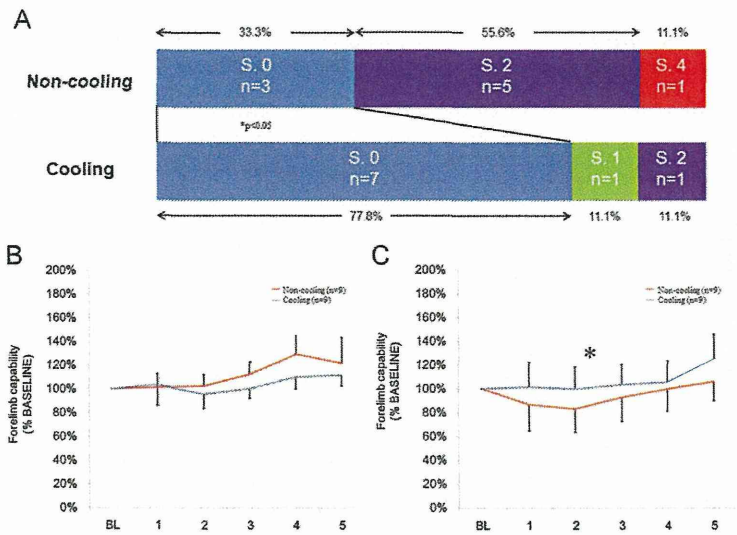

Fig. 6 – (A) Neurobehavioral test at 24 h post-ischemia, the neurologic score of the cooling group was better than that of the non-cooling group ($n=9$ $*p<0.05$ by Mann–Whitney *U* test. S. indicates scores of motor function. Score 0: best, score 4: worst. N: number of rats.). Forelimb capability (B. ipsilateral forelimb; C. contralateral forelimb) measured by a grip strength meter from 5 days before surgery to 5 days after surgery. Note that in the contralateral forelimb (bottom tracing), a significant difference was observed between the cooling and non-cooling groups at 2 days after surgery. Data are expressed as mean±SD ($n=9$ $*p<0.03$ by paired *t*-test. BL=baseline average grip strength 5 days before the surgery.)

Motor function was restored to baseline at 3 days after the ischemic event and there was no statistically-significant difference between the cooling and non-cooling groups. This phenomenon is supported by previous studies showing that most animals recover from their impaired function, more or less after brain injury because of active neuroplasticity (Alexis et al., 1995).

While the neuroprotective effect of FBC was confirmed in our study, further proof is still necessary to confirm the optimal cooling temperature, cooling period and rewarming times, as well as comparison of the efficacy of FBC and systemic hypothermia (Steiner et al., 2001; Clark et al., 2008; Colbourne et al., 2000; Yanamoto et al., 2001).

In our institute, we have initiated development of an implantable focal cooling system, including a cooling component, temperature control system, battery, and a fail-safe system. Recently, a focal cooling system, the ChillerPad™, was applied to a monkey model of traumatic brain injury and in patients with stroke or aneurysm (King et al., 2010; Wagner and Zuccarello, 2005). However, several issues remain and must be resolved from the medical engineering point of view before this neuromodulation can be used clinically. With the continued development of such apparatuses, clinical application of this implantable local cooling system may thus be realized in the near future.

4. Experimental procedure

4.1. *Animals*

Healthy adult male Wistar rats (350±50 g) housed in a temperature-controlled room (23.0±2.0 °C) were used in the study ($n=30$ in total). Animal experiments were performed using protocols that were approved by the Institutional Animal Care Committee at Yamaguchi University School of Medicine.

4.2. *Surgical procedures and the focal cerebral ischemia model*

Rats were anesthetized with sevoflurane (3% for induction via a face mask, 2% after intubation and maintenance in an 80%/20% mixture of oxygen and air by a ventilator (A.D.S.1000 Engler Engineering Corporation. USA). During the procedure, rectal temperature was monitored and kept constant at 37±0.2 °C by a temperature-controlled heating pad (NS-TC10, Neuroscience Inc., Japan). The femoral artery and femoral vein were cannulated for continuous monitoring of arterial blood pressure, obtaining blood gas samples and drug administration. Systemic blood pressure was maintained at 100–120 mmHg, and blood-gas data in the non-cooling and cooling groups were controlled at pH 7.44±0.04 and 7.43±0.03, pO_2 of 202.6±36.0 and 215.3±37 and pCO_2 of 40.7±2.2 and 40.5±3.0.

The skull was fixed using a stereotactic apparatus (SR-6N, Narishige, Japan). A scalp incision was performed at the midline following injection of lidocaine (2%), and both the Bregma and Lambda points were exposed after dissection of the pericranial tissue. A small burr hole, 3 mm in diameter, was made 4 mm lateral to the right and 0.5 mm anterior to the Bregma and the dura matter remained intact. Focal cerebral ischemia was achieved by the photothrombotic method (Yao et al., 2003; Grome et al., 1988; Fujioka et al., 2010b), illumination was initiated through the burr hole with

a fiber optic bundle of a cold light source (Fiber-Lite series 180 Dolan-Jenner Industries. Inc., USA) just after the Rose Bengal injection (1.3 mg/100 g body weight in 0.9% sterile saline) via the right femoral vein, and lasted for 20 min. In this manner, focal cerebral ischemia of the primary sensorimotor (SI-MI) cortex of the right hemisphere (2.5–5.5 mm lateral, 2.0 mm anterior, 1.0 mm posterior to the Bregma) was photochemically-induced (Fig. 1).

4.3. *Focal brain cooling*

An additional craniotomy, including the burr hole, was made over the ipsilateral SI-MI cortex (1.0–7.0 mm lateral, 3.0 mm anterior, 4.0 mm posterior to the Bregma) just after induction of the focal cerebral ischemia. A cooling device composed of a thermoelectric chip that was originally developed in our laboratory, and has been described previously (Fujioka et al., 2010a, 2010b), was placed on the SI-MI cortex (Fig. 1). We focally cooled the SI-MI cortex above the dura matter to a temperature of 15 °C for 5 h from 1 h after the development of focal ischemia. After the cooling period ended, the cortex was spontaneously rewarmed and the temperature was maintained for 1 h. The 15 °C cooling was selected because it represents the borderline temperature affecting neurobehavioral function (Fujii et al., 2012). Sham-operated rats underwent craniotomy and placement of the cooling device.

The focal cooling experiments were composed of short- and long-term studies. The short-term study evaluated the periodic epileptiform discharges in the border zone of the ischemic focus and of the infarct area. The long-term study assessed the impact on neurobehavioral function (Fig. 2).

4.4. *ECoG recordings and the spectral analysis*

We placed a pair of ball-type electrodes (impedance 500 kΩ at 500 Hz) to detect the ECoG on the cortex at the boundary of the ischemic area after placement of the cooling device (Fig. 1). Thereafter, ECoGs were recorded continuously for 8 h in two channels (monopolar recording; with a reference electrode inserted in the scalp) and analyzed using PowerLab Chart 5 software (PowerLab Chart5; AD Instruments) at a sampling rate of 2 kHz (low-cut filter 5 Hz, high-cut filter 60 Hz) for visual examination. To quantify the frequency profiles, the power spectrum was calculated by fast Fourier transform (FFT) analysis, which was performed for ECoG data in 60 s intervals at 1 h (precooling), 3 h (cooling) and 7 h (rewarming) after the development of focal ischemia (Fig. 2). We analyzed the ECoG components in all conventional frequency bands, including delta, theta, alpha and beta (delta=1–4 Hz; theta=4–9 Hz; alpha=9–14 Hz; beta1=14–25 Hz; beta2=25–30 Hz). Six rats were used in each of the cooling and non-cooling groups.

4.5. *Measurement of the infarct area*

All of the rats in both the cooling and non-cooling groups ($n=6$) were sacrificed after the recording of ECoG for 8 h. The brains were immediately removed and incubated in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC Lot KWG 6634, Wako Pure Chemical Industries, Ltd., Japan) at 4 °C for 12 h (Hatfield et al., 1991). Three serial sections from each brain were cut at 1 mm intervals from the center of the infarct zone and photographs of the sections were taken at the middle of the 3 sections.

4.6. *The impact on neurobehavioral function*

After the focal cooling for 5 h, cortices were naturally rewarmed and the temperature was maintained at the baseline for 1 h (cooling group, $n=9$). Thereafter, the scalp was closed without repairing the cranial window. The rats awakened from anesthesia were placed back in the cages. To distinguish the effect of the sensorimotor injury, appropriate control experiments were also conducted (non-cooling group, $n=9$).

Neurobehavioral function of each rat was evaluated after surgery by an observer who was blinded to the experimental procedure. Neurobehavioral function was assessed by five categories of motor neurological findings, as follows: score 0: no observable deficit, score 1: forelimb flexion, score 2: forelimb flexion and decreased resistance to lateral push, score 3: forelimb flexion, decreased resistance to lateral push and unilateral circling, and score 4: forelimb flexion, unable or difficult to ambulate (Lee et al., 2002). Neurobehavioral function was assessed 24 h after the induction of focal ischemia in the cooling and non-cooling groups (Fig. 2).

Grip strength test was also employed in the assessment of neurobehavioral function. An inverted T-type bar 63 mm in length and 103 cm wide connected to a grip strength meter (Ugo Basile Comerio (VA), Italy) was used to measure graded changes in the forelimb grip strength of the rats. Rats held the bar and were gently pulled away from it in a smooth manner, by grasping of the tail, in a steady motion, until they released the bar. The grip strength meter measured the force [g] required to break the rat's grip. Prior to the surgery, rats were trained on the apparatus for 5 days ($n=9$). Each rat was allowed to grasp the apparatus for three consecutive times, to determine the strength of the forelimbs of the left, right and both sides, respectively. The average grip strength for all patterns was used as the baseline force. From 1 to 5 days after surgery, three readings were taken for each rat and the average force required was recorded as the individual grip strength score for that rat (Fig. 2) (Bertelli and Mira, 1995).

4.7. *Statistical analyses*

The Mann–Whitney U test was used for comparison of the infarction area, neurobehavioral test between groups. The grip strength test was performed by paired t-test. The power spectrum was analyzed with one-way analysis of variance (ANOVA), followed by a LSD post-hoc test. These tests were performed using SPSS 17.0 for Windows (SPSS Inc, Chicago, IL, USA). The data are expressed as the mean±SD. p-values <0.05 were considered significant.

Disclosure

None of the authors have any conflicts of interest to disclose.

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Focal Brain Cooling: Revisiting a Potential Therapeutic Option for Intractable Epilepsy

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Abstract Seizure control is not achieved in approximately one-third of patients with epilepsy, even with the best available medications. Surgical treatment can be performed for these patients, however this is also not always successful. Under these circumstances, the potential for seizure suppression by focal brain cooling has gained attention. Brain cooling was first proposed about 50 years ago, and has come into the spotlight in recent years with advances in technology. Recent studies indicate that focal cooling of the brain to a cortical surface temperature of 20 to 25°C terminates epileptic discharges without inducing irreversible neurophysiological dysfunction or neuronal damage. These results have promoted development of implantable focal cooling devices, but some aspects of the hardware in these devices require optimization. However, advances in precision machining have enabled optimization of an implantable focal cooling system, and this suggests that brain cooling therapy may become a reality in the near future.

Key words: epilepsy, focal brain cooling, seizure, device, neuromodulation

Introduction

Epilepsy is usually treated with medication, but approximately one-third of epilepsy patients do not attain seizure control, even with the best medications.[1] Surgical treatment is also used, but is not always successful. Furthermore, surgical resection is impossible if the epileptogenic focus is in critical areas such as the motor and speech cortices. Under these circumstances, several clinical trials of neuromodulation technology for treating refractory epilepsy have recently been performed. Vagal nerve stimulation has been used for the past decade[2] and electrical stimulation of the brain has been proposed as an alternative to surgical resection. The anterior nucleus of the thalamus or hippocampus has been chosen as a stimulation target. Clinical pilot stimulation studies have been performed, but the results remain unsatisfactory.[3-5] A unique clinical study with an implantable, responsive, closed-loop stimulation system is currently in progress.[6] This device can terminate seizures by delivering a burst of stimulation after detecting a seizure with an electroencephalogram (EEG) algorithm through an implanted electrode.[7] The preliminary efficacy of this method was demonstrated in a feasibility trial, but further clinical investigation and optimization are required.

Focal cooling of the brain is another attractive and nondestructive approach for treatment of patients with epilepsy. Brain cooling was first proposed about 50 years ago as an effective method for suppressing epileptic discharges (EDs),[8,9] and has recently been revived with advances in technology and medical engineering.[10,11] At our institution, we have obtained interesting results in practical use of brain cooling as a new therapy, which we refer to as "thermal neuromodulation", for patients with intractable epilepsy.[12,13] In

this review, we discuss the historical background of focal cooling, the influence of focal cooling on epileptic seizure and the normal brain, the mechanisms of seizure termination due to focal cooling, and the practicality of use of an implantable cooling system based on our experimental data and results published in the literature.

Historical background

The therapeutic value of focal cooling initially gained attention in the 1950s. At that time, local cooling of the nervous system was achieved in animal models using perivascular methods.[14,15] Local cooling was also used to treat patients with head trauma, cancer, and pain, and the findings emphasized the utility of this method.[16] The effect of cooling on epilepsy was first demonstrated by suppression of EDs in the primate temporal lobe using systemic hypothermia.[8] Thereafter, local cooling with the gas method was shown to suppress EDs in human.[9] Ventricular irrigation with cold Ringer's solution was also found to suppress seizures.[17] Another early study indicated that systemic hypothermia suppressed seizures in patients with refractory epilepsy.[18]

Despite these initial studies indicating that brain cooling has the potential to terminate seizure activity, the method was not optimized for clinical use because of the difficulty in improving the cooling system. Initial cooling methodologies such as local refrigeration with gas and cold water or ventricular irrigation had many problems for clinical use. These methodologies increased the chance for infection and are difficult to use over long periods or permanently. Severe systematic hypothermia can suppress seizures,[18,19] but also has fatal complications including infection, cardiac arrhythmia, and blood coagulation disturbances.[20]

Focal brain cooling has recently gained attention because of advances in technology. In recent studies, evidence for an anticonvulsant effect of focal cooling has been obtained in neocortical and hippocampal epilepsy models[10,11,21-23] and in humans.[24,25] Clinically, Sartorius et al. found that focal seizure activity induced by direct cortical stimulation mapping was rapidly halted by irrigation of the brain surface with cold Ringer's solution.[26] In recent studies, including our work, a thermoelectric device has been used because of its small size and strong cooling effect.[10-13,27] This kind of focal-cooling device is implantable and can be combined with a seizure detection system.[28] Use of this technology has caused new interest in focal brain cooling as a therapy for patients with intractable epilepsy.

Inhibitory effect of focal cooling on epileptic seizure

We investigated the effect of focal brain cooling on EDs in rat neocortical and hippocampal seizure models.[12,13] A Peltier chip was used as the basis of the thermoelectric device. This chip consists of two conductors, which are connected in parallel. Passing an electric current between the conductors causes cooling of one conductor and heating of the other because of the electronic refrigeration phenomenon (Peltier effect). A heat sink made of aluminum with a water channel is attached to the chip to help dissipate the heat generated. Two silicone tubes are connected to the heat sink to circulate water through the channel.[12]

A neocortical seizure model was made in adult male Sprague-Dawley rats. After craniotomy, a cooling device was placed on the surface of the sensorimotor cortex. Kainic acid (KA) was injected into the cortex just beneath the cooled area to provoke EDs. Reduction of the temperature of the cortical surface to 30°C, 28°C, and 25°C caused the frequency of EDs to decrease as the temperature of the cortex was lowered, with final disappearance of EDs at 25°C during the cooling period.[12] Rapid termination of EDs by focal cooling of the neocortex has previously been shown in rats with 4-aminopyridine-induced epilepsy.[11] Our results are also consistent with reports showing that the optimum temperature of the cortical surface for terminating seizures is approximately 20 to 25°C.[11,27]

We also investigated the inhibitory effect of selective hippocampal cooling on KA-induced hippocampal seizures in rats.[13] Control of the temperature of the cooling site at 20°C caused significant suppression of the amplitude of the EDs. These results are also con-

sistent with previous findings.[21-23]

Influence of focal cooling on brain tissue and neurophysiological function

Focal brain cooling has an inhibitory effect on EDs and a protective effect on brain tissue.[29] However, the mechanisms underlying the influence of focal cooling on brain tissue and neurophysiological function have not been investigated in detail. Therefore, we examined the pathological and neurophysiological consequences of focal cooling in the neocortices of rats.[30] Pathologically, focal cortical cooling at -5°C for 1 hour caused irreversible histological changes that were consistent with cryoinjury. However, focal brain cooling above 0°C for 1 hour did not cause histological damage of the cortex. Yang et al. found that cooling of the rat brain to 5°C every 2 minutes for 30 seconds for a total duration of 2 hours and cooling of the cat brain to 3°C for 1-2 hours every day for 7-10 months had insignificant pathological consequences.[31] These findings agree with our results, and we also showed that irreversible neuronal damage was not caused by focal brain cooling above 0°C for 1 hour.[30]

Several studies have described the effects of cooling on the electrophysiology of the normal brain. Cooling of cortical tissue to temperatures between 0 and 20°C disrupts local synaptic activity without causing permanent injury to brain tissue.[32] The motor response is preserved after cold saline is applied for termination of EDs caused by cortical stimulation mapping.[24] Focal cooling of the somatosensory cortex in rats at 20°C for 5 minutes induces recognizable changes of somatosensory evoked potentials, but these are fully reversible after warming the tissue.[33] These studies suggest that reversible neurophysiological dysfunction is induced at a threshold temperature of approximately 20°C.

Mechanisms of seizure termination

Focal brain cooling is generally thought to reduce transmitter release,[34] alter the kinetics of voltage-gated ion channels,[21,35] and cause network desynchronization.[36] The precise antiepileptic mechanisms remain to be determined, but it is generally recognized that suppression of synaptic transmission is involved in reduction of seizures.

In our study, EDs were selectively inhibited, but motor function was preserved when the cortical surface was cooled to 20-25°C.[30] An explanation of this phenomenon is needed. An *in vitro* study showed that synaptic transmission begins to decrease below 20°C.[35] 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. Selective suppression of synaptic transmission due to a cooling-induced thermogradient in the cortex may have contributed to the vulnerability of somatosensory processing, as indicated by the reduction of receptive fields during cooling. Since the motor cortex lies deep in the sensorimotor cortex (layer V), selective transmission failure may have occurred during surface cooling.[37]

Practicality of use of an implantable cooling system

Our previous studies and those of others have demonstrated termination of EDs by focal brain cooling and indicate the therapeutic potential of this method for patients with intractable epilepsy, as an alternative to invasive surgery. Focal brain cooling may be applied for patients with an epileptic focus on the eloquent cortex (i.e., motor or language area). In our institute, we have initiated development of an implantable focal cooling system including a cooling component, an automatic electrocorticogram (ECoG) analytical system, a heat processing system, a rechargeable battery, and a fail-safe system (Fig. 1). However, several hardware issues remain to be resolved before this system can be used clinically on a large scale. First, an optimal fluid is required for use as the circulating fluid for heat dissipation. Second, the cooling device with Peltier chips requires large amounts of electricity, and development of electricity supply technology for the device is required. Third, miniaturization of the cooling device may be necessary. Smaller ancillary devices such as the electric power sup-

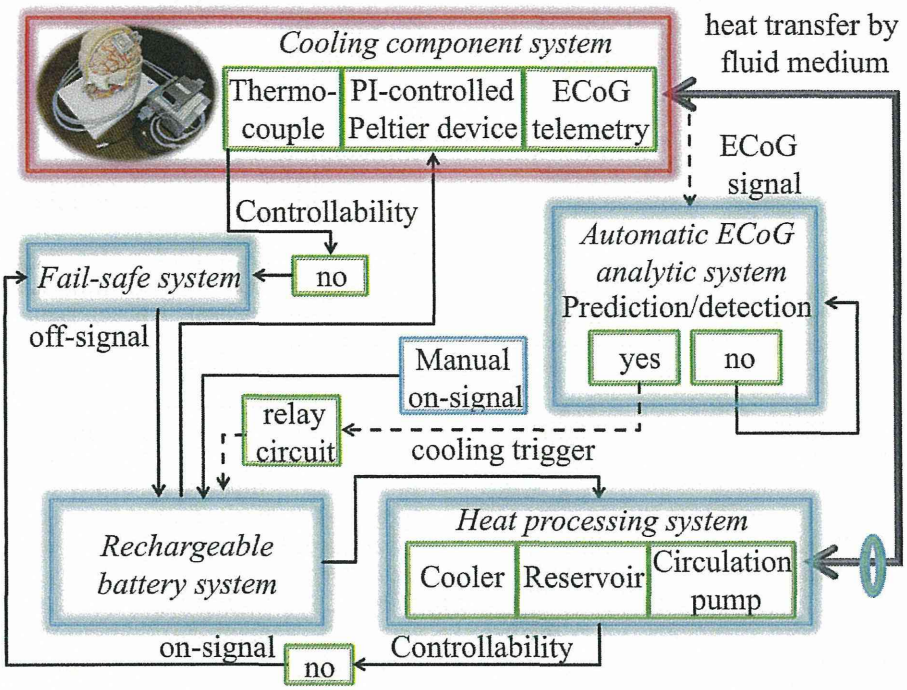

Fig. 1 Flow chart of an implantable focal brain cooling system for intractable epilepsy. ECoG; electroencephalogram.

ply, EEG detection system, and thermometer are also required. However, precision devices and micro-electromechanical technology have made remarkable advances that are likely to facilitate development of micropumps, micro-batteries, and microcharging systems. The continuing development of this equipment suggests that an implantable local cooling system may become available in the near future.

Proposal for "thermal neuromodulation"

In this review, we discussed brain cooling for treatment of intractable epilepsy. However, clinical demand for a focal-cooling device will not be limited to the epileptic field; other potential applications include treatment of cerebrovascular diseases in post-stroke rehabilitation,[38,39] neurotrauma,[38] and pain,[40] all of which depend on "thermal modulation" of neuronal excitability. Therefore, thermal neuromodulation has considerable potential as a new therapy for serious neurological disorders.

Conclusion

Focal brain cooling terminates EDs and modulates seizures. These findings have promoted development of implantable focal cooling devices with a closed-loop system (seizure detection and focal cooling) for use in neuromodulation. However, several hardware components of these devices require optimization before clinical use can be considered.

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Conflict of Interest

The author states no conflict of interest.

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光トポグラフィー装置を用いた無侵襲言語優位半球の同定法について ～Wada testとの比較～

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要旨　光トポグラフィーを無侵襲性言語優位半球同定に応用し，Wada testの結果との一致状況を検討した。対象は，13歳から81歳の患者11例（脳動脈瘤3例，脳腫瘍6例，てんかん1例，脳動静脈奇形1例），男性7例，女性4例である。課題はブロックデザインの単語想記記述課題を用いた。左右6種のROI（関心領域）を設定し，LI（Laterality Index）算定によりWada testの結果との対比から判定に至適なROIの決定を行った。その後，至適ROIのLIに基づき，光トポグラフィー法で言語優位半球の判定を行った。光トポグラフィー法の結果は，左側優位8例，右側優位2例，両側優位1例，判定不能0例であり，Wada testの結果は左側優位8例，右側優位1例，両側優位1例，判定不能1例であった。両検査法の一致率は，90%（9/10例）であった。また，光トポグラフィー法では，Wada testの実施が困難であった1例についても評価が可能であった。以上より，本法は言語優位半球の同定法として臨床応用が可能と考えられた。

はじめに

脳神経外科治療に際して，術前に脳機能局在を同定し，機能の温存を図ることは，患者のQOL保持においてきわめて重要である。そこで，近年，各種脳機能評価技術によって感覚野・運動野・聴覚野・視覚野などの同定法が確立されてきている[1~5)]。

現在実施されている言語優位半球の決定法の標準試験は，Wada test[6)]であるが，侵襲性の問題から，非侵襲性言語優位半球決定法の確率が切望されている。これを受けて，機能磁気共鳴画像（fMRI）[7)]や脳磁図記録（MEG）[8)]といった脳機能画像を中心とした言語優位性評価が試みられている。ところが，上述の技法では，認知能の低下した患者や幼児といった，安静状態の維持が困難な症例では，実施が困難である。このため，我々は，体動による影響を受けにくく，特別な設備や薬物の使用を必要としない近赤外分光法（NIRS）に着目し，光トポグラフィー（多チャンネル近赤外線分光計測）による非侵襲性言語優位半球決定法の有効性を検証した。併せて，Wada testの結果と比較し，その特性について報告する。

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対象と方法

1. 対象

対象は当院当科にて，開頭手術を施行した患者11例である。年齢は13歳～81歳（平均57歳），男性7例，女性4例である。疾患内訳は，脳腫瘍6例，脳動脈瘤3例，脳動静脈奇形1例，てんかん1例である（表1）。

本研究は，山口大学医学部附属病院治験・臨床研究

表1 症例のまとめ
Table 1 Summary of the patients

症例	性別	年齢	臨床診断
1	M	39	右中大脳動脈動脈瘤
2	F	80	右後頭部悪性膠腫
3	M	78	左蝶形骨平面髄膜腫
4	M	63	右頭頂後頭部神経膠芽腫
5	F	50	右動静脈奇形
6	F	53	右前頭葉乏突起膠腫
7	M	72	右前大脳動脈巨大動脈瘤
8	M	41	右内頸動脈巨大動脈瘤
9	F	57	左前頭葉神経膠芽腫
10	M	13	左症候性てんかん
11	M	81	右頭頂後頭部神経膠芽腫

審査委員会の承認を得て実施し，患者本人もしくは患者の判断が困難な場合には，患者家族から同意を得て実施した。

2. 方法

(1) Wada Test

大腿動脈を穿刺し，左右の内頸動脈に対して個別にカテーテルを挿入後，両腕を挙上させた状態で，1，2，3…とカウントさせながらプロポフォール（1 mg/mL）をゆっくり，麻痺が出現するまで動注した。その後，5つの物品を見せて呼称をしてもらった。優性大脳半球の評価は，物品呼称・カウント停止をスコア化し，スコアが小さい方を優位半球とした。

(2) 光トポグラフィー

課題は，定常課題として風景画の模写を30秒行った後に，標的課題として，提示したひらがな一文字に対する，単語想起記述（時間内複数回答）15秒を1セッションとする5セッションのブロックデザインを採用した。この単語想起時に提示する一文字は，その都度異なる文字を提示した。

課題の提示は，パソコン画面にWatanabeらが開発した単語想記課題ソフトを使用して行った[9]。この際，患者には絵は書きたい部分から自由に書き始めるように指示し，模写が難しいという訴えがあった場合には，絵のなかの好きな部分の四角でも三角でも良いので書くように指示を与えた。

計測は，光トポグラフィ装置ETG7100（日立メディコ社製）で行った。プローブは3×5個のプローブホルダー（縦10 cm 横16 cm プローブ接地間隔3 cm）を用いて，ホルダー内側下部をFp1，Fp2に相当する領域に，外側下部が耳の上部（T3，T4）にかかる位置に配置し，左右それぞれ22チャンネル（図1）からサンプリング周波数10 Hzで記録を行った。

得られたデータに対し，まずintegral解析（Fittingによるベース補正処理，つまりデータをTask区間毎に切り出し，PreとPost区間に対して最小二乗法近似でFitting線を引き，次にタスクに伴うHb濃度変化をFitting線からの変化として最小二乗法近似で補正し平均加算波形として表示したもの）を行った。

次に，外耳孔と眼窩下外側縁の2点を底辺とする正三角形の頂点（B点）が，area 45と一致する[11]ことを利用して，B点（本設定のROI6とほぼ一致）周辺に，図1に示す左右の6か所の関心領域ROIを設定した。この6種のROIに対して，課題遂行中のmean Oxy-Hb値から，偏性指数laterality index（LI）を算出した。LIは，課題遂行中の左右のmean Oxy-Hb値をそれぞれ，L，Rとするとき，LI=(L−R)/(L+R)の式で計算した。このとき，LもしくはRがマイナスを示した場合には，これを0とした。また，L，Rが共に0の場合のLIは，判定不能とした。

優位半球の判定は，Rutenらの判定法[12]に従い，0.25より大きい場合を左優位，−0.25以上0.25以下を両側性，−0.25より小さい場合を右優位と判定した。優位半球決定に用いる至適ROIの選定は，Wada testとの一致率が高く，MRI上area 45の近傍に位置することが確認できたものとした。

結果

測定により得られた11例の光トポグラフィー画像を図2に示す。一見して活性化の状況を確認することは可能である。しかし，実際の優位半球の判定に際しては，例え視認により判定するとしても，半球全域の評価ではなく，機能分担領域で行う必要がある。また可能な限り主観を排除した方法で判定すべきである。そこで，LIを客観的指標として用いることで機能局在部位の明示を試みた。

1. LI判定に用いるROI解析用チャンネルの決定

光トポグラフィー法による6か所のROIでの判定結果とWada testによる判定結果の一致状況を表2に示す。各ROIに対する一致率は，それぞれ，40%，

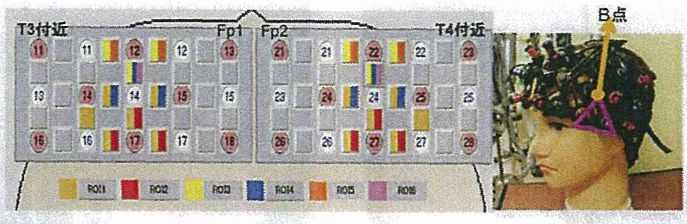

図1　プローブの配置とROIの位置関係
プローブは3×5個のプローブホルダーを用いて，内側下部をFp1，Fp2に相当する領域に，外側下部が耳の上部（T3，T4）にかかる部分に配置した。そして，図に示す6か所のROIを設定した。図に示す三角は，先行文献のプローブ配置のメルクマールであり，B点がarea 45付近と考えられている。

Fig. 1　The placement of the probe and relations of ROI. Using the 3×5 unit probe folders, medial inferior probe was located at Fp1 and Fp2, and external inferior probe was located at superior region of the ear (T3 and T4). And we set six places of ROI to show in the figure. The point B was marked on the top of equilateral triangle, and supposed to be introductive of area 45. The triangle was plotted as the prior literature[10].

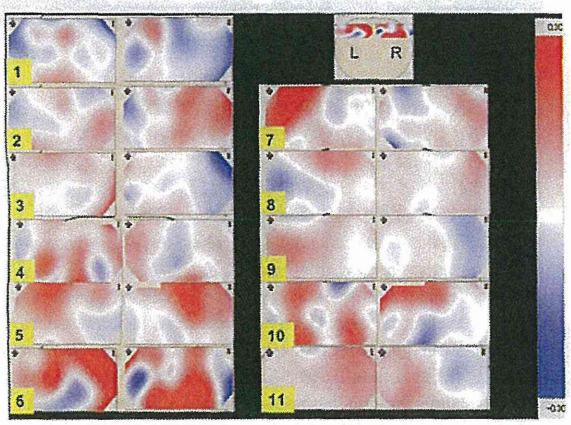

図2　課題遂行中のOxy-Hbの光トポグラフィー2D画像
integral解析後，Oxy-Hbが最大値を示した時間帯での全患者の光トポグラフィー2D画像を図に示す。一見して，優位半球を決定するのは，難しい。

Fig. 2　2D images of optical topography based on Oxy-Hb during task accomplishment. The figure shows the 2D optical topography images of all patients with maximum Oxy-Hb value after integral analysis. It is difficult to determine the dominancy at a glance of these pictures.

表2　各ROIにおけるlaterality index (LI) 値およびWada testとの一致率

Table 2　The laterality index (LI) value in each ROI and agreement rate with Wada test

	ROI1	ROI2	ROI3	ROI4	ROI5	ROI6	Wada
1	L(0.66)	L(0.78)	X(0)	L(1)	X(0)	L(1)	L
2	R(−1)	R(−0.33)	R(−0.56)	R(−1)	B(−0.03)	R(−0.81)	R
3	B(−0.09)	R(−0.6)	L(0.97)	L(1)	L(1)	L(0.61)	L
4	L(0.84)	L(0.56)	L(0.81)	L(1)	L(0.94)	L(0.5)	L
5	R(−0.42)	L(0.75)	R(−0.29)	R(−0.44)	B(−0.21)	R(−0.48)	L
6	R(−0.86)	R(−1)	L(0.58)	B(−0.12)	L(0.56)	L(0.65)	L
7	B(0.14)	L(0.30)	L(0.84)	B(0.03)	L(0.96)	B(0.12)	L
8	R(−1)	R(−1)	L(1)	R(−0.82)	L(1)	L(0.73)	L
9	L(0.77)	B(0.06)	L(1)	L(0.48)	L(1)	L(0.33)	NOT
10	L(1)	L(1)	B(−0.18)	L(1)	B(0.06)	R(−1)	L=R
11	L(0.33)	R(−0.36)	L(1)	L(0.86)	L(1)	L(0.46)	L
一致率 (%)	40%	50%	80%	50%	70%	80%	

50%，80%，50%，70%，80%であり，ROI3，ROI5，ROI6で高い一致をみた。次に課題に反応し，Oxy-Hbの増加が認められた領域が，脳皮質上どの部位に相当するかを確認するために，3例の患者に対して光トポグラフィーとMRIの合成画像を作製した。3例とも，ROI3とその構成領域であるROI5，ROI6の領

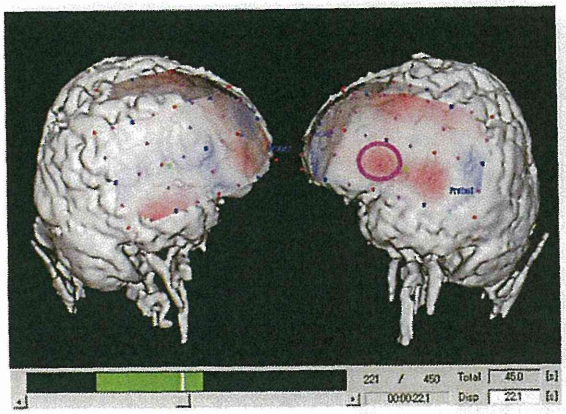

図3 課題遂行中のOxy-Hbの光トポグラフィーとMRIの合成画像
41歳男性，右内頸動脈巨大動脈瘤（判定：左優位；LI＝0.94）。integral解析後，Oxy-Hbが最大値を示した時間帯での代表患者の光トポグラフィー3D MRI合成画像を図に示す。ROI3, 5, 6に一致した左area 45近傍に血流の増加を認めた。

Fig. 3 The 3D fusion image of optical topography based on Oxy-Hb and MRI during task accomplishment. The case is a 41 year-old male with right internal carotid giant aneurysm (determination: left dominant; LI＝0.94). The figure shows the 3D optical topography images of the typical patient with maximum Oxy-Hb value after integral analysis. The cerebral blood flow (CBF) increase at ROIs 3, 5 and 6 where were close to the area 45.

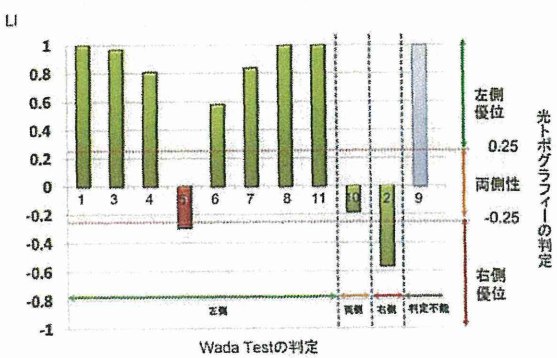

図4 各症例のWada testと光トポグラフィーの結果の一致状況
*X*軸方向にWada testの結果を，*Y*軸方向にLI値を示す。Wada testと光トポグラフィーの結果が一致したものは緑で，不一致であったものは赤で，Wada test判定不能例は青で示した。

Fig. 4 The agreement situations of each case between Wada test and optical topography. *X*-axis direction indicates the results of Wada test, *Y*-axis indicates LI values. The green bar indicates agreement of the optical topography and Wada test, the red bar indicates mismatch, and blue bar indicates inability case of Wada test.

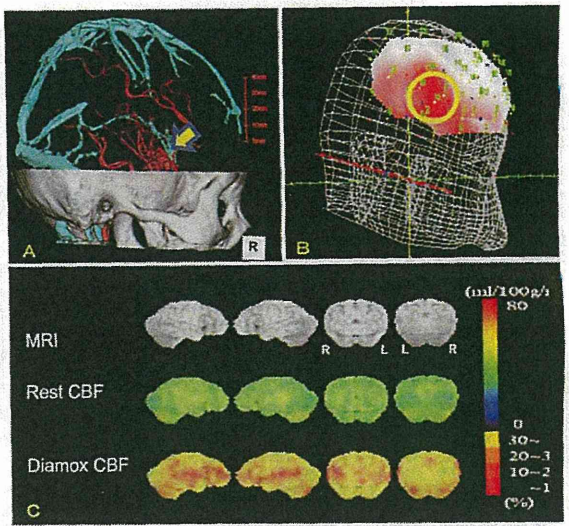

図5 判定不一致症例の脳血流評価
50歳女性，area 45に近接する動静脈奇形。
5-A：area 45近傍に異常血管が存在している。
5-B：異常血管の存在する部位の近傍に血流の増加がみられる。
5-C：ダイアモックス負荷試験においても同部位に容易に血流の増加が認められた。

Fig. 5 The cerebral blood flow evaluation of the case with determination mismatch. The case is a 50 year-old female with arterio-venous malformation (AVM) adjacent to the area 45. 5-A: An abnormal blood vessels are located adjacent to the area 45. 5-B: The CBF increases at the area of AVM. 5-C: The CBF after acetazolamide administration has been enhanced at the area of AVM.

域のOxy-Hbの増加が認められた。また，この領域はarea 45とほぼ一致していた（図3）。

以上より，一致率が高くarea 45近傍と考えられたROI3，ROI5，ROI6を至適ROIとした。また，優位半球決定は，3箇所の至適ROIの全領域をカバーするROI6から算出したLI値をもとに行うこととした。ただし，判定に際し，ROI6の波形にアーチファクトの混入がある場合には，アーチファクトの混入の最も少ない残りの至適ROIからLI値を計算し，優位半球を決定した。

2. Wada testとの比較

全症例の光トポグラフィーとWada testの一致状況を図4に示す。Wada testが実施可能であった10例では症例5を除く9/10例（90％）でWada testの結果との一致がみられた。また，Wada testの実施が困難であった1症例（症例9）においても，光トポグラフィー

では優位半球の決定が行えた。

3. 代表症例

(1) Wada testで判定の乖離がみられた症例

症例は50歳女性で，area 45近傍の動静脈奇形に対して手術を施行した。本例でのLIは−0.29で右優位半球と判定したが，Wada testでは左半球優位であった。本症例では，area 45近傍に異常血管が増生していた。また，同部位は，ダイアモックス負荷試験において，明らかな血流増加が認められた（図5）。

(2) Wada testの実施が困難であったが光トポグラフィーで言語優位半球が決定できた症例

症例は57歳女性で，左前頭葉膠芽腫の症例である。本例に対してWada testを試みたが，麻酔導入に伴い患者が不穏状態となり，実施が困難であった。そこで，光トポグラフィー検査を実施したところROI3でLIが1，ROI5，ROI6でLIが0.33となり左優位と判定した。

考察

1. Wada testの非侵襲性代替法としての光トポグラフィー検査

頭動脈内アモバルビタール法/Wada testは，危険と不快を伴う観血的な試験であり，加えて再検査が困難である。また，その評価に際しても患者の意識水準の変動，行動および情動反応により結果が不明瞭となるなどの問題を有する[13]。現在，Wada testの代替法として，言語皮質の非活性化を利用した方法，構造的非対称を利用した方法，言語作業によって直接的に事象関連脳活性化を検出する方法および言語皮質の血行力学応答を検出する方法などが報告されている（表3）。これらの報告での，RTMS（Repetitive transcranial magnetic stimulation），MRI，MEG，光トポグラフィー，PET，fMRIとWada testの一致率は，それぞれ，71%(12/17)，100%(12/12)，87%(74/85)，88%(9/11)，96%(23/24)，91%(91/100)である[8,9,14~17]。代替法に求められる絶対条件としては，一致率の高さに加えて，臨床応用にあたって各種方法の特性を考慮する必要がある。臨床的観点から，PETでは安全に投与できる放射能量が制限される。このため，時間分解能は1~2分程度で，加えて再検査が容易に行えないなどの問題がある。fMRIとNIRSは無侵襲であるので，安全面からの制限は少ない。

次に，rCBVの変化に着目してfMRIとNIRSの違いを考察する。rCBVは血管床の面積とrCBFの速さの積で表される。この点で，rCBFの増加には2種類のパターンが想定される。1つは血管床の面積の増加に起因したrCBVの増加が起こる場合である。この場合には，Oxy-Hbとdeoxy-Hbの双方の増加が見込まれる。もう1つは，rCBFの速さの増加に起因したrCBVの増加である。この場合には，流入した動脈血によりdeoxy-Hbはwash outされてしまい，Oxy-Hbの増加とdeoxy-Hbの減少が見込まれる。光トポグラフィーでは，Oxy-Hbとdeoxy-Hbの両者の増減による変化を検出可能である。これに対して，fMRIでは後者の変化をとらえたBOLD効果そのものを検出原理としているため，前者のrCBVの変化の検出は困難である。この理由により，感度においては光トポグラフィーの方が高いことが想定される。事実，fMRIにより検出不能であった脳活動を，同時記録のNIRSで検出したとする報告がなされている[10]。したがって，光トポグラフィーは，空間分解能ではfMRIには及ばないものの，時間分解能と賦活部位の検出感度において，fMRIより優勢であると考えられる。加えて，MEGやfMRIのように磁場を必要としないため，人工内耳やペースメーカー装用者への検査も可能である。

表3　各種言語優位半球決定法の原理とWada testとの一致状況

Table 3　The determination methods of language lateralization: the principle and agreement situation with Wada test

方法	判定原理	直接性	和田試験一致率	著者
RTMS	電気干渉による非活性化法	直接的	71%（12/17）	Epstein et al, 2000[14]
MRI	形態的優位性と関連	間接的	100%（12/12）	Foundas et al, 1994[15]
MEG	直接活性化と関連した磁性束密度法	直接的	87%（74/85）	Papanicolaou et al, 2004[8]
光トポグラフィー	活性化に対する血行力学反応法	間接的	88%（9/11）	Watanabe E et al, 1998[9]
PET	活性化に対する血行力学反応法	間接的	96%（23/24）	Tatlidil et al, 2000[16]
fMRI	活性化に対する血行力学反応法	間接的	91%（91/100）	Woermann et al, 2003[17]

また，NIRSは体動に比較的強い検査法であるとされ[18]，安静状態保持が困難な症例に対しても測定できる可能性を有する。我々の事例においても右後頭部に悪性膠腫を有した80歳の認知機能低下患者（症例2）および13歳の自閉症患児（症例10）でも検査が遂行でき，麻酔により不穏状態となった症例においても優意半球の決定ができた点は臨床的意義が大きい。これらを総合的に考えるとき，光トポグラフィー法はWada testの有力な代替法になりうると考える。

2. LIの判定値の設定

Rutten らはfMRIでLIを算出し，Cohen's kappaテストで0.25，0.50，0.75の場合のカットオフ値の妥当性について検討している[12]。この結果として，LI 0.25のカットオフで最善の一致が示され，強い左側方化（LI>0.50），わずかに左側方化（0.25<LI≦0.50），両側性（−0.25<LI≦0.25），弱い右側方性（−0.50<LI≦−0.25），強い右側方性（LI<−0.50）を規定した。我々は，これをもとに，LIの判定基準として「0.25」を採用したが，実際にはさらに症例を増やして0.25の妥当性の評価をする必要がある。

3. 光トポグラフィー検査の標準化

渡辺らは，外耳孔と眼窩下外側縁（infraorbital point）の2点を底辺とする正三角形の頂点（B点）を同じデータで作製した脳表像の上に投射することで，area 45の後端周辺に集まることを確認し[11]，これをメルクマールとしてプローブを配置している。今回我々は，さらに簡便な方法として図1のプローブ配置法を採用した。この部分は，渡辺らの示したarea 45を含む正三角形領域と重複しており，また彼らが設定したROIと本検討のROI3は，ほぼ一致している。本検討では，MRI画像と光トポグラフィーの合成画像を作製した3例全例でROI3にOxy-Hbの増加が認められ，9/10例（90%）でWada testの結果との一致がみられた。以上より，プローブの配置法と判定法は，適切であると考えられ，本プローブの配置法は，簡便であるため検査の標準化に寄与することが考えられた。

4. 判定乖離例から考えられる光トポグラフィーの特性

本研究においては，1例（11%）の患者にWada testと乖離がみられた。その原因は，脳動静脈奇形が右前頭側頭部に存在しており，中大脳動脈の灌流異常が結果に反映されたものであると考えられる。同様の事例として，Lehéricy らは動静脈奇形の患者の血流異常がfMRIの言語優位性を妨げることを報告している[19]。光トポグラフィー検査の誤判定については，上述の原因以外にも，腫瘍，発達時の問題に伴う言語野の偏移などが考えられる。また，Wada testとの乖離については，麻酔薬による脳機能抑制を利用したWada testに対して，言語皮質の血行力学応答を間接的に検出する光トポグラフィーの原理的違いが表現された可能性も，不一致の要因として否定できない。

結語

1. プローブ配置の簡便化とarea 45に相当する適切なROIの決定を行い，設定ROIの有効性を確認した。
2. 光トポグラフィー装置による無侵襲性言語優位半球の同定はWada testと90%（9/10例）の一致率をみた。
3. 中大脳動脈領域の病変の存在は，判定に影響を及ぼす可能性がある。
4. 本法の導入によりWada testの判定不能症例であっても優位半球の決定できる可能性が示唆された。

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Non-invasive determination of language dominance with optical topography: comparison with the intracarotid amobarbital procedure

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Background; During neurosurgical treatment, it is extremely important to localize of cerebral function to preserve cerebral function and maintain the quality of life of the patients. Recently, hemispheric dominance for language has been assessed using the Wada test in which amobarbital is injected into the carotid artery. However, this is an invasive technique with considerable risk of complications. Herein, we attempted optical topography (OT) along with the findings of the Wada test.

Methods; Eleven patients who underwent craniotomy in this hospital were tested with optical topography during a word generation task. These patients included 3 patients with cerebral aneurysms, 6 with brain tumor, 1 with epilepsy, 1 with cerebral arteriovenous malformation, who were from 13 year to 81 years of age, and comprised of 7 men and 4 women.

Word generation task: Each subject was given 15 seconds to write down as many words as possible, beginning with a randomly presented letter on a computer monitor. In between presentation of letters, subjects were instructed to focus on copying a picture for 30 seconds during which the NIRS baseline was established.

NIRS measurements: We measured the relative changes in oxygenated (Oxy-Hb) deoxygenated hemoglobin (deoxy-Hb) and total hemoglobin, which were calculated by combining the two parameters following collection of NIRS data (ETG-7100; Hitachi Medical Corporation, Tokyo, Japan) during performance of the Word generation task. We subsequently used a region of interest (ROI) and laterality index (LI). Six ROIs were set to determine the useful ROI, and the agreement rate with the Wada test was calculated. The LI, for Oxy-Hb was calculated from L and R, the sum of the concentrations for the activated ROIs over the left and right inferior frontal regions bilaterally, according to the following formula: $LI = (L - R)/(L + R)$. The LI ranged from -1 to 1, where a positive value (0.26 to 1) indicated left language lateralization and a negative value (-1 to -0.26) indicated right language lateralization. A value between -0.25 and 0.25 inclusively was considered to reflect bilateral language dominance.

Results; The results indicated a high agreement ratio in ROI 3, 5, and 6, of which 5 and 6 were included in ROI 3. We subsequently determined the language dominant hemisphere from the foregoing ROI and LI. The results based upon the optical topography were eight left-sided predominance, right predominance in two, and one case of bilateral predominance. Meanwhile, the results of Wada test were eight left-sided predominance, one case each of right and bilateral predominance, and inability to determine in one. The agreement between the techniques was 90% (9/10 case). One evaluation that was impossible by determination using Wada test was possible.

Conclusions; Therefore, this study demonstrates that OT is a feasible clinical application for the identification of the language dominant hemisphere.

Key Words : near-infrared spectroscopy, optical topography, Wada test, language dominance, non-invasive measurement

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Focal brain cooling terminates the faster frequency components of epileptic discharges induced by penicillin G in anesthetized rats

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- Epileptic discharges (EDs) in superficial layers were induced with penicillin G.
- Focal brain cooling preferentially terminated the faster frequency components of EDs.
- Frequency analysis demonstrated that cooling below 25 °C may be an effective treatment for epilepsy.

ABSTRACT

Objective: The goal of the study was to investigate the effects of focal brain cooling on epileptic discharges (EDs) and background rhythms in the sensorimotor cortex of anesthetized rats using spectral analysis of electroencephalography (EEG).
Methods: Penicillin G was administered intracortically into superficial layers of the left sensorimotor cortex and EDs were induced. Focal brain cooling was achieved using a cooling device attached to the cortical surface. The cortical surface was cooled to 25 °C, 20 °C and 15 °C, and EEG was continuously recorded just beneath the cooling device. EEG spectral powers were determined using fast Fourier transform before and during cooling.
Results: Penicillin G induced EDs and increased the Alpha and Beta power spectra. Cooling suppressed EDs with an effect that depended on the brain temperature. Cooling to 25 °C attenuated Beta powers, cooling to 20 °C attenuated Alpha and Beta powers, and cooling to 15 °C suppressed spectral powers ranging from Delta to Beta bands.
Conclusions: These results suggest that focal brain cooling can terminate EDs in the cortex and suppress spectral powers with a temperature-dependent effect.
Significance: These findings may contribute to development of a new clinical treatment for patients with epilepsy.

1. Introduction

Epilepsy is a neurological disorder characterized by recurrent brain abnormalities that result in seizures and can be detected by electroencephalography (EEG). Epilepsy is usually treated with medication, but approximately one-third of epilepsy patients do not attain seizure control (Guidelines for epidemiologic studies on epilepsy, 1993). Surgical treatment is also used, but is not always successful. Brain cooling has been proposed for suppression of epileptic discharges (EDs) for over 50 years (Baldwin and Frost, 1956; Ommaya and Baldwin, 1963; Sartorius and Berger, 1998; Yang and Rothman, 2001; Rothman, 2009). Our previous studies demonstrated that use of a focal brain cooling device could suppress EDs induced by cerebral infusion of kainic acid without causing histological damage in rats (Imoto et al., 2006; Oku et al., 2009). However, little is known about the profile of the EEG frequency spectrum during suppression by focal brain cooling.

Experimental epilepsy induced by penicillin is a classical model of epileptic activity mediated by GABA A receptor antagonism and has been widely used in animal experiments (Schwartzkroin and Prince, 1977; Chen et al., 1986; Fisher, 1989; Bertsche et al., 2010). Recently, it was reported that intracerebroventricular (i.c.v.) infusion of penicillin G potassium shifted the EEG spectral

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