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Limitations of Local Brain Cooling on Generalized Motor Seizures from Unknown Foci in Awake Rats

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Abstract

Local brain cooling of an epileptic focus at 15°C reduces the number of spikes on an electrocorticogram (ECoG), terminates seizures, and maintains neurological functions. In this study, we attempted to suppress generalized motor seizures (GMSs) by cooling a unilateral sensorimotor area. GMSs were induced in rats by intraperitoneal injection of bicuculline methiodide, an antagonist of gamma-aminobutyric acid. While monitoring the ECoG and behavior, the right sensorimotor cortex was cooled for 10 min using an implanted device. The number of spikes recorded from the cooled cortex significantly decreased to 71.2% and 62.5% compared with the control group at temperatures of 15 and 5°C (both P <0.01), respectively. The number of spikes recorded from the contralateral mirror cortex reduced to 61.7% and 62.7% (both P <0.05), respectively. The ECoG power also declined to 85% and 50% in the cooled cortex, and to 94% and 49% in the mirror cortex by the cooling at 15 and 5°C, respectively. The spikes regained in the middle of the cooling period at 15°C and in the late period at 5°C. Seizure-free durations during the 10-min periods of cooling at 15 and 5°C lasted for 4.1 ± 2.2 and 5.9 ± 1.1 min, respectively. Although temperature-dependent seizure alleviation was observed, the effect of local cortical cooling on GMSs was limited compared with the effect of local cooling of the epileptic focus on GSMs.

Key words: epilepsy, generalized motor seizure, bicuculline methiodide, brain temperature, implantable device

Introduction

Focal excision is an established surgical technique for medically intractable epilepsy. However, this technique carries a risk of severe postoperative neurological deficits if the foci are located in a functionally eloquent area. We are developing a focal brain cooling treatment for patients with foci in eloquent cortices. This treatment terminated seizures and epileptic discharges both in animal models[1–9] and epilepsy patients.[10–12] This method corrected the uncoupling of brain metabolism and cerebral blood flow, inhibited the release of excitatory neurotransmitters, and was beneficial for the treatment of neurological diseases.[13–16] The optimal temperature at which seizures could be terminated without suppressing neurological function or causing histological brain damage was 15°C.[4,17] Temperature controlling devices were implanted appropriately in the epileptic foci in these studies.

Our aim is to extend local brain cooling treatments to epilepsy patients with multiple or unknown foci. Even if the foci cannot be cooled directly, local

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cooling may disconnect the seizure propagation network either within the foci,[18,19] between the cortex and thalamus,[20–23] or within the thalamus. The unilateral sensorimotor cortex was chosen as the location where the device would be implanted because it has been implicated in generalized motor seizures (GMSs).[21]

We induced seizures originated from random regions in freely moving rats by the intra-abdominal injection of bicuculline methiodide, an antagonist of gamma aminobutyric acid-A receptor. The aims of our study were to investigate whether cooling of the cortex without involving epileptic foci can terminate GMSs, and to determine the temperature at which the cooling should be performed.

Materials and Methods

Animals

Among the male Sprague–Dawley rats weighing 350 ± 50 g used in the experiments, data from 12 rats which survived and completed three cooling and one control studies were analyzed. The Institutional Animal Care Committee at Yamaguchi University School of Medicine approved all surgical procedures.

Implantation of the cooling device and electrodes

Animals were topically anesthetized in a bell jar with diethyl ether. The trachea was intubated followed by mechanical lung ventilation with 2% sevoflurane in 21% oxygen using a ventilator (A.D.S 1000, Engler Engineering. Hialeah, FL, USA). The rectal temperature was maintained at 37 ± 0.5°C using a heating pad (NS-TC10, Neuroscience, Inc., Bunkyo-Ku). A cooling device initially developed in our laboratory was used in this study. It has been described in detail in our previous reports.[4–9,11,12,14–17] The rat was fixed in a stereotaxic apparatus (SR-6N, Narishige Co., Ltd., Tokyo). Craniotomy was performed on the right side of the skull (1.0–7.0 mm lateral, −3.0 to 4.0 mm from Bregma), and the cooling device was implanted over the right sensorimotor cortex (4.0 mm lateral, −0.5 mm from Bregma). A temperature sensor was implanted under the cooling device on the right cerebral cortex. We did not measure the temperature of the contralateral hemisphere. One electrocorticogram (ECoG) electrode (Ch 1, thickness: 10 μm) was attached to the bottom of the cooling device to record from the cooled hemisphere. A small burr hole was made in the equivalent position on the left side of the skull, and another ECoG electrode (Ch 2) was implanted for recordings from the uncooled hemisphere. Two stainless steel screws were fixed on the occipital and nose bone as the reference and ground electrodes, respectively. All devices were secured using resin (Unifast II; GC Corporation, Bunkyo-Ku, Tokyo).

Induction of generalized motor seizures and brain cooling

To allow complete recovery from surgery, experiments on seizure generation were performed on the fifth day after implantation. A high dose of bicuculline methiodide (BM; 10 mg/ml; BML-EA149, Enzo Life Sciences, Farmingdale, NY, USA) solution was injected intraperitoneally to induce seizures.[24] Rats exhibited generalized seizures approximately 10 min after injection of the BM solution. Ten minutes after the initiation of the seizures, the temperature of the cortical surface was decreased to either 15, 10, or 5°C for 10 min using the implanted cooling device. Brains were naturally rewarmed after the 10-min cooling period and restored to the precooling temperature levels within 10 min. Body temperature was measured by thermography during the experiments. Every rat was subjected to four experiments involving cooling at 15, 10, 5°C, and the control temperature. The four experiments were performed in random order every 3 days.

Recording and analysis of ECoG and observation of seizure

Baseline activities were observed for 10 min to ensure stability of the ECoG. ECoG signals were fed into a biological amplifier (SYNAX1200; NEC-Sanei, Tokyo) with a bandpass filter (0.1–3 kHz). After amplification, data were recorded by a PowerLab system (AD Instruments, Sydney, Australia) with a sampling rate of 2 kHz. Spectral analysis of ECoG based on fast Fourier transformation was performed using LabChart 7 (AD Instruments). The beta-l band of 14–24 Hz was extracted and the power was determined. The power detected during every one-min period was divided by the average power measured over the 10-min period before cooling started.

Seizure progression was evaluated by rating the seizures on a scale of 0–5 using Racine stages[25]: (0) no convulsive activity, (1) mouth and facial movements, (2) head nodding, (3) forelimb clonus, (4) rearing, and (5) rearing and falling. Seizure (Racine stages 1–5) and seizure free (Racine stage 0) periods were measured by two physicians, one of whom was blinded to the purpose, methods, and classification of the rat group.

Histological evaluation

Upon completion of the experiments, the animals were euthanized with an overdose (60 mg/kg i.p.) of pentobarbital (Sumitomo Dainippon Pharma, Osaka).

The hemispheres ipsi- and contralateral to the cooling device were sectioned in the coronal plane at a thickness of 4 μm. Hematoxylin and eosin (H&E) staining was performed.

Data analysis

Data analysis included the quantification of the following GMS features: (i) brain and body temperatures, (ii) number of spikes, (iii) power in ECoG, (iv) Racine stages, and (v) seizure free duration. Intergroup comparisons of the number of spikes was performed using paired sample *t*-test. The ECoG power and seizure free duration were assessed using the one-way analysis of variance (ANOVA) with a least significant difference test. Racine stages were assessed using ANOVA with a Tukey post-hoc test for pairwise comparisons. Differences were considered significant at $P < 0.05$. All analyses were performed using the SPSS Statistical software (Ver. 17.0, IBM, New York, USA).

Results

Effects of seizure on brain temperature, and of brain cooling on body temperatures

In BM-induced GMSs, the brain temperatures increased significantly from 36.57 ± 0.14°C to 36.69 ± 0.13°C ($P < 0.05$) with an increase in ECoG power (Fig. 1). The brain temperature was successfully lowered during the cooling periods and rose to precooling levels in the post-cooling periods. Body temperatures were not influenced by the seizures or local brain cooling procedure (Table 1).

Effects of local brain cooling on ECoGs

In ECoGs of the cooled cortex, the number of spikes per 10 min during local brain cooling at 15, 10, and 5°C was 222 ± 23 (71.2%, $P < 0.01$), 229 ± 61 (73.4%, $P < 0.05$), and 195 ± 42 (62.5%, $P < 0.01$), respectively, which was significantly lower in comparison to 312 ± 52 in control rats (Table 2). These reductions were also seen in the mirror cortex with spike numbers of 195 ± 26 (61.7%, $P < 0.05$), 219 ± 57 (69.3%, $P < 0.05$), and 189 ± 48 (62.7%, $P < 0.05$) during the contralateral brain cooling at 15, 10, and 5°C, respectively, while in control rats the spike number was 316 ± 58, which was comparable to those in the mirror cortex (Table 2). Typical results for local brain cooling-induced suppression of ECoG power are shown in Fig. 2, and averaged results for serial changes in ECoG power are shown in Fig. 3. Epileptic discharges were maintained in the cortex bilaterally for more than 30 min in controls although there was a constant decrease in ECoG power (Figs. 2 and 3, *bottom rows*). The ECoG power declined in the cooled cortex to 85%, 64%,

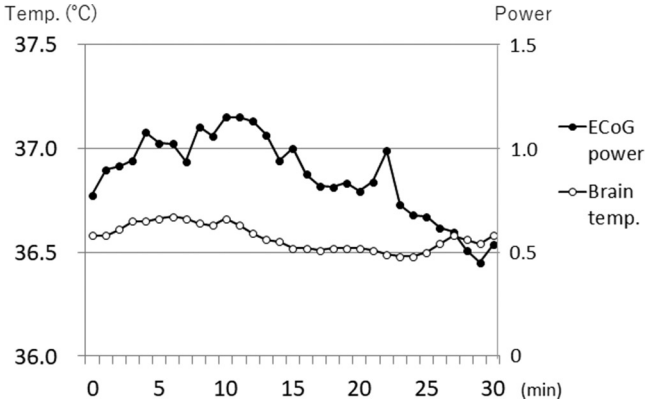

Fig. 1 Serial changes in electrocorticogram (ECoG) power and brain temperature. By the induction of epileptic discharge, both the power of the ECoG and the brain temperature increased. Both of them decreased after the peaks. *Solid circle*: ECoG power. *Open circle*: brain temperature.

Table 1 Serial changes in body and brain temperatures

Region	Cooling condition (*n*)	Temperature (°C)			
		Seizure-free period	Seizure period		
			Precooling	Cooling	Rewarming
Body	5°C (12)	36.34 ± 0.13	36.46 ± 0.12	36.24 ± 0.19	36.32 ± 0.10
	10°C (12)	36.45 ± 0.11	36.41 ± 0.12	36.25 ± 0.12	36.24 ± 0.10
	15°C (12)	36.33 ± 0.16	36.42 ± 0.16	36.24 ± 0.19	36.50 ± 0.18
	Control (12)	36.48 ± 0.09	36.50 ± 0.09	36.42 ± 0.09	36.49 ± 0.12
Brain (cooled hemisphere)	5°C (12)	36.52 ± 0.14	36.64 ± 0.12*	5.00 ± 0.10***	36.10 ± 0.09*
	10°C (12)	36.45 ± 0.16	36.63 ± 0.15**	10.00 ± 0.10***	36.13 ± 0.10*
	15°C (12)	36.51 ± 0.11	36.69 ± 0.10*	15.00 ± 0.10***	36.07 ± 0.08**
	Control (12)	36.57 ± 0.14	36.69 ± 0.13*	36.72 ± 0.10*	36.69 ± 0.12

Values are shown as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. seizure-free period in each group (paired sample *t*-test).

Table 2 Serial changes in spike numbers

Brain hemisphere	Cooling condition (*n*)	Spike numbers (per 10 min)		
		Seizure period		
		Precooling	Cooling	Rewarming
Cooled hemisphere	5°C (12)	403 ± 50	195 ± 42*	269 ± 46
	10°C (12)	345 ± 52	229 ± 61**	281 ± 79
	15°C (12)	360 ± 40	222 ± 23*	223 ± 38
	Control (12)	320 ± 48	312 ± 52	227 ± 39
Uncooled hemisphere	5°C (12)	394 ± 54	189 ± 48**	279 ± 45
	10°C (12)	335 ± 45	219 ± 57**	283 ± 88
	15°C (12)	365 ± 39	195 ± 26**	205 ± 37
	Control (12)	306 ± 50	316 ± 55	201 ± 32

Numbers of spikes per 10 min are shown as mean ± SEM. *P <0.01, **P <0.05 vs. precooling for each temperature condition (paired sample t-test).

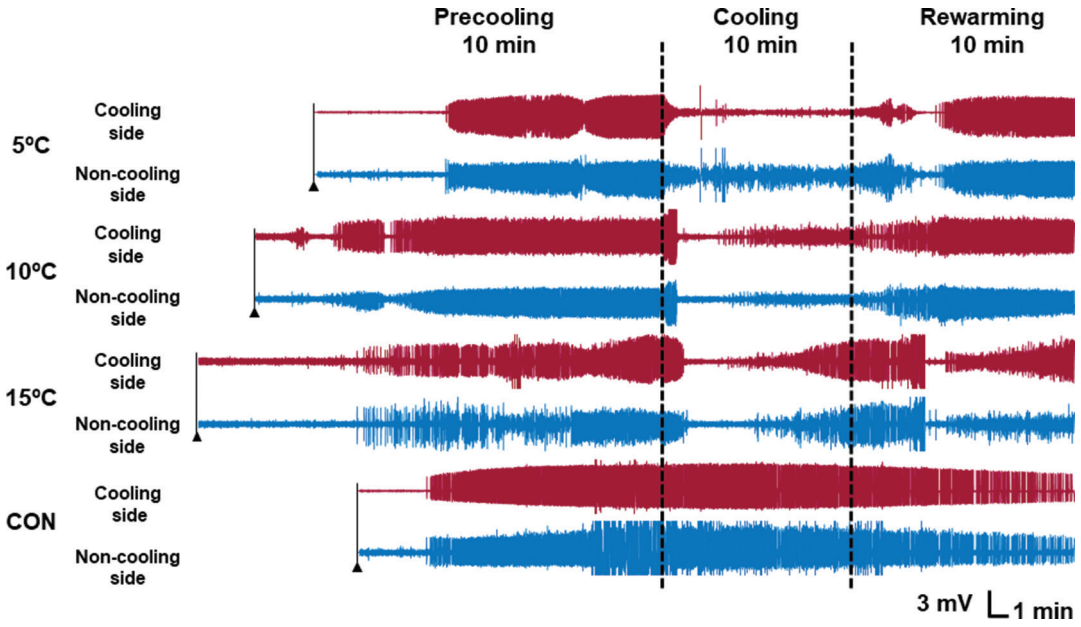

Fig. 2 Serial changes in representative rat electrocorticograms. Amplitudes of both the cooling and the non-cooling sides of ECoG increased after intraperitoneal injection of bicuculline methiodide (*arrowheads*) and decreased during cooling at 5°C. During cooling at 10 and 15°C, the decreased ECoG amplitudes increased in the middle of the cooling phase. Ten-minute durations of the precooling, cooling, and post-cooling periods were recorded.

and 50% during local brain cooling at 15, 10, and 5°C, respectively, in comparison to those in control rats. The power recovered to 83%, 83%, and 65% in the post-cooling periods. The ECoG power in the mirror cortex declined to 94%, 91%, and 49% during the cooling period and recovered to 73%, 101%, and 73% during the post-cooling period after local brain cooling at 15, 10, and 5°C, respectively. The ECoG power significantly declined in the first 5 min during the cooling periods, but increased again in the middle of the cooling periods during cooling at 15 and 10°C and in the late period during cooling at 5°C. These trends were observed in the mirror cortex as well.

Effects of local brain cooling on GMSs

During cooling periods, the Racine stage scores in rats with local brain cooling at 15, 10, and 5°C were 1.00 ± 0.17, 1.00 ± 0.17, and 0.75 ± 0.18, respectively. These scores were significantly lower compared to that in control rats (2.58 ± 0.23) (all P <0.001). These scores reversed during the

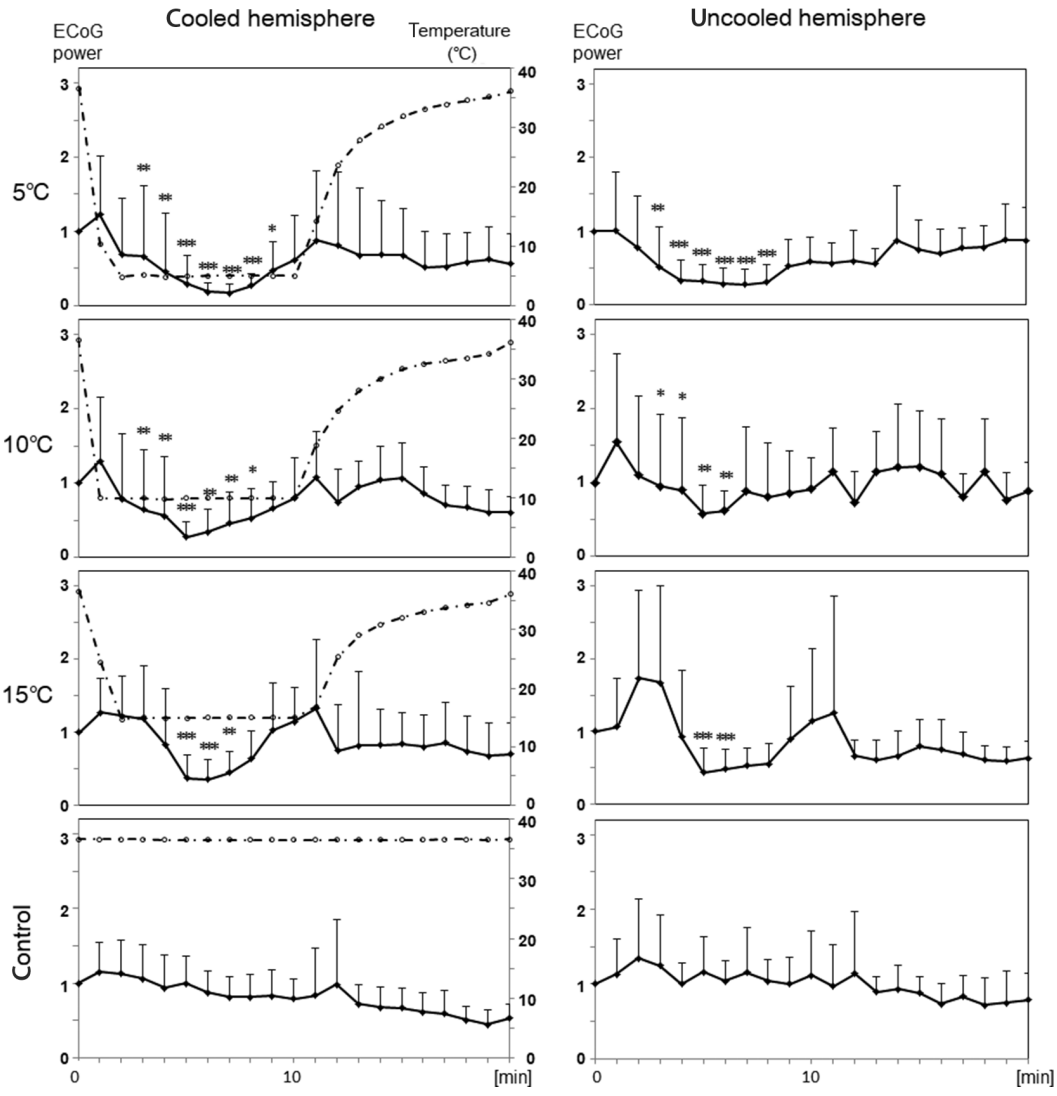

Fig. 3 Relationship between temperature and ECoG power. The ECoG power of both the cooled and uncooled hemispheres decreased significantly during cooling at 5°C. The decrease in power become statistically insignificant in the middle of the cooling periods during cooling at 10 and 15°C. Values are shown as means ± SEM; $^{*}P$ <0.05, $^{**}P$ <0.01, $^{***}P$ <0.001 vs. power in precooling periods.

post-cooling periods to 1.52 ± 0.14, 1.75 ± 0.03, and 2.17 ± 0.27, respectively. In control rats only, the scores declined slightly to 1.92 ± 0.19. Individual scores in any local brain cooling group remained stable during the cooling periods within the range of 0 and 2. In other words, unilateral seizures possibly indicated only by Racine 3, characterized by forelimb clonus, were not observed in any rat during local brain cooling. Unilateral local brain cooling suppressed motor seizures either bilaterally or not at all.

As shown in the timeline of ECoG powers (Fig. 3), seizures were suppressed in the first half of the cooling periods but this suppression was lost in the second half. Seizure free durations during the 10-min periods of cooling at 15, 10, and 5°C were 4.1 ± 2.2, 4.3 ± 1.2, and 5.9 ± 1.1 min. Each of these durations were significantly longer (P <0.0001) than the seizure free duration in the control rats. These rats had no seizure free periods (Fig. 4).

Effect of local brain cooling on brain histology

The cortical surface under the cooling device, which was implanted for 20 days and was used to induce local brain cooling three times in this period, presented mild arachnoid fibrosis, but detrimental changes were not confirmed through H&E staining.

Discussion

We reported that local brain temperature was elevated by epileptiform activity induced by topical administration of penicillin in rats.[26] A similar result was seen in freely moving rats during seizures induced by intraabdominal injection of bicuculline methiodide (Fig. 1). Additionally, since increased activation of neuronal metabolism in seizure elevates temperature, the temperature may influence epileptic discharge.

The unilateral local brain cooling of the sensorimotor cortex suppressed GMSs and bilateral epileptic discharges in the ECoG. The suppression

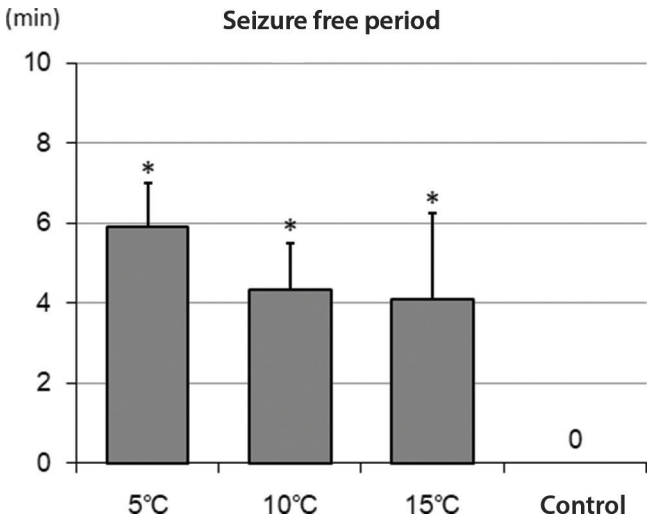

Fig. 4 Seizure free duration during the cooling period. Seizure free durations during the 10-min cooling period at 5, 10, 15°C and control temperature are indicated. Significantly longer durations of seizure suppression were achieved during cooling at the three temperatures. However, no complete termination was achieved in any group. Seizures presented continuously in the control rats. Values are shown as means ± SD; *P <0.0001 vs. duration in the control group.

was observed at the beginning of cooling, but reversed in the middle of the cooling period. The effect was less than that of focal brain cooling.[11,12] Although insufficient, mechanisms of seizure suppression by contralateral cooling, especially at 5°C, are discussed. Cooling disrupts neuronal network synchrony since Javedan et al.[27] reported that cooling a hippocampal slice to 21°C abolished the synchronization of epileptic discharges induced by bicuculline methiodide. Local brain cooling at 15°C cools the cortical layers I–IV. Therefore, epileptic discharges generated by intra- and inter-cortical networks are inhibited. In addition, pyramidal and extrapyramidal tracts projecting from layers V and VI are inhibited at 5°C.[17] To inhibit connections responsible for GMSs such as commissural fibers and ipsilateral cortico-thalamic tracts, layers V and VI have to be cooled to 5°C. This was the reason why local brain cooling at 5°C was more effective compared with cooling at 15°C. Other possibilities may be that cooling to lower temperatures stabilizes membrane properties,[28,29] changes the balance between excitatory and inhibitory neurotransmitters, and alters glial modification of neurons, although we obtained no evidence to support these speculations in this study.

The cortical temperature never reached 15°C as a result of contralateral cortical cooling at 5°C. We analyzed temperature distributions under the cooling device.[6,7] The temperature in the brain was different from that in an agar phantom because the brain is continuously warmed by heat from systemic circulation. For example, at 15°C, the temperature at a depth of 5 mm from the device is 25°C while the temperature in the ipsilateral thalamus and the contralateral hemisphere is 37°C.

Even though seizure suppression was not achieved completely and neuronal activities were temporally sacrificed, local brain cooling at 5°C may help patients with severe intractable epilepsy. The principle behind the treatment with local brain cooling is similar to that behind the administration of diazepam or barbital which terminates seizure and produces a side effect of temporal sedation. For this purpose, a device for the continuous monitoring of ECoG, an on-demand cooling device, and a detection or prediction system for epileptic discharges must be developed.

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Conflicts of Interest Disclosure

The authors declare no conflicts of interest.

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