures were induced, experiments were performed within 30 min. The frequency of seizures was stable over this time window. To investigate the effect of cooling when the seizure focus extends out of the forelimb area, >1,200 IU (up to 2,200 IU) of PG solution was also applied (n = 3).

Focal epilepsy was induced by direct application of cobalt powder on the same area of the cortex (Dow et al., 1962). Following a small craniotomy made with a dental drill, cobalt powder (8 mg; Sigma-Aldrich Co. LLC., Tokyo, Japan) was applied on the dura over the eloquent

cortex. A sterilized cotton sheet was placed and the skin was sutured. Following a recovery period of 3 days, the rat was reanesthetized and the cooling device was implanted using dental resin at the center of the forepaw area, which became an epileptic focus. Cooling experiments were performed at 9 ± 2 days after implantation.

Neurologic assessments

The effect of cooling on the frequency of seizures before and during cooling was evaluated by the number of involuntary lifts of the forepaw from the floor in 3 min for both the focal seizure and epilepsy models. All tests were recorded by video camera (60 frames/s) and forepaw lifting was evaluated blindly by at least two of four researchers. Comprehensive neurologic functions before and during cooling were assessed on a 21-point neurologic scale, which was originally developed for assessment of a cerebral ischemia model in rodents (Hunter et al., 2000). This scale comprises a battery of 10 items: assessment of paw placement, righting reflex, ability to grip a horizontal bar, time on an inclined platform, rotation, visual forepaw reaching, circling, contralateral reflex, motility, and general condition. This kind of scale is commonly used in behavior assessments of rats (McGill et al., 2005). The assessment was performed three times within 30 min in the current study.

Sensorimotor functions of the limbs were investigated in foot-fault tests and according to the receptive field size in the forepaw area. Foot-fault tests were evaluated using the following formula: (foot faults *per* limb/steps *per* limb) × 100 (Soblosky et al., 1996). The rat was placed gently on an elevated grid and the number of slips into the grid (i.e., foot faults) in 25 paired steps was calculated. The trial was performed three times and mean scores were calculated. Sensory function was evaluated by measuring the receptive field (RF) size of the forepaw area contralateral to the cooling cortex (layer iv; depth 450–800 μm) under the ketamine anesthesia described above (n = 5) (Fujioka et al., 2004). Tactile stimuli were applied on the forepaw areas of the skin (40 points) with von Frey hair-type probes (calculated force of 0.6 g) before and during cooling. The number of reaction fields was counted and defined as the RF size. All behavioral experiments were performed at cooling temperatures of 20, 15, and 10°C. All tests were also recorded by video camera (60 frames/s) and were evaluated blindly by at least two of four researchers.

Electrocorticography

An electrocorticography (ECoG, 1 Ch) over the epileptic focus in all rats was differentially recorded using a pair of needle-type electrodes (impedance 500 kOhm at 500 Hz) attached to the bottom of the cooling device (Fig. 1C). Data were amplified and recorded in Powerlab (ADInstruments, Colorado Springs, CO, U.S.A.) with a sampling rate of 2 kHz (low-cut filter 5 Hz, high-cut filter 100 Hz).

Electrocardiography (ECG)

Before implantation of the device, the skin of the right chest was incised in the supine position and a telemetry system (PhysioTel, DSI, St. Paul, MN, U.S.A.) was implanted in normal rats (n = 4) and in normal sham rats with a cooling device implanted in the brain (n = 4). Two-lead ECG was sampled at 2 kHz with a duration of 1 min and recorded in a PC via a Powerlab instrument (ADInstruments).

Histology

Following the experiments, the rats were sacrificed and hematoxylin and eosin (H&E) staining was performed (5-μm sections).

Statistics

Statistical analyses were performed by paired Student t-tests, Dunnett post hoc tests, or Steel-Dwass tests using the R software package (see the homepage; http://www.R-project.org.). $p < 0.05$ was considered to be statistically significant. Analysis of variance (ANOVA) was performed to evaluate the significance of differences between the means of all groups. A Dunnett test or Steel–Dwass post hoc test for multiple comparisons was used to compare groups with parametric or nonparametric data and unequal sample size or sample variance. Data are shown as the mean ± standard deviation (SD) in Student t-tests and Dunnett tests, and as the mean ± standard error of the mean (SEM) in the Steel–Dwass test.

RESULTS

Temperature gradient of the cooling area

The temperature gradient under the cooling device was evaluated thermographically on an agar surface warmed to 37°C. This surface was cooled to 20°C with the cooling device (6 × 6 mm). The cooling effect was limited to the contact area and did not reach the perimeter (Fig. 2).

Effects of cooling on focal seizures

Device implantation

The implanted device (Fig. 1A–C) did not influence ordinary behaviors in sham rats, such as eating, moving, grooming, or sleeping. ECG did not show any cooling-associated changes in rate rhythms (194 ± 6.32 in normal rats vs. 198.5 ± 14.73 in normal sham rats, p = 0.65 by Student t-test) and did not induce arrhythmia before, during, or after cooling. Although cooling to a target temperature was achieved without overshooting or undershooting, such precise temperature control was generally obtained at the cost of time. The times to reach the target temperatures of 20, 15, and 10°C were 9 ± 0.2, 12 ± 0.4, and 20 ± 0.4 s, respectively (mean ± SD, each n = 4).
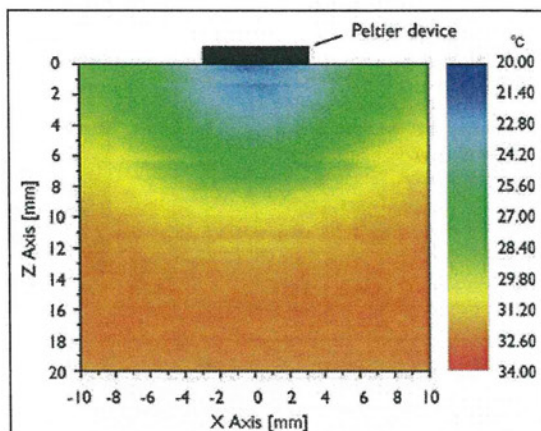

Figure 2.
Temperature gradient under the cooling device. The temperature of the cortical surface was controlled at 20°C. Note that the cooling effect was limited to the contact area and did not reach the perimeter.
Epilepsia © ILAE

Focal seizure model

Intracortical application of a PG solution reliably induced focal seizures limited to the unilateral forelimb area. Cooling of the seizure focus immediately and significantly reduced the frequency of seizures per minute at surface brain temperatures of 20°C (48.7%, p < 0.0001), 15°C (11.8%, p < 0.0001), and 10°C (0%, p < 0.0001), in comparison to the noncooling ictal group (100%, n = 6, Fig. 3A). The reduction of the seizure frequency was coincident with the suppression of epileptic discharges (EDs) in the ECoG (Fig. 4A,D). The effect of cooling-induced seizure suppression was continuous and was not diminished as long as cooling was performed. There was no apparent difference in the extent of suppression between rapid and slow cooling to a target temperature.

The significant reduction of the seizure frequency was also associated with improvement of neurologic scores within the range of 20–15°C. Induction of seizures caused a significant deterioration of neurologic scores (15.6 ± 0.43, p = 0.023). These scores were improved to 18.33 ± 0.21 at 20°C (p = 0.027) and 19.5 ± 0.22 at 15°C (p = 0.029), compared to those of the noncooling group (Fig. 3B). These effects disappeared soon after cessation of cooling. Cooling to 10°C also achieved a seizure-free condition, but neurologic scores remained low (15.93 ± 0.21, p = 0.979). Additional injection of a PG solution (>1,200 IU) induced seizures outside the forelimb area, which made it impossible to inhibit seizures in the forelimb area, as well as in areas outside the forelimb (Fig. 4B,E).

Implantation of the device for 1 month with 1 h cooling per day did not result in detrimental changes in H&E

Figure 3.
Frequency of seizures (**A**) and neurologic scores (**B**) as a function of temperature at the seizure foci. PG, penicillin G; NC, noncooling. Error bars: SD in **A**, SEM in **B**. *p < 0.05. **p < 0.01 versus noncooling groups (n = 6). *Epilepsia* © ILAE
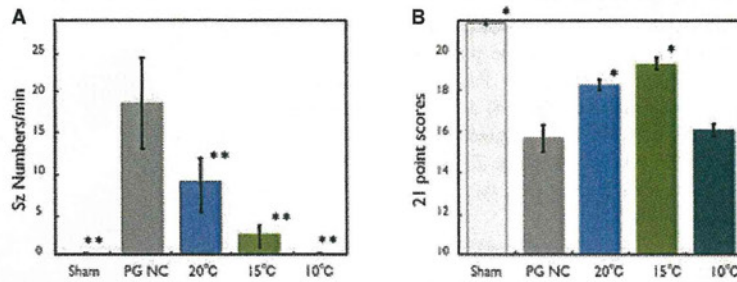
Figure 4.
Epileptic discharges (EDs) before (**A–C**) and during cooling at 15°C (**D–F**) at the seizure focus in penicillin G (PG)–treated (**A,B,D,E**) and cobalt-treated (**C, F**) rats. Suppression of EDs was associated with amelioration of seizure frequency when a seizure focus was in a cooled area—before (**A**) and during (**D**) cooling at 15°C. When the seizure focus extended outside the cooled area due to excess application of PG, EDs were not completely terminated at temperatures down to 15°C (**B, E**). Suppression of EDs also occurred during cooling in a cobalt-treated rat (**C, F**). *Epilepsia* © ILAE
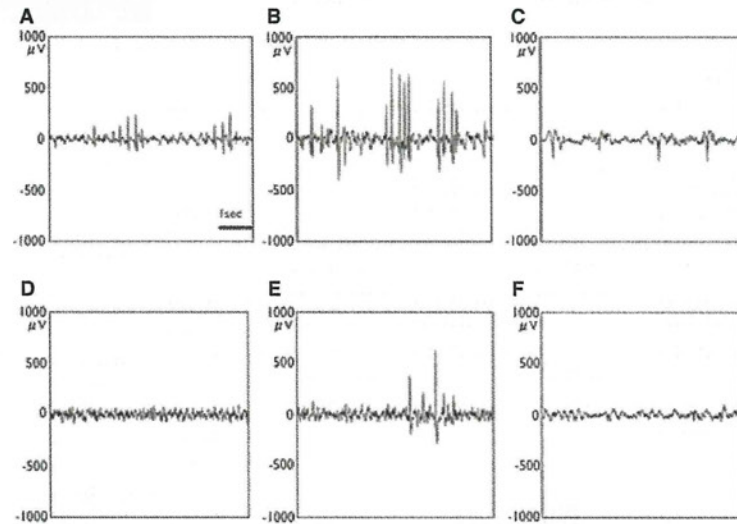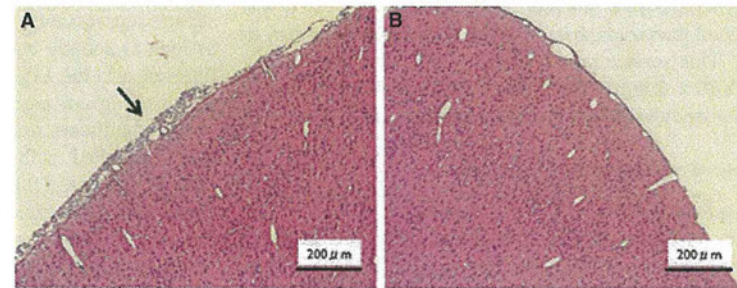
Figure 5.
Following implantation of the device for 1 month, partial fibrosis was observed in the subarachnoid of the cortex, but was limited to the area under the device (shown by an arrow). No histologic changes were observed in the contralateral homologous area. *Epilepsia* © ILAE
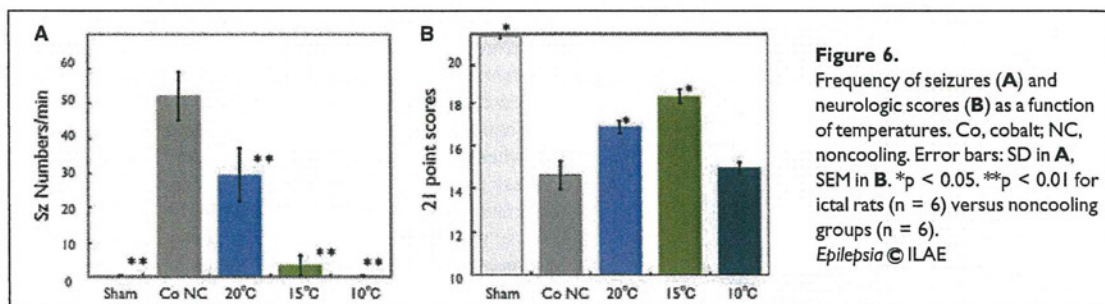
staining (n = 5), except for partial fibrosis of the subarachnoid region under the device (Fig. 5).

Focal epilepsy model

Cobalt-induced epileptic seizures limited to the unilateral forelimb area were sufficiently severe and continuous to be suggestive of a state of epilepsia partialis continua. The number of seizures per minute in the cobalt model was double that in the PG model. Seizure frequency was reduced in association with improvement of neurologic scores in a cooling range of 20–15°C and was coincident with suppression of EDs during cooling (Fig. 4C,F). The frequency of seizures per minute was reduced by 54.4% at 20°C (p < 0.0001), 3.9% at 15°C (p < 0.0001), and 0% at 10°C (p < 0.0001) (Fig. 6A). The significantly lower neurologic scores under noncooling, ictal conditions (14.5 ± 0.34,

Figure 6.
Frequency of seizures (**A**) and neurologic scores (**B**) as a function of temperatures. Co, cobalt; NC, noncooling. Error bars: SD in **A**, SEM in **B**. *p < 0.05. **p < 0.01 for ictal rats (n = 6) versus noncooling groups (n = 6).
Epilepsia © ILAE

p = 0.023) were improved by 16.88 ± 0.28 at 20°C (p = 0.027) and 18.38 ± 0.2 at 15°C (p = 0.029, Fig. 6B), in comparison with the noncooling group (n = 6). As in the PG model, cooling to 10°C did not improve the neurologic scores (14.88 ± 0.41, p = 0.979, Fig. 6B). The therapeutic effect was not diminished as long as cooling was performed.

Histology in cobalt-treated rats shows bowl-shaped necrotic changes (Dow et al., 1962), which were limited to the shallow cortex in our study. There were no other particular cooling-associated changes.

Effects of cooling on neurologic functions

Because neurologic improvement was limited in the cooling ranges of 20–15°C, we hypothesized that cooling below 15°C induced excessive blockage of synaptic transmission. Therefore, we investigated the effects of cooling on normal neurologic functions in sham rats. Apparent neurologic deficits in ordinary behaviors (walking, eating, grooming, and so on) were not observed by cooling to 15°C. Neurologic functions (n = 6) were robust with cooling to 20°C (20.9 ± 0.06, 99.8%, vs. sham group, p = 0.92), but a trend for deterioration began at 15°C (20.6 ± 0.20, 98.1%, p = 0.089), and these changes reached statistical significance at 10°C (18.2 ± 0.21, 86.7%, p = 0.012), in comparison to the noncooling group (20.95 ± 0.05, 100%) (Fig. 7A).

A subsequent investigation of sensorimotor functions invariably revealed significant deterioration at 15°C. In foot-fault tests (n = 6), cooling to 20°C did not induce substantial changes (% error of 1.1 ± 1.31%, p = 0.957), but the findings reached statistical significance at 15°C (3.34 ± 0.73%, p = 0.018) and 10°C (18 ± 3.45%, p < 0.0001, Fig. 7B). In anesthetized sham rats (n = 5), RFs of the forepaw under the cooling area began to diminish at 20°C (84.1%, p = 0.099) and reached statistical significance at 15°C (30.6%, p < 0.0001) and 10°C (1.2%, p < 0.0001), in comparison to the noncooling group (100%) (Fig. 7C).

DISCUSSION

This study provided important evidence for a therapeutic effect of low temperature on focal seizure and epilepsy in an animal model. The results build on findings in previous studies (Yang & Rothman, 2001; Rothman et al., 2005; Yang et al., 2006; Rothman, 2009). Temperatures from 20°C down to 15°C significantly suppressed seizures and were associated with improvement of neurologic function. The effect was powerful, instantaneous, and continuous, which suggests advantages over other existing epileptic therapies.
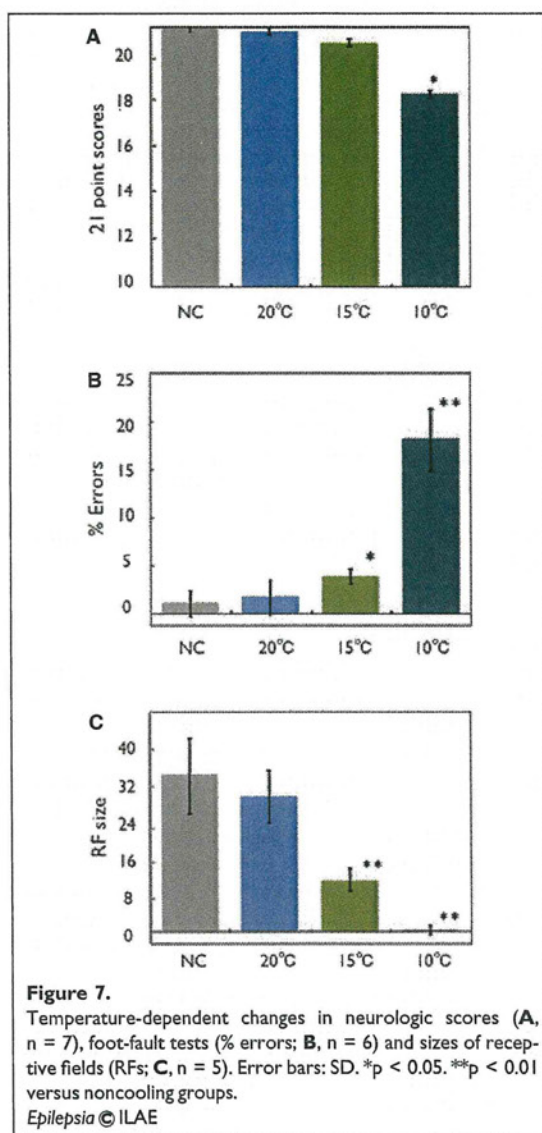
Limitations and feasibility of focal brain cooling for epilepsy treatment

An indication of focal brain cooling for focal epilepsy requires accurate identification of epileptic foci of a size that is well within the cooling area. These conditions were produced in two animal models. The PG model of focal seizure allows adjustment of the size and extent of seizures (Elger & Speckmann, 1983). Therapeutic effects were obtained when the focal seizure was within the cooling area. However, application of PG >1,200 IU caused the focal seizures to no longer be limited to the forelimb, but to extend over the hind limb and body. In such cases, the suppressive effects of focal cooling were limited, even in the epileptic focus (Fig. 4B,E).

In our study, the epileptic focus in the cobalt model was clearly identifiable (Chang et al., 2004). The epileptic seizures in this model were more severe than those in the PG model, but seizure control was as prominent as that in the PG model. Therapeutic temperatures were also identified in the cobalt model, suggesting the feasibility of focal brain cooling as therapy for focal epilepsy.

Factors influencing the therapeutic cooling temperature

The results of the study show that therapeutic temperatures are not uniquely defined, but are changed by factors such as seizure severity and the size of the focus. Other factors that can influence the therapeutic temperatures include antiepileptic drugs (AEDs) and neuroplasticity. We did not use AEDs in the study, but the assumed synergistic effects of AEDs during cooling (Sourek & Travnicek, 1970) may increase the upper limit of the therapeutic temperature. Another important aspect of focal brain cooling is the involvement of functional compensation, presumably due to

Figure 7.
Temperature-dependent changes in neurologic scores (**A**, n = 7), foot-fault tests (% errors; **B**, n = 6) and sizes of receptive fields (RFs; **C**, n = 5). Error bars: SD. *p < 0.05. **p < 0.01 versus noncooling groups.
Epilepsia © ILAE

scores), but a detailed investigation of sensorimotor functions (foot-fault tests and receptive field size) in normal sham rats revealed significant deterioration. Clinicians who place an emphasis on seizure suppression may prefer lower therapeutic temperatures at the cost of functional deterioration, whereas those who wish to avoid neurologic dysfunction may prefer higher therapeutic temperatures. Therefore, determining the therapeutic temperature in patients with epilepsy will depend not only on objective criteria but also on subjective criteria that maximize the quality of life.

Mechanism of seizure suppression by focal brain cooling

Focal brain cooling is generally considered to induce reduction of transmitter release (Eilers & Bickler, 1996), kinetic alteration of voltage-gated ion channels (Traynelis & Dingledine, 1988; Hill et al., 2000; Volgushev et al., 2000), and network desynchronization (Javedan et al., 2002). Although the precise antiepileptic mechanisms remain to be determined, it is generally recognized that suppression of synaptic transmission is involved in reduction of seizures. An in vitro study showed that synaptic transmission begins to decrease below 20°C (Volgushev et al., 2000). In a case in which the temperature is <20°C at 1 mm under the cortical surface, but >20°C at a depth of 2 mm, it is reasonable to assume that synaptic transmissions and EDs in the shallow cortex (layer II/III) are selectively suppressed because of the spread through neurons in the shallow layer with horizontal connections to the ipsilateral or contralateral cortex (Nolte, 2009). Selective suppression of synaptic transmission due to a cooling-induced thermogradient in the cortex may contribute to the vulnerability of somatosensory processing, as indicated by the reduction of RFs during cooling. Because the neurons that form a pyramidal tract (layer V) lie deep in the sensorimotor cortex, selective transmission failure may have occurred during surface cooling.

Histologic assessment

Pathologic changes due to cooling were not observed in the PG and cobalt models. Although partial fibrosis under the cooling device did occur, this was probably not caused by cooling, given the histologic tolerance to focal brain cooling even down to 5°C (Yang et al., 2006; Oku et al., 2009). Rather, it is likely that this histologic change was caused by direct contact with the cooling part of the device (i.e., pure silver) because inflammation of the contact area cannot be avoided under free-moving conditions.

Clinical advantages and requirements of the cooling device

Temperature control is of crucial importance in therapeutic applications, given that the range of therapeutic temperatures is narrow and that a small deviation from this range may lead to neurologic dysfunction. Furthermore, varying brain temperatures in the ictal stage may further complicate temperature control. In this regard, thermoelectronic

behavioral adaptation or neuronal plasticity. This property has been reported in a series of studies in normal monkeys, wherein cooling-induced functional deficits began to be ameliorated over a time course of months (Sasaki & Gemba, 1984; Brinkman et al., 1985). Identification and evaluation of these factors are important issues that remain to be addressed.

Determination of the therapeutic cooling temperature

Our data showed that cooling to 15°C reliably suppressed focal seizures and improved neurologic function (21-point

devices have an advantage over traditional circulatory-cooling devices, since the thermoelectronic devices are small but have sufficient cooling power and precise temperature control. An alternative approach using systemic hypothermia has been used in refractory status epilepticus (Corry et al., 2008), but clinical use of this method is limited by adverse effects and limitations on the cooling temperature (31–35°C) and period.

Clinical demand for an implantable cooling device will not be limited to the epileptic field. Other potential applications include treatment for cerebrovascular diseases, including poststroke rehabilitation (Clark & Colbourne, 2007), neurotrauma (Clark & Colbourne, 2007), and pain (Fujioka et al., 2010), all of which will depend on thermal modulation of neuronal excitability.

Application of the cooling device for treatment of epilepsy

Focal brain cooling may be applied therapeutically for patients who have an epileptic focus on the eloquent cortex (i.e., motor or language area) or those who cannot be treated with AEDs. Cooling may also be used as a diagnostic tool in intracranial ECoG monitoring of patients with potential neurosurgical indications, but in whom the focus cannot be clearly defined. In such cases, the final surgical indication would be decided by preliminary application of cooling to the focus. There are several physiologic and technical issues to be solved before the device can be applied in intractable epilepsy. However, this study is an important step toward medical use of an implantable hypothermal device for treatment of focal epilepsy and other neurologic disorders.

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DISCLOSURE

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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