プを評価したいところである．

高解像度の焦点局在診断，機能マッピングは，通常，頭蓋内電極（特に硬膜下電極）を留置して行う．機能マッピングのみなら，覚醒下手術で術中に行う方法もある．

硬膜下電極による大脳皮質電気刺激マッピング

てんかん焦点局在，機能局在ともに高解像度の情報が得られる（❸）．電極留置期間は通常4週間以内である．機能局在診断に関しては，時間的に余裕をもってさまざまな課題を施行できる．一方，頭蓋内電極留置のための手術が必要であり，留置中の感染リスクの問題がある．

■覚醒下手術による言語機能マッピング

開頭し脳表を露出してから患者を覚醒させ，電気刺激マッピングを行う．術中ゆえに時間的制約，課題の制約がある．一方で，切除を進めながら機能を確認できる点，白質線維の刺激マッピングもできる点が利点である．

■電気刺激の方法

刺激パルスは，パルス幅0.2 msec，周波数50 Hzの二相性矩形波で，刺激電流値は3〜10 mAが標準である．筆者らは，双極刺激で，1回の刺激時間を5秒間としている．

（川合謙介）

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第4章　管理・治療

迷走神経刺激療法

要旨

迷走神経刺激療法（VNS）は，てんかんに対する植込型電気刺激療法として最初に臨床応用された．欧米ではすでに補助的治療としての位置付けが確立しており，2010 年から保険適用となった日本でも徐々に普及している．左頸部迷走神経を常時刺激し，薬剤抵抗性てんかん発作を減少・軽減する緩和的治療である．てんかん分類，発作分類，年齢の制限はなく，幅広い患者で発作を約 50％減少させる．

● キーワード

てんかん
薬剤抵抗性てんかん
難治性てんかん
迷走神経刺激療法
ニューロモデュレーション

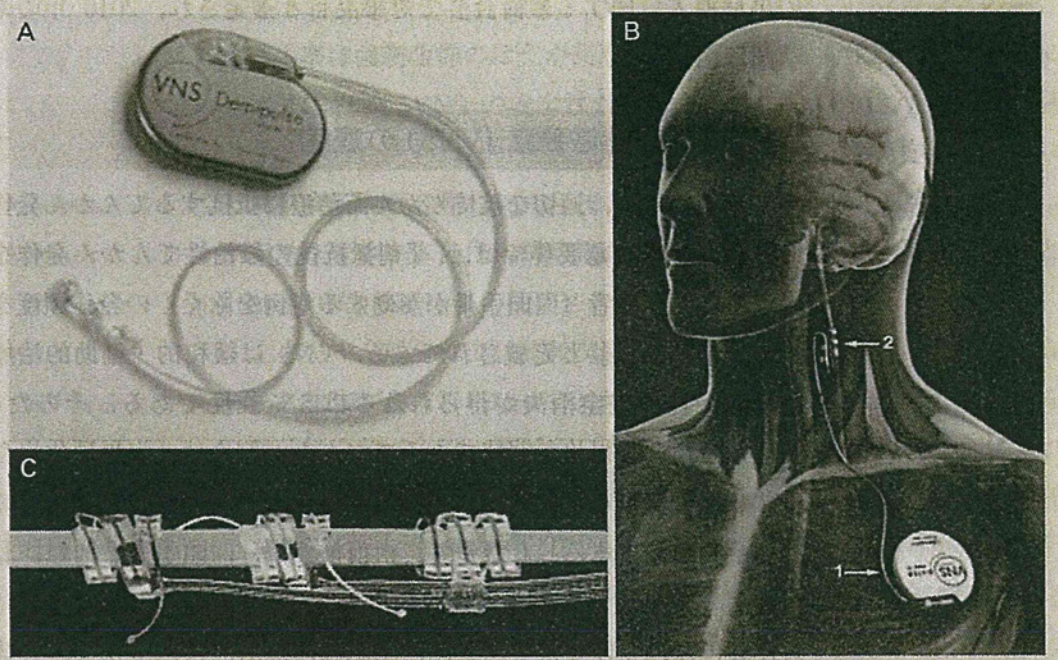
はじめに

迷走神経刺激療法（VNS）は，体内植込型の電気刺激装置で左頸部迷走神経を慢性的・間欠的に刺激して，てんかん発作を緩和する治療法である（図1）．開発から約 20 年，欧米での臨床導入から約 15 年が経過し，薬剤抵抗性てんかんに対する緩和治療としての位置付けが確立している．日本では 2010 年に薬事法の承認を得て，保険適用となった．

てんかん分類，てんかん発作分類，年齢などに適応の制限はなく，薬剤抵抗性てんかん発作に対して幅広い適応を有する．発作減少率は約 50％，発作消失率は約 5％で，治療効果は経時的に漸増する．発作減少とは独立した QOL 改善効果がある．刺激に伴う副作用は，咳・嗄声・咽頭部違和感などで，刺激条件の調整によって予防可能である．

神経疾患に対する植込型デバイスとしては最初のもので，これまでのてんかん治療のコンセプトとは，かなり異なる特徴を有する．日本でも，薬剤抵抗性てんかんに対する緩和治療として今後普及する可能性が見込まれるので，幅広い領域の臨床医を対象に，本治療法の基本的事項について紹介する．

図1 迷走神経刺激法（VNS）



A：迷走神経刺激装置一式は体内植込型の刺激装置で，ジェネレータ・らせん電極・リードから成る．
B：ジェネレータは電源と信号発生回路を内蔵しており，前胸部皮下に埋設する．約5年ごとに交換が必要である（1はジェネレータ，2はらせん電極）．
C：らせん電極の拡大図で，長期にわたり末梢神経を安定して刺激できる．左迷走神経に巻き付けて留置する．

迷走神経刺激療法（VNS）の歴史

VNS の開発者 Zabara らは，迷走神経刺激の中枢神経安定作用に着目してんかん治療への応用を着想し[1]，長期に安定した刺激を可能とする植込型の末梢神経刺激電極を開発した（図1）．動物実験を経て，1988 年にヒトへの臨床応用，1993 年には 12 歳以上の部分発作に対する多施設臨床治験と進み，1994 年に欧州で，1997 年米国で認可を受けた．1999 年の米国神経学会指針では，有効性と安全性が保証されている[2]．米国では，小児や全般発作も公的および民間保険でカバーされており，これまでに世界で行われた 60,000 件以上の VNS 植込・交換手術のうちの 90％ が米国で行われている．包括的てんかん治療において，薬剤抵抗性・開頭手術非適応症例に対する緩和的治療としての地位が，すでに確立していると言える．

日本でも 1993 年から多施設治験が行われたが，承認には至らなか

った[3]．2008 年になって厚生労働省「医療ニーズの高い医療機器などの早期導入に関する検討会」で対象品目と選定され，2010 年の薬事承認に至った．

迷走神経刺激療法（VNS）の適応

治療の対象は，適切な抗てんかん薬治療に抵抗するてんかん発作である．薬事法承認要件では，「薬剤抵抗性の難治性てんかん発作を有するてんかん患者（開頭手術が奏効する症例を除く）の発作頻度を軽減する補助療法」と定義されている．VNS は緩和的・補助的治療であり，発作の完全消失が得られる率は 5％程度である．そのため，開頭手術による根治が期待できるてんかん，例えば，海馬硬化症や海綿状血管奇形など限局性病変に伴う焦点性てんかんでは，原則的に開頭手術を優先する．したがって，術前検査は，開頭手術と同様，長時間ビデオ脳波を含めた非侵襲的焦点診断一式を行うことが望ましい．具体的には，発作症候学，脳波，脳磁図，MRI，核医学検査，神経心理学的検査などである．

適応に関して，患者の年齢，てんかん分類や発作型による制限はない．後述のように，無作為化比較試験によって有効性が検証されたのは 12 歳以上の部分発作だが，欧米では 10 年以上にわたり幅広い年齢層の患者に施行されてきており，患者群による有効性や合併症の差は認められていない．

迷走神経刺激療法（VNS）の効果

VNS の開始により直後から発作が減少する患者も存在するが，多くは徐々に発作が減少する．治療の継続により，平均的には 2 年後までに 50～60％ 減少し，その後は長期安定して発作減少した状態が続く[4]．治療 5 年後では 50％ 以上発作が減少する患者の率は約 60％ である[5]．約 5％ の患者では発作が消失する．無効な患者は 10～20％ である．

米国での承認の根拠となった臨床治験は，1990 年代に行われた 2 つの無作為化二重盲検試験で，共に多施設共同・付加的・無作為化二重盲検・実対照試験である[6][7]．VNS 治療 3 ヵ月後の発作減少率と安全性を強刺激条件と弱刺激条件で比較した結果，おのおのの平均発作

減少率は25～28％，6～15％で，発作が50％以上減少した患者の率はおのおの31％，13％であった．声の変調や呼吸苦などの副作用発生率は強刺激群のほうが高かったが，心肺・消化器の生理検査に変化は認めず，VNSの安全性が確認された．これら2つの比較試験は3ヵ月の短期評価だったが，その後の追跡評価で，発作が50％以上減少する患者の率は，1年で37％，2年で43％，3年で43％と，年単位で徐々に発作抑制効果が高まり，およそ50％に達することが示されている[8]．

小児や全般発作では比較試験は行われていないものの，多くのシリーズ報告がある[9]．これらのほとんどがLennox-Gastaut症候群など難治性全般てんかんを対象に含めているが，成人と同等またはそれ以上の有効性を報告している．さらに，細かい年齢層による効果の差や発作型による効果の差は認められていない．孤束核から広汎な上行性伝導路を介して大脳皮質をモデュレートする機構や，VNSによる血流変化が脳幹や視床にも見られることなどを考慮すると，VNSが全般性発作に対しても有効性を発揮するのは肯ける．しかし，その臨床効果は，ランダム化比較試験での検証が望まれる．

VNSは単に発作を減少させるだけでなく，発作重症度を軽減してQOLを改善する[10]．Lennox-Gastaut症候群や強直発作はVNSに反応することが多く，発作重症度，発作持続時間，発作からの回復時間の減少が期待できる．また，副次的効果として，覚醒度の上昇[11]，記憶機能の改善[12]，自覚的・他覚的な情動改善効果など[13][14]，さまざまな形でQOLを改善するが，この効果は発作抑制の程度や時間経過と必ずしも相関しない[10]．また，小児では発達改善の報告もある[15]．このような，成人および小児における認知機能・発達・QOLの改善についても後方視的シリーズ報告がほとんどなので，より科学的な検証が望まれる．

迷走神経刺激療法（VNS）の副作用

VNSの植込手術に関連する合併症は極めてまれだが，創部感染や迷走神経損傷による一過性声帯麻痺，テスト刺激に伴う一過性心停止が報告されている．感染を起した場合には，装置を抜去し，数ヵ月待ってから再植込手術を行う．装置抜去を必要とする感染の発生率は約

2％である．植込み手術操作に伴う左反回神経麻痺は，1％程度の発生率ですべて一過性である．術中のテスト刺激により，まれに一過性の徐脈や心停止が発生する．不適切な電極設置部位による頸部心臓枝の直接刺激，術野に貯留した血液や洗浄用の生理食塩液による漏電，陰陽電極を逆に留置する単純ミス，ジェネレータ本体の異常などの機構が想定されている．電極位置の補正や装置の交換などにより徐脈や心停止が消失すれば，その後問題なく刺激治療が行なえるが，消失しない場合には植込みは中止せざるをえない．その発生率は0.2％である[16]．

刺激治療に伴う副作用は，咳，嗄声，咽頭部不快感，嚥下障害などである．このような刺激に関連した症状は，可逆的で治療継続とともに減少する．刺激条件調節の際には，これらの症状が出現するよりも1段階弱い条件（電流値，周波数，パルス幅）に設定しておくと，2～3ヵ月後の次の外来診察時には1段階上げても症状が出現しなくなることが多い．

自律神経支配を受ける血圧，心拍数，Holter心電図，呼吸機能などは影響を受けない．また，VNS施行中のてんかん患者の原因不明の突然死（SUDEP）の発生率は年間4.1／1,000で，難治性てんかん患者全体のSUDEP発生率4.5／1,000よりも低い[17]．

迷走神経刺激療法（VNS）の解剖生理と作用機序

迷走神経は，運動・知覚・内臓運動・内臓知覚に対応する混合神経である．無髄で細いC線維が主体だが，VNSでは主に，有髄性で太いA線維が神経インパルスを生じ，上行性に孤束核へと伝導する．迷走神経の頸部心臓枝は，頸部迷走神経本幹または上喉頭神経から複数の枝として分かれるが，これらを直接刺激しないよう，VNSの電極は，上下の頸部心臓枝の分岐部よりも遠位（心臓側）に留置する．また，左迷走神経は房室結節から心室主体に，右迷走神経は洞房結節から心房主体に線維を送っており，左刺激で徐脈を誘発しやすいことが動物実験で確認されており，VNSでは，刺激電極を必ず左側の迷走神経に装着する．

VNSのてんかん発作抑制効果は，さまざまな動物モデルを用いて検証されてきた．用いられたてんかんモデルは，イヌのストリキニー

ネやペンチレンテトラゾル誘発けいれんモデル，サルのアルミナゲルモデル，ラットのペンチレンテトラゾル誘発けいれんモデル，遺伝性欠神てんかんラットなどであり，すでに起始した発作に対する急性の発作終息効果，刺激トレインの間欠期にも発作頻度と重症度を軽減させる急性の予防効果，慢性長期刺激の後に発作頻度と重症度を軽減させる慢性進行性予防効果，すでに抗てんかん薬を使用していてもさらに得られる付加的な発作抑制効果，などが検証された．

ラットの迷走神経を刺激すると，脳幹，間脳，終脳のさまざまな部位に，Fos タンパクが発現する．孤束核のシナプス伝導が易けいれん性に影響することが確認されており，VNS の効果はまず求心性に孤束核を経て，その後，脳幹の複数の経路を経て大脳に及ぶと考えられる．この上行性の大脳モデュレーションの経路には，ノルアドレナリン系，セロトニン系，アセチルコリン系が想定されている．VNS 施行中の患者では，大脳皮質のみならず視床や脳幹にも脳血流変化が見られ，視床を介して広範な大脳皮質活動の修飾が行われているのであろう．

大脳皮質レベルでは，VNS は広汎な安定化作用をもたらし，異常興奮性を抑制して，抗てんかん作用を発現すると考えられている．ラットの迷走神経刺激では，大脳皮質ニューロンが緩徐に過分極し，自発放電が減少する[18]．また，VNS 治療中患者の経頭蓋磁気刺激による運動誘発電位計測では，治療開始前や VNS 休止中よりも大脳皮質の局所抑制機能が上昇している[19]．

VNS 治療患者の脳脊髄液では，エタノラミンの上昇が認められ，細胞膜構成要素のターンオーバーにも影響している可能性がある．ラット VNS モデルでは，海馬の細胞新生や神経栄養因子の増大，樹状突起の形態学的複雑性の長期持続的増大が認められている[20]．VNS の臨床効果が長期漸増的に発揮されることからは，中枢神経系において何らかの形態的変化が誘発されている可能性も推察される．

迷走神経刺激療法（VNS）の実際

刺激条件の設定に入院の必要はなく，外来の診察で薬剤の調整と同様に行える．通常，0.25 mA，500 μsec，30 Hz，30 秒刺激，5 分休止，という条件から開始し，副作用の出現しない範囲で，発作に対す

る効果を見ながら1～2 mA まで電流値を上げていく．2 mA 以上の電流でも効果がない場合，副作用のために電流値が上げられない場合には，60 秒刺激，3分休止など1サイクルのうち，刺激時間の占める割合を上げていく．ただし，神経損傷を避けるため，刺激時間は1サイクルの 50％ 未満とする．至適条件は患者によって異なり，試行錯誤が必要である．

自動的な間欠性刺激のほかに，マグネットをジェネレータ表面にかざして任意に刺激を開始できる．マグネット刺激は単発なので，マグネットの電流値は通常刺激の電流値よりも1段階（0.25 mA）高くしておくと良い．こうするとマグネット刺激のときのみ嗄声・咳などの副作用が出現することになるが，多くの場合，2～3ヵ月後には出現しなくなる．

VNS 植込み患者での MRI 検査は，3テスラまでの頭部コイルを用いた頭部撮像は，迷走神経刺激を中断し推奨条件下に行えば問題はない．なお，体部コイルによる撮像は避けるべきである．家電製品，携帯電話，空港の金属探知機や商店の盗難防止センサーなどからは，VNS 装置は影響を受けない．ただし，非常に強い磁石は刺激開始や中止の誤指令を出す可能性があり，患者マニュアルでは，大きなスピーカーやバイブレーターなど，強い磁石を内蔵する機器からは，15 cm 以上離れるよう推奨している．

パルスジェネレータの電源寿命は約5年で，治療継続のためにはパルスジェネレータの交換手術が必要である．電源寿命による装置停止による発作の再発や悪化がありうるので，装置停止前に警告に従って，パルスジェネレータを交換する．有効性の得られない患者では，装置の抜去も可能である．パルスジェネレータの抜去は容易で，合併症リスクは極めて低い．一方，迷走神経に留置した電極の抜去は，神経損傷のリスクがあるが，技術的には可能である．

おわりに

VNS は薬剤抵抗性てんかんに対する有用な緩和的治療である．その特徴を十分理解し，てんかん診療の選択肢として使いこなしてほしい．

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Simultaneous Recording of Single-neuron Activities and Broad-area Intracranial Electroencephalography: Electrode Design and Implantation Procedure

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Abstract

Background: There has been a growing interest in clinical single-neuron recording, to better understand epileptogenicity and brain function. It is crucial to compare this new information, single-neuronal activity, with that obtained from conventional intracranial electroencephalographyduring simultaneous recording. However, it is difficult to implant microwires and subdural electrodes during a single surgical operation, since the stereotactic frame hampers flexible craniotomy.

Objective: We describenewly designed electrodes as well as surgical techniques to implant these with subdural electrodes that enable simultaneous recording from hippocampal neurons and broad areas of the cortical surface.

Methods: We designed a depth electrode that does not protrude into the dura and pulsates naturally with the brain. The length and the tract of the depth electrode were determined preoperatively between the lateral subiculum and the lateral surface of the temporal lobe. A frameless navigation system was used to insert the depth electrode. Surface grids and ventral strips were placed before and after the insertion of the depth electrodes, respectively. Finally, a microwire bundle was inserted into the lumen of the depth electrode. We evaluated the precision of implantation, the recording stability, and therecording rate with microwire electrodes.

Results: Depth-microwire electrodes were placed with a precision of 3.6mm. The mean successful recording rate of single- or multiple-unit activity was 14.8%, which was maintained throughout the entire recording period.

Conclusion: We achieved simultaneous implantation of microwires, depth electrodes and broad-area subdural electrodes. Our method enabled simultaneous and stable recording of hippocampal single-neuron activities and multi-channel intracranial electroencephalography.

Running title: Simultaneous Recording with Intracranial Electrodes

Key words: Depth electrode; Epilepsy; Hippocampus; iEEG; LFP; Microwire; Single-unit

Introduction

Intracranial electroencephalography (iEEG), recording the electrical activity of an ensemble of neurons using electrodes placed directly onto the brain surface or into the brain tissue, has been widely used and playsan important role in evaluating candidates for epilepsy surgery.[1-4]A unique feature of iEEGis that it achieves millisecond-order temporal resolutionand centimeter-order or finer spatial resolution, making it an ideal technique not only for detecting epileptic foci, but also for functional brain mapping. In recent years,monitoring of single-unit activity and local field potential (LFP) with microelectrodes during an epileptic seizure has begun to be investigated in the human hippocampus or cerebral cortex.These kinds of

recordings have not been popular because of its unknown benefit and possible additional risk. However, it is expected that the data from these recordings will contribute to predicting epileptic seizures and to revealing the neural mechanism underlying seizure initiation,propagation and prediction.[5-7]

Simultaneous recording ofsingle-unit activity, LFP and iEEGin animalshave shed new light on the relationship between evoked surface potential and single-neuron activity, as well as the cooperative behavior of neurons.[8,9]It is also expected that a study of the multilevel neural activities in the human brain would contribute to a better understanding of epileptogenecity and brain function, particularly of higher cognition.[8]Simultaneous recording of single-unit activities and broad-area iEEG are expected to provide more precise information of the foci and propagation of seizures.

In spite of theirpotential importance,[10] these recordings have been performed separately in clinical settings because of surgical difficulties.[11-14]While the use of frame-based stereotactic devices allows highly accurate implantation, itrestricts the size and areas of craniotomy,access to surgical fields,and intraoperative adjustment of trajectory.It is crucial to develop surgical techniques forthe flexible placement of a microelectrode with simultaneous implantation of subdural electrodes, covering abroad area of the brain surface, not only to approach the precise neural mechanisms of an epileptic seizure, but also to accommodate for various lesions associated with epilepsy.

The combined use of subdural surface electrodesand depth electrodes has been employed in many epilepsy centers.[15]While the accurate placement of a depth electrode without a stereotactic frame has proven difficult in the past, frameless stereotactic systems enable this today.[16-18]We combined these techniques and further designed new electrodes, and refined the implantation procedure to allow the simultaneous recording of single-neuron activity andbroad-area iEEG. In this technical report, we describe our electrodes and implantation procedure, along with their evaluation and representative recordings.

Methods

Subjects

Nine patients (5 males and 4 females; mean age 29.4 years; range 15 to 41 years) with pharmacologically intractable epilepsy participated in this study. Preoperative MRI findings revealedhippocampal sclerosis, focal cortical dysplasia, periventricular nodular heterotopia, cystic tumor, and non-lesional cases(Table1).

The protocols for implantation, recording and analysis described here were reviewed and approved by the institutional ethical committee of the University of Tokyo Hospital (#2954 for the development of intracranial electrodes for simultaneous recording of multichannel iEEG and single-unit activity, #1797 for research on a functional neural network by

intracranial electrodes). Written informed consent was obtained from all patients and their families.

Electrodes

We designed microwireelectrodes for single-unit/LFP recording combined with a polyamide depth electrode for iEEG recording (Unique Medical, Tokyo, Japan) (Fig. 1). While the basic concept of the electrodes draws on Behnke-Fried electrodes,[11]the main difference here was the design of a depth-iEEG electrode. We made it of polyamide, which is stiffer and less flexible than silicon. The electrode-microwirelead complex was L-shaped as a whole. Thus, handling it in the craniotomy field was easier and the positional stability after implantation was better. We fabricated the inner stylet to be attached firmly to the pointer probe of the navigation system (Fig. 1). We put a silicon fringe at the end of the electrode to prevent subduction into the brain in patients later in the series (Fig. 1C). The depth-iEEG electrode has two or six 2-mm-wide platinum contacts with inter-contact separations of 2 mm.The lengths of the depth electrodes were preoperatively determined according to the planned trajectory in 3-D T1-weighted MRIs of the subject's brain, which consisted of 136 sequential 1.4-mm-thick axial slices with a resolution of 256 x 256 pixels in a field of view of 240mm, using medical imaging software (OsiriX).For each hippocampus, we planned two depth electrodes whose tips were located in the lateral area of the subiculum in the head and body of the hippocampus. The entry point and trajectory were determined to avoid passing through the sulci. In most cases, the entry point was at the middle temporal gyrus, but in some cases it was at the superior or inferior temporal gyrus. We avoided passing through the ventricle in principle, but could not in some cases, particularly when the entry point was at the superior temporal gyrus. We chose a non-orthogonal trajectory that was as vertical as possible to the brain surface.

In addition, eightmicrowires made of 80-ìm platinum/iridium were inserted through the lumen, extending 1.5 to 5 mm beyond the tip of the depth electrode to record neuronal activity from various layers of the hippocampus or subiculum. The impedance of each microwire was in the range of 300 to 500 kÙ. We fixed the end of the microwires and depth electrodes with an instant adhesive immediately after insertion of the former into the latter, so that the whole electrode-microwirelead complexwould pulsate with the brain, with the expectation of potential long-term micro-recording stability.

For surface iEEG, we used grid, strip and trapezoidelectrodes (Unique Medical, Tokyo, Japan) with either 3-mm-diameter platinum contactsand a 10-mm separation or 1.5-mm-diameter contacts with a 5-mm separation. The number and location of the recording sites were determined solely based on clinical factors.

Surgical procedures

Under general anesthesia, the patients' headswere fixed with a Mayfield radiolucent skull clamp. 3-D-fast spoiled gradient echo (SPGR) MRIs of the subject's brain, which consisted of 1.5-mm-thick axial slices with a resolution of 256 x 256 pixels in a field of view of 240mm, was integrated into a frameless navigation system (Signa HDxt 3.0Tesla; GE Healthcare, Milwaukee,WI,USA). Then the three-dimensional spatial coordinate was registered using superficial markers and a surface mergefor a navigation system (Stealth Station S7 Navigation; Medtronic Inc, MN, USA). Following craniotomy and dural opening, subdural grid/strip electrodes for surface iEEG were temporarily placed on the surface of the brain. We intraoperatively readjusted the entry point and trajectory of the depth electrodes according to the sulcal pattern so as to spare the cortical vessels and surrounding tissues. The placements of the surface grids were adjusted as well, and holes for depth-electrode insertion were made in the grids if necessary. The depth electrode was then placed. It was advanced to the target point manually under the guidance of a navigation system. The inner stylet was removed from the depth electrode. Then, strip and trapezoid electrodes for the ventral temporal surface and parahippocampal gyrus were placed under fluoroscopy.[19] We always put the parahippocampal surface electrode under fluoroscopy so that its most anterior contact is located just posterior to the sella turcica. We believe this method enables the consistent placement of the electrode in all patients.Because this step caused a significant loss in cerebrospinal fluid (CSF), we always placed the depth electrodes before placing the ventral strip/trapezoid electrodes. After all iEEG electrodes had been placed and fixed (Fig. 1D), we gently introduced the microwires through the lumen of the depth electrode using a preset thin insertion cannula (Fig. 1C). The depth electrode and the microwires were firmly glued together with medical-grade cyano acrylate(Aron alpha A, Toa-Gosei, Tokyo, Japan)immediately after the insertion. After all the procedures had beencompleted, the dura was tightly sutured and sealed with fibrin glue to prevent CSF leaka

Electrode localiz

All pati underwe olution 3 Tesla T1-weighted volumetric MRI scans prior to electrod ion, and high-resolution 0.5-mm-slice computerized tomography (CT) scans 3 to 5.3 days) after surgery (Fig. 2A). MRI and CT images were then co-registered u 3-D constructing software (Dr.View; Asahi-kasei, Tokyo, Japan) (Fig. 2B). The location of the microwires were inferred from their relative positions to the depth electrodes.

Recording and Data analysis

Continuous video-EEG monitoring was performed from post-operative Days 1 to 25 until a sufficient number of habitual seizures were recorded.The signals recorded from the microwireswere referenced to an electrode placed againstthe internal surface of the dura or in

the intracranial space outside of the epileptic focus. The signalswere analog-filtered between 0.3 and 7.5 kHz and sampled at 30 kHz(Cerebus; Blackrock Microsystems, UT, USA). For single-unit recording, the sampled data was digitally high-passfiltered at 750 Hz, and the local waveforms for whichthe negative peak fell below the 3.7 SD of the background signal were sorted offline using the T-distribution expectation-maximization paradigm (Offline sorter; PlexonInc, TX, USA). For LFP recording, the original data sampled at 30 kHz was down-sampled at 2 kHz. iEEG was referenced to an electrode placed on the scalp or against theinternal surface of the dura, filtered between 0.55 and 150 Hz and sampled at 400 Hz (NicoletOne, Care Fusion, CA, USA). The in-house Matlab (MathWorks, Natick, MA, USA) programs, with statistical toolbox, signal processing toolbox, and the open source Matlab toolbox EEGLABwere utilized for data analysis.[20]

Evaluation of the procedures

To assess the feasibility of our procedures, we examined the data in four ways. First, wecalculated the spatial error of our frameless-navigated implantation surgery. Three-dimensional coordinates of the target point, which was retrieved from the stored data of the navigation system, and the implanted point, which was determined from the overlay image of postoperative 3-D-CT, were measured; the gaps were calculated and then plotted on a 2- or 3-D display(Figs.2C, 3).Second, we evaluatedthetemporal stability of single-unit recording. The recording yield of the microelectrode was divided and analyzed every 5 days and then tested for significant differences among each recording period with the Kruskal-Wallis test. Third, we confirmed whether simultaneoussingle-unit, LFP and iEEG recording were successfully performed or not. The sorted single-unit data were transformed into a firing rate for a period of approximately 65 min and displayed as a histogram. iEEG data was Fourier-transformed for the correspondingperiod, which was worth 131,072 samples. These data and the raw LFP signal were displayed and compared in the same time series. Fourth, we recorded visually evoked responses and confirmed whether our methodology was applicable for cognitive study or not. The patients performed a familiar face detection task while gray-scaled photographs of 36 different faces, including four familiar faces of the patients' sister, father, mother and grandmother, were presented in random order on a PC monitor at a viewing distance of 100 cm. Each stimulus was presented for 1000 milliseconds (ms), followed by a 3000 ms interval period,using a Stimuli Output Sequencer (NoruPro Light Systems Inc., Tokyo, Japan).

Results

Post-operative CT revealed that depth-microwire electrodes implanted with a frameless navigation system were placed within a margin of error of 3.6 ± 2.3 mm (mean ± SD) (Figs. 2, 3). No surgical complications (e.g.,intracranial bleeding, CSF leakage,infection) were noted in anypatients in this series. We recorded single-unit activity, LFP and iEEG from 9

subjects in a chronic and/or subacute phase. One subject was excluded from recording rate and temporal stabilityanalysis because of damage to the ground/reference wire. The rates of successful recording of single- or multiple-unit activity with microwires ranged from 0 to 40.6% (mean: 14.8%)(Table 1). In the evaluation of recording stability, no significant differences were observed in the recording rate among each recording period (P=0.65; Kruskal-Wallis test) (Fig. 4). Finally, we compared the interictal-ictal neural activity recorded with a singleunit, LFP and iEEG in a local brain region of one patient(Fig. 5). In this epileptic seizure, firing rate and LFP, recorded from the microwire, and spectral power showed increasing activity prior to the clinical seizure onset. The earliest change was observed inLFP (Fig. 5, second row),followed by an increase in firing rate. We observed a trough in both firing rate and LFP immediately after the first local peak.

We also recorded a visually evoked single-unit response in some of the patients. Figure 6 shows a representative response recorded from the microwire in the hippocampus of Subject 2. This neuronal activitywas in response to the photograph of a particular family member, but not to those of the other members. The latency of the peak response was 400 ms, and the firing rate at the peak exceeded the mean + 2 SD of the rate in the pre-stimulus period.

Discussion

Single-unit recording hasa high temporal resolution of up to 30kHz, allowing us to evaluateunitary output events of neuronal activity.Although the studies using single-unit recording were applied to the very restricted regions of the brain, ithas provided fundamental and important information on the mechanism of human brain function.[21-25] By contrast, iEEG can cover a broad area of the cortical surface,allowing us tolocalize the functional and diseased area,as well as to clarify the association of multiple areas and the spread pattern of the disease.[26,27]To elucidate the relationship between the single-unit and ensemble activity of neuronsis crucial but has not been examined in humans because of technical difficulties.

In the present study, we fabricated a depth-microwire electrode for use with aframeless navigation system, which enabled the simultaneous placement of broad-area subdural electrodes. While the well-known Behnke-Fried electrodeshave already been commercially available in the United States, they were designed to be implantedwith a stereotactic frame and to be fixed to the skull. A frameless navigation system enabled us to implant depth-microwire electrodes at desired positions in a surgical setup without a stereotactic frame.We made our depth electrode shorter and stiffer for more precise implantation without bending of the shaft. We made the depth-microwire lead complex L-shaped so as to enable it to sit under the dura without protruding from the brain surface, with the expectation of easier handling in the craniotomy field and a natural pulsation with the brain. The expectations were achieved in two aspects. First, there was no complication associated with depth-electrode placement. Second, recording stability was maintained for longer than 3 weeks. Thislong-term stability

provided us enough time to record a sufficient number of habitual seizures and for the subjects to perform cognitive tasks. Some hippocampal neurons showed a preference for a particular member of the family. These characteristics matched the property of previously reported human hippocampal neurons.[28,27]

In terms of the implantation procedure, we paid special attention to the following three points: First, the trajectory of the depth electrodeswas determined to completely avoid the sulci and to be as vertical as possible to the brain surface in order to make the length of the electrode shorter and to decrease placement error. Second, we made an effort to minimize CSF loss and, thereby,the subsequent brain shift that affectsthe spatial accuracy of the depth-electrode implantation. We always placed the temporobasal electrodes after placing the depth electrodes, since placement of the former causes significant CSF loss.While the opening of the dura and placement of the surface grid before the depth electrodes did not cause significant CSF loss as long as they were performed smoothly and promptly, we adjusted the tilt of the bed and paid attention to the cotton used in the surgical field, ensuring that it did not cause a siphon effect. Third, it was important to determine the final entry point and implant the depth electrodes after placing the grid electrodes on the lateral temporal surface. We were afraid that placement of the depth electrodes in the first step of the procedure would increase the risk of extra injurious force to the brain and insufficient placement of surface grid electrodes.

In comparison with a frame-based stereotactic procedure, the spatial error of 3.6 mm waslarger. At the start of the present study, our objective was to place the tip of the microwires in the neuronal layers, and we had assumed that adepth-tip spatial error of up to 5 mm was acceptable because we could not control how the microwires would protrude and spread from the tip of the depth electrode. Therefore, we did not employ the Point Setter arm, which may have allowed forthe more steady advancement of the depth electrode and a smaller spatial error.[16] However, since the error was distributed almost equally in all directions, we attributed it mainly to the manual advancement of the depth electrode. If the error had been caused by brain shift, it would most likely have deviated in one direction. The employment of the arm may be efficient, particularly when the target is set in a specifically restricted region within the hippocampus. We are preparing such a system and will verify whether it improves spatial accuracy and the rate of successful micro recording.

Other factors that influence the micro-recording rate may include the learning curves of the implantation procedure and recording procedure, particularly regarding noise reduction techniques,microelectrode properties, and the nature of the target neuronal areas. We found a tendency in patients later in the series; namely, they showed higher recording rates and spatial accuracy even though we were using the same electrodes. Our microelectrodes had a larger diameter and a tapered tip with higher impedance than a Behnke-Fried electrode. An appropriate diameter and impedance must be determined in the future. Micro-recording from

the primary motor cortex using the Neuroport system renders a higher recording rate.[29]Since itintegrates an amplifierinto the electrode, providing a higher signal-to-noise ratio, we must clarify whether the employment of the technology improves the recording rate. We must clarify,as well,how differences in neuronal density, and distance from the surface, affect the recording rate.

Although increasing the type and number of electrodes may theoretically lead to a possible increase in complications, such as intracranial bleeding or infection, we did not experience such complications in the present series.However, we were able to confirm the safety of this techniquein a larger number of patients. Furthermore, recording stability for a much longer period than one month may be required in the future for use in epilepsy treatment and brain-machine interfaces. The presently available electrodes, including ours, are not sufficient for that purpose.[30]The effort to develop next generation electrodes must be continued.

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