VI. 班構成員名簿

「反復磁気刺激によるパーキンソン病治療の確立研究班」

平成20年度　班員

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Ⅶ. 業 績 別 刷 り

脳波・筋電図の臨床

Triple stimulation techniqueを用いた脊髄小脳変性症，パーキンソン類縁疾患での皮質脊髄路機能の検討

Triple stimulation technique in patients with spinocerebellar ataxia and parkinsonism

佐久間研司* 村上 丈伸 中島 健二**
SAKUMA Kenji MURAKAMI Takenobu NAKAJIMA Kenji

古典的な臨床神経生理学的手法を用いた皮質脊髄路機能評価法は感度が低い場合がある。
Triple stimulation technique（TST）は下降性 volley の非同期性を回避することができ，上位運動ニューロンの異常をより顕著に検出できる。
これまで客観評価の困難であった皮質脊髄路の機能異常の検出においてTSTは有用であり，変性性神経疾患，免疫性神経疾患の鑑別および治療選択に有効である可能性がある。

KEY WORDS Triple stimulation technique（TST），SCA6，PARK2，皮質脊髄路機能

はじめに

臨床的には皮質脊髄路機能異常は腱反射の亢進と病的反射の出現によって判断をする．皮質脊髄路機能異常を客観的に評価する試みはいくつか行われている．臨床神経生理学的手法を用いた皮質脊髄路機能評価法としては中枢伝導時間（central motor conduction time；CMCT）が古典的に用いられているが，この手法は直接的な上位運動ニューロンの機能障害を反映しないとされており，CMCTは筋萎縮性側索硬化症（ALS）のような上位運動ニューロンの変性疾患の病期進行に応じて延長する傾向を呈さないことが報告されている[1]．またALSにおいてCMCT異常が認められるのは13.4%に過ぎないとの報告もあり[2]，その感度の低さも問題である

経頭蓋磁気刺激（Transcranial magnetic stimulation；TMS）で発生させた運動誘発電位（motor evoked potential；MEP）で最大上刺激が可能であれば大脳皮質刺激のMEP振幅と末梢神経刺激による複合筋活動電位（Compound muscle action potential；CMAP）振幅の比は正常では1となり，異常があれば定量化できるはずである．しかし，実際は正常でもMEP amplitudeとCMAP amplitudeの比は20～70%となってしまう．では，TMSでは最大上刺激ができていないのかというとそうではなく，1983年のMarsdenの報告では，筋収縮力で比較すると，運動や刺激でも末梢神経刺激でも差はないので，実際はTMSでも最大上刺激はしている[3]．しかし，TMSではMultiple descending volleyが発生するために時間的分散が

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起きて振幅低下が起きてしまう．さらにALSなどの病的状態では特に下降性volleyの非同期性が増大することも報告されている．このMultiple descending volleysの影響がなくなれば単純な振幅比で皮質脊髄路機能を定量化できるだろうとして考えられたのが1998年にMagistrisにより始めて報告されたTriple stimulation technique（TST）である[1]．IMSでは避けられない下降性volleyの非同期性を回避することができること，上位運動ニューロンの異常をより顕著に検出できる中枢伝導時間の計測などによる方法と比較して2.75倍の検出率があるとされる[5]．本稿ではTSTを脊髄小脳変性症，パーキンソン類縁疾患に応用し，皮質脊髄路機能異常を検出しその病態生理について考察する

脊髄小脳変性症，パーキンソン類縁疾患での皮質脊髄路機能の検討

P/Q型Caチャンネル遺伝子のCAGリピート伸長が確認された脊髄小脳失調症6型（SCA6）7例（56－77歳；68.4±7.0歳），Parkin遺伝子変異が確認されたPARK2 3例（63－67歳；64.3±2.3歳）と年齢をマッチさせた健常成人（35－84歳；66.0±15.8歳）を対象とした．

被験者はベッド上で仰臥位とし，不必要な筋収縮を防ぐために右3～5指をテープで固定し，さらに前腕部には2kgの砂嚢を置いた．前腕部は10×40cmの固定板上にベルクロテープで固定した．CMAPの記録は0背側手骨間筋から行った．筋電図は5Hz～10kHzのband passフィルターで取り込み増幅した．ADコンバータ（micro 1401；Cambridge Electronic Design）を介して1試行につき刺激前25msと刺激後400msを取り込み，保存した．

TSTはIMS，Erb's点電気刺激，手首部末梢神経電気刺激を適切な時間差で組み合わせることにより皮質脊髄路機能を定量する方法である．IMSは8の字コイルを用い左大脳半球の手の運動野に与えた．Erb's点電気刺激はErb's点をカソードとし肩甲上窩をアノードとし1.5×2.0cmのディスポーザブル電極で刺激を与えた．手首部末梢神経電気刺激は尺骨神経手首部をバー電極で刺激を与えた．それぞれの刺激のタイミングは3チャンネルの外部タイマー（SEN-7203, Nihon Koden）を用いて行った．最初に運動野へのIMSを行い，その下行性volleyが手首部に到達する直前の時間を見計らって（Delay Ⅰ）手首部を刺激した．さらに手首部刺激で発生した上行性volleyがErb's点に到達する直前を見計らって（Delay Ⅱ）Erb's点を電気刺激した．各々の時間差は以下の計算式で決定した[4]

Delay Ⅰ＝MEP潜時－手首部刺激CMAP潜時

Delay Ⅱ＝Erb's点刺激CMAP潜時－手首部刺激CMAP潜時

対照としては第1刺激をErb's点刺激に置き換えて得られた振幅比でTSI ratioを計算した．

また，古典的な皮質脊髄路評価法としてのCMCTは以下の計算式で行いTSIとの比較を行った[6]

CMCT＝MEP潜時－（手首部刺激CMAP潜時＋手首部刺激F波潜時－1）/2

結　果

TSI ratioはSCA6では0.77±0.15と健常対照群の0.95±0.11と比べて有意に低値であった（図1，2）．CMCTはSCA6では7.1±1.7msに対して健常対照群では6.1±1.2msと有意差はなかった．PARK2においては検索した3例全例においてTST ratioは有意に低値であった．疾患対照として計測したパーキンソン病7例のうち6例ではTSI ratioは正常であった（図2）．

考　察

今回検討したSCA6とPARK2においては錐体路障害は主たる症候とは考えられないが，こうした疾患においてTSTを用いて客観的に皮質脊髄路機能障害を検出しえたことが新しい．第1刺激であるTMSはdescending volleys同期性が十分でな

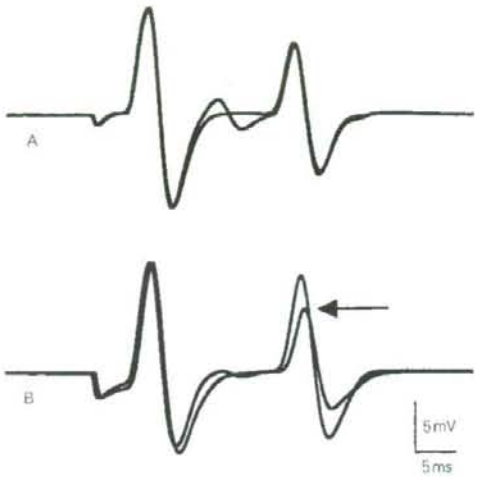

図1　健常対照（A）とSCA6患者（B）から記録したTSTの典型例

この図では第1刺激をErb s点にしたTST control curveと第1刺激を大脳皮質運動野へのTMSにしたTST test curveを重畳してある．健常対照では両者は完全に一致し，TST ratioは0.96であるが，SCA6では0.66となる（矢印）．ISTでは2つのピークをもつ波形が得られる．第1のピークは第2刺激の末梢神経手首部電気刺激による第1背側手骨間筋から導出したCMAPである．第1刺激のTMSによるMEPは第2刺激により相殺されるので，第1背側手骨間筋からは導出されない．第2のピークは第3刺激であるErb's点を電気刺激した場合の第1背側手骨間筋から導出したCMAPである．この第2のピークは皮質脊髄路機能障害時には振幅が低下（矢印）する．第1刺激をErb's点電気刺激に置き換えたものを対照として振幅比（TST ratio）を算出する．

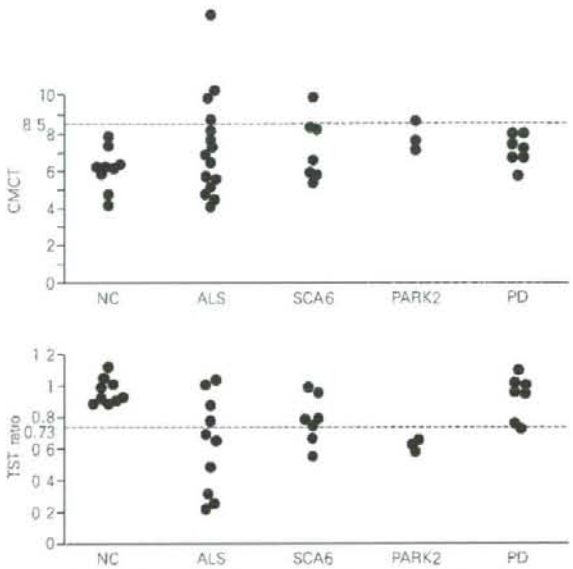

図2　各疾患におけるCMCT（<8.5）とTST ratio（>0.73）

SCA6およびPARK2においては有意にTST ratioが異常である．疾患対照のPDではPARK2に見られる異常はない．点線はCMCTおよびTSI ratioにおいてそれぞれmean＋3SDおよびmean-3SDをあらわす

いため単純にその振幅で皮質脊髄路機能を定量することは実際上できない．しかし，この3者刺激を行うことでMultiple descending volleysの要素が相殺され振幅比で皮質脊髄路機能を定量できる結果としてTST異常は上位運動ニューロン数の減少，中枢性軸索障害，中枢性の伝導ブロック

TMSに対する低応答性を反映する[4]. TSTの欠点としては3チャンネルの刺激制御装置など一般の臨床現場に常備されていない機器が必要なことやErb's点刺激において上肢全体が飛び跳ねるように動くため，被験者に不快感を与えることがあるので，前もって十分に患者に説明が必要な点があげられる．欧米ではTSTがプログラムを組み込まれた臨床筋電計が発売されており，今後のルーチン検査としての普及が期待される

1．SCA6における皮質脊髄路機能異常

SCA6は電位依存性カルシウムチャネルのα1Aサブユニット遺伝子内（CACNA1A）のCAGリピートの異常伸長により診断され，平均発症年齢は45歳（20～66歳）である．臨床的には，ほぼ純粋に小脳症状のみを呈する（pure cerebellar syndrome）とされており，MRIで小脳虫部および上面の高度の萎縮が認められる．しかし錐体路症状が33%から53%に認められると報告されている．病理学的には小脳皮質でのプルキンエ細胞の脱落，顆粒細胞の軽度脱落，分子層の軽度のび薄化が報告されており，障害が皮質脊髄路に及んだとの報告はない．それでは臨床的に，また，今回検討したTSTで明らかな錐体路障害がとらえられるのはなぜだろうか．そこでCrossed cerebellar diaschisisによる運動野の機能障害とCACNA1A mutationsが運動野の機能障害をきたすという2つの仮説を立てて考察する．

Crossed cerebellar diaschisisはテント上の局在性の病変，多くは脳梗塞により反対側の小脳の血流あるいは代謝の低下をきたすものである[1]. SCA6では大脳の糖代謝の低下がPETで示されたとの報告があり天幕下に主病変があってもreversed crossed cerebellar diaschisisとして運動野の機能障害をきたす可能性があり，今回の皮質脊髄路機能異常となったと考えられる[8][9].

もうひとつの仮説としてCACNA1A mutationsとの関連も注目される．CACNA1A mutationに関連する疾患のひとつとして家族性片麻痺性片頭痛がある．前兆を伴う片頭痛の一種として分類され，片頭痛発作の前兆として錐体路障害，すなわち片麻痺があるのが特徴である．1996年にOphoffらによりP/Q-type Ca2＋ channel α1 subunit (CACNA1A)の点変異であることが証明された．

もうひとつのCACNA1A mutation関連疾患としては反復発作性失調症2型がある．これは発作性の小脳失調を呈する疾患であり6週から40歳で発症し優性遺伝形式をとる．ストレスや運動，コーヒーなどの飲用がtriggerとなる．めまい，眼振，頭痛をきたすものもあり，頭痛，失調が固定するものもある．画像では小脳虫部の萎縮を認める．これら，CACNA1A mutations関連疾患3種のphenotypeには似た部分があることがわかる[10][11]．すなわち，家族性片麻痺性片頭痛のような錐体路障害の症状をきたすものの存在からCACNA1A遺伝子そのものに錐体路に障害をきたす性質が存在することを推定させる[12].

2．PARK2における皮質脊髄路機能異常

PARK2はparkin遺伝子変異による疾患で常染色体劣性遺伝形式の若年性パーキンソニスムを呈する．ParkinはE3ユビキチンリガーゼとして機能し，その障害はユビキチン－プロテアソーム系の障害をきたすことが知られている．通常のidiopathic Parkinson diseaseとの相違点としてPARK2では発症年齢が若いこと，腱反射亢進が40%に見られること，足のジストニアが多く，認知症がまったくない，感覚障害を認めること，睡眠効果といった特殊な症状変動を認めること，L-Dopaの効果がパーキンソン病より良いこと，進行が遅く，Yahr重症度も低値であることがあげられる．

イタリアのグループが最近4例のPARK2においてCMCTを測定したところ3例で異常であったと報告している[13]．われわれの検討ではそこまでの異常はCMCTでは検出しえないが，同様に皮質脊髄路機能異常を考える意味で興味深い結果といえる．

病理学的にはPARK2では黒質以外にも変化が見られることが判っている．大脳皮質や脳幹に神

経原線維変化，銀親和性のアストロサイトが見られるとの報告 spinocerebellar system の細胞脱落 Meynertやamygdalo-hippocampal regionにLewyが出現すると言う報告 MRIで内包後脚にT2高信号を認めた例等の報告があり[13]，少なくともパーキンソン病とは異なる病理変化が存在する疾患対照のパーキンソン病では明らかでない皮質脊髄路機能異常はこのような病理変化を反映しているものと考えることができる

3．TSTの展望

TSTはALSや多発性硬化症の診断および評価に用いた研究報告が多い 特殊な応用例では通常の方法では伝導ブロックの検出できない多巣性運動ニューロパチーにおいてTST法で近位側の伝導ブロックを確認したとの報告があり これまでは軸索型の多巣性運動ニューロパチーと考えられていた症例で免疫グロブリン大量静注療法が著効したとの報告がある[14] またMSA-pでは70%で異常 IPDとの鑑別に有用との報告もある[15]

これまで客観評価の困難であった皮質脊髄路の機能異常の検出においてTSTは有用であり 変性性神経疾患，免疫性神経疾患の鑑別および治療選択に有効である可能性がある

本稿で述べた要旨は第36回日本臨床神経生理学会(2006年11月 横浜)において発表した

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RESEARCH ARTICLE

Changes in somatosensory-evoked potentials and high-frequency oscillations after paired-associative stimulation

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Abstract Paired-associative stimulation (PAS), combining electrical median nerve stimulation with transcranial magnetic stimulation (TMS) with a variable delay, causes long-term potentiation or depression (LTP/LTD)-like cortical plasticity. In the present study, we examined how PAS over the motor cortex affected a distant site, the somatosensory cortex. Furthermore, the influences of PAS on high-frequency oscillations (HFOs) were investigated to clarify the origin of HFOs. Interstimulus intervals between median nerve stimulation and TMS were 25 ms (PAS_{25}) and 10 ms (PAS_{10}). PAS was performed over the motor and somatosensory cortices. SEPs following median nerve stimulation were recorded before and after PAS. HFOs were isolated by 400–800 Hz band-pass filtering. PAS_{25} over the motor cortex increased the N20–P25 and P25–N33 amplitudes and the HFOs significantly. The enhancement of the P25–N33 amplitude and the late HFOs lasted more than 60 min. After PAS_{10} over the motor cortex, the N20–P25 and P25–N33 amplitudes decreased for 40 min, and the HFOs decreased for 60 min. Frontal SEPs were not affected after PAS over the motor cortex. $PAS_{25/10}$ over the somatosensory cortex did not affect SEPs and HFOs. $PAS_{25/10}$ over the motor cortex caused the LTP/LTD-like phenomena in a distant site, the somatosensory cortex. The PAS paradigms over the motor cortex can modify both the neural generators of SEPs and HFOs. HFOs may reflect the activation of GABAergic inhibitory interneurons regulating pyramidal neurons in the somatosensory cortex.

Keywords Somatosensory-evoked potentials (SEPs) · High-frequency oscillations (HFOs) · Paired-associative stimulation (PAS) · Somatosensory cortex · Plasticity

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Introduction

Plasticity in the human motor cortex can be elicited using an intervention shaped after a model of associative long-term potentiation (LTP) in experimental animals (Stefan et al. 2000). Median nerve stimulation is paired with transcranial magnetic stimulation (TMS) over the contralateral motor cortex representing the abductor pollicis brevis muscle (APB). This protocol, termed paired-associative stimulation (PAS), rapidly induces a long-lasting, reversible, and topographically specific increase in motor-evoked potentials (MEPs), when applied repeatedly. Additional studies have found that the LTP or long-term depression (LTD)-like phenomena of the MEPs depended on the interstimulus intervals (ISIs) between the two modalities, and this LTP was blocked by an *N*-methyl-D-aspartate (NMDA) receptor antagonist (Stefan et al. 2002; Wolters et al. 2003; Ziemann et al. 1998). PAS over the somatosensory cortex could modulate the cortical components of the somatosensory-evoked potential (SEP), and these effects also depended on the timing of the stimulation (Wolters et al. 2005).

Low-amplitude, high-frequency oscillations (HFOs) of 500–800 Hz superimposed on the ascending slope of the N20 primary response following stimulation of the median nerve have been reported (Curio et al. 1994; Hashimoto et al. 1996). Several candidates for the generator of HFOs

have been proposed, including the thalamus (Eisen et al. 1984; Klostermann et al. 2002), the thalamocortical presynaptic action potentials (Gobbele et al. 1998), and the somatosensory cortex (Curio et al. 1997; Hashimoto et al. 1999; Sakuma and Hashimoto 1999; Sakuma et al. 1999, 2004; Shimazu et al. 2000), but the precise anatomical location of the generator remains unclear. Hashimoto and colleagues hypothesized that HFOs represented a localized activity of the GABAergic inhibitory interneurons in layer 4 of area 3b, whereas the N20 component was considered to be generated by excitatory postsynaptic potentials (EPSPs) of pyramidal neurons (Hashimoto et al. 1996).

Previous PAS studies reported changes in synaptic efficacy in the stimulated site (PAS over the motor cortex via MEP assessment, PAS over the somatosensory cortex via SEP assessment). In the present study, we investigated the changes in synaptic efficacies in a site distant from the stimulated site (PAS over the motor cortex via SEP, HFO assessment), comparing them to the effect of PAS over the somatosensory cortex. We hypothesized that the LTP/LTD-like phenomena obtained by PAS over the motor cortex occurred not only in the motor cortex but also in the ipsilateral somatosensory cortex, because dense cortico-cortical connections exist between the motor and somatosensory cortices (Enomoto et al. 2001). Furthermore, we investigated how HFOs were influenced by the bidirectional LTP/LTD-like phenomena following PAS to clarify their anatomical and physiological origins.

Materials and methods

Subjects

We studied a total of 54 right-handed healthy volunteers (23 women, 31 men), 20–39 years of age (mean age, 24.4 ± 3.6 years). None had a history of physical or neurological illness. Some subjects took part in more than one experiment. This study was approved by the Human Ethics Committee of Tottori University and was conducted in accordance with the Declaration of Helsinki. All participants gave their informed consent prior to participation.

PAS protocol

PAS consisted of combined electrical median nerve stimulation and TMS over the sensorimotor cortices (Stefan et al. 2000; Wolters et al. 2005). For peripheral nerve stimulation, electrical stimuli 0.2 ms in duration were delivered to the right median nerve at the subjects' wrists. The stimulus intensity was set at three times the sensory threshold. Peripheral nerve stimulation was followed by TMS. TMS was applied using a Magstim 200 stimulator (Magstim Co., Whitland, Dyfed, UK) and a figure-of-eight coil with external loop diameters of 80 mm. The coil was held tangentially to the skull, with the handle pointing backward and laterally at a 45° angle to the sagittal plane. In the "PAS over the motor cortex" paradigms, the center of the linear contiguous segment of the coil was placed over the hand area of the left motor cortex. In the "PAS over the somatosensory cortex" paradigms, the coil was placed over an area 2 cm posterior to the hand area of the left motor cortex (Wolters et al. 2005). Intensities were expressed as a percentage of the maximum output of the stimulator. Intensities used for PAS were determined to produce a peak-to-peak MEP amplitude of approximately 1 mV in the resting right APB muscle. One stimulus pair was given every 20 s (0.05 Hz stimulation rate) over 30 min (total of 90 stimulus pairs), at ISIs of 25 ms (PAS_{25}) and 10 ms (PAS_{10}). These ISIs were chosen because they were shown to be effective in producing an increment/decrement in the MEP amplitude (Stefan et al. 2000; Wolters et al. 2003). Throughout the experiment, complete muscle relaxation and the MEPs caused by PAS over the motor cortex from the APB muscle were monitored appropriately by electromyogram.

Experiment 1: effects on SEPs and HFOs after PAS over the motor cortex

Subjects and SEP/HFO recordings

Twenty-six subjects participated in the SEP experiments. Eleven subjects participated in the "PAS_{25} over the motor cortex" paradigm, and 11 subjects participated in the "PAS_{10} over the motor cortex" paradigm, with four subjects participating in both experiments on different days. These experiments were conducted at least one month apart.

Subjects lay supine on the bed and were instructed to stay awake with their eyes closed and to pay no attention to the stimuli to avoid sleepiness (Ogawa et al. 2004). Alertness was monitored by electroencephalography (EEG) recording. When subjects were drowsy or sleepy, recordings were stopped, and they were re-started when subjects were completely awake after a nap (Mochizuki et al. 2003). Electrical stimuli of 0.2 ms duration were delivered alternately to the bilateral median nerves at the wrists (cathode proximal). The stimulus intensity was adjusted to three times the sensory threshold so as to induce a small muscular twitch in the thenar muscles. The stimuli were delivered at irregular intervals, with ISIs between 211 and 262 ms. Recording electrodes were placed on C3′ (2 cm posterior to C3), C4′ (2 cm posterior to C4), Fz, F3, and A2 of the International 10–20 System. Electrode impedance was maintained below 5 kΩ. EEGs were recorded from C3′–Fz, C4′–Fz, and F3–A2 using a 0.3 Hz low-frequency filter and a 3,000 Hz high-frequency filter, then digitized with an ana-

logue-to-digital converter (micro1401, CED, Cambridge, UK) at a sampling rate of 20 kHz and stored on a disk for further analysis. We used a C3′–Fz and C4′–Fz montage for recording HFOs, because it is known from previous studies to be an appropriate method (Mochizuki et al. 1999; Sakuma et al. 2004). SEPs with an epoch of 50 ms duration (from 5 ms pre-stimulus to 45 ms post-stimulus) were recorded before and immediately after PAS. It took about 20 min to record each session of SEPs. Responses to 5,000 stimuli were averaged offline using SPIKE2 software (CED, Cambridge, UK). For separation of HFOs from the underlying N20, the digitized wide-band signal was band-pass filtered (400–800 Hz) digitally and averaged. In wide-band recordings, the SEP peak-to-peak amplitudes were measured and analyzed over C3′–Fz and C4′–Fz for the P14-, N20-, P25- and N33-components (Fig. 1a) and over F3–A2 for the P22- and N30-components. The latency of the N20-component from C3′–Fz was also recorded and analyzed. The size of the HFOs was calculated from their root-mean-square (RMS) amplitude from their onset to their endpoint. Onset/endpoint criteria for HFOs were when they exceeded the averaged background noise level for the subject's control session by three standard deviations. All of these parameters were separated into two parts: (1) early HFOs (onset to N20 peak) and (2) late HFOs (N20 peak to endpoint), as shown in Fig. 1b.

The individual SEP and HFO values for each subject were evaluated using a three-way analysis of variance (ANOVA) of mixed design with the within-subject factors of Time (before PAS vs. just after PAS) and Recording Site (C3′ vs. C4′) and the between-subject factor of Intervention (PAS_{25} vs. PAS_{10}). In addition, the effects of each PAS on SEPs and HFOs were evaluated using two-way or one-way, repeated-measures ANOVAs as a within-subject factor.

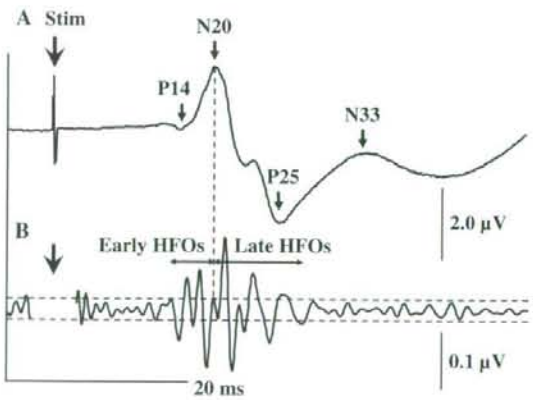

Fig. 1 **a** Typical wide-band (0.3–3,000 Hz) and **b** narrow-band (400–800 Hz) somatosensory-evoked potentials (*SEPs*) from C3′–Fz following right median nerve stimulation in a subject. **b** The narrow-band trace shows a short burst of high-frequency oscillations (*HFOs*) around N20

Experiment 2: time courses of changes in SEPs and HFOs after PAS over the motor cortex

Subjects and time courses of SEP/HFO recordings

In 15 of the 26 subjects who participated in Experiment 1, SEPs following PAS were monitored over time (8 subjects with PAS_{25}, 7 subjects with PAS_{10}). To study the time courses of changes in SEPs and HFOs after PAS, we recorded them for about 1 h, and the responses to 5,000 stimuli from C3′–Fz, C4′–Fz, and F3–A2 were averaged every 20 min after each PAS session (just after, 20 min after, and 40 min after).

The time course of each PAS-induced effect on the SEPs and HFOs was studied using a one-way, repeated-measures ANOVA with Time (before PAS vs. just after PAS, 20 and 40 min after PAS) as a within-subject factor.

Experiment 3: comparison of the effects of PAS over the motor cortex with those of PAS over the somatosensory cortex

Subjects and stimulation sites

In 14 subjects, PAS over the somatosensory cortex was performed (7 subjects with PAS_{25}, 7 subjects with PAS_{10}). A magnetic coil was positioned over an area 2 cm posterior to the motor hot spot (Wolters et al. 2005). The effects of PAS over the somatosensory cortex on SEPs and HFOs were compared with those after PAS over the motor cortex (Experiment 1).

To compare the differences between PAS over the motor cortex and PAS over the somatosensory cortex, we performed a two-way ANOVA of mixed design with the within-subject factor of Time (before PAS vs. after PAS) and the between-subject factor of Stimulation Site (motor cortex vs. somatosensory cortex).

Experiment 4: effect of PAS over the motor cortex on motor cortical excitability

Subjects and time courses of MEP recordings

Motor cortical excitability was assessed in 14 subjects (7 subjects with PAS_{25}, 7 subjects with PAS_{10}). TMS was performed with a round coil (external diameter, 130 mm) connected to a Magstim 200 stimulator to obtain MEPs. The coil was positioned over the vertex in the optimal scalp position to elicit motor responses in the right APB muscle. The MEP amplitude was measured by using the stimulator

intensity sufficient to evoke a peak-to-peak amplitude of 1 mV in the relaxed APB muscle. MEPs were recorded from the APB muscle using a pair of 2 × 2-cm Ag–AgCl disposable surface electrodes in a belly tendon montage. Each parameter was measured before the PAS session, just after the PAS session, and 15, 30, 45, and 60 min after the PAS session.

Each PAS-induced effect on the MEP was studied using a two-way ANOVA of mixed design with the within-subject factor of Time (before PAS vs. after PAS) and the between-subject factor of Intervention (PAS_{25} vs. PAS_{10}).

Statistical analysis

Data were analyzed using ANOVAs in SPSS for Windows, version 11.5. Whole analyses could assume the sphericity was not violated. When the effect was significant, a post hoc Dunnett's paired *t*-test was performed on the data. The statistical analyses were carried out on absolute amplitude values to compare variables before and after PAS, whereas normalized amplitudes were described in Figs. 3, 4, 5, and 6. A value of $P < 0.05$ was considered to be statistically significant. Data were expressed as mean ± standard error of the mean.

Results

Experiment 1: effects on SEPs and HFOs after PAS over the motor cortex

Figure 3 reveals the comparison between changes in SEPs and HFOs after PAS_{25} and PAS_{10} over the motor cortex. A three-way ANOVA of mixed design revealed no significant three-way interactions, but did reveal significant two-way interactions for Time × Intervention (early HFOs: $F_{(1,56)} = 9.648$, $P = 0.003$; late HFOs: $F_{(1,56)} = 18.224$, $P < 0.001$; total HFOs: $F_{(1,56)} = 16.339$, $P < 0.001$; P14–N20: $F_{(1,56)} = 5.847$, $P = 0.019$; N20–P25: $F_{(1,56)} = 4.684$, $P = 0.035$; P25–N33: $F_{(1,56)} = 15.549$, $P < 0.001$). Separate two-way, repeated-measures ANOVAs on SEPs and HFOs following PAS_{25} and PAS_{10} revealed no significant Time × Recording Site interactions, whereas the ANOVAs on SEPs and HFOs following PAS_{25} showed significant main effects of Time (early HFOs: $F_{(1,28)} = 8.463$, $P = 0.007$, late HFOs: $F_{(1,28)} = 19.615$, $P < 0.001$; total HFOs: $F_{(1,28)} = 15.574$, $P < 0.001$; P14–N20: $F_{(1,28)} = 5.051$, $P = 0.033$; N20–P25: $F_{(1,28)} = 5.967$, $P = 0.021$; P25–N33: $F_{(1,28)} = 13.126$, $P = 0.001$). Neither SEPs nor HFOs following PAS_{10} revealed significant main effects. One-way, repeated-measures ANOVA revealed that HFOs and N20–P25 and P25–N33 amplitudes from C3′–Fz increased significantly following PAS_{25} relative to the pre-PAS_{25} values (early HFOs: $F_{(1,14)} = 14.356$, $P = 0.002$; late HFOs: $F_{(1,14)} = 22.561$, $P < 0.001$; total HFOs: $F_{(1,14)} = 24.291$, $P < 0.001$; N20–P25: $F_{(1,14)} = 6.179$, $P = 0.026$; P25–N33: $F_{(1,14)} = 14.747$, $P = 0.001$), and there was a significant decrement in the P25–N33 amplitude from C3′–Fz following PAS_{10} relative to the pre-PAS_{10} value ($F_{(1,14)} = 11.501$, $P = 0.004$), as shown in Figs. 2, and 3. There were no significant main effects on the P14–N20 amplitude from C3′–Fz. SEPs and HFOs recorded from C4′–Fz did not show significant main effects, either. For the P22–N30 amplitude from F3–A2, two-way, repeated-measures ANOVA revealed no significant main or interaction effects.

The averaged latency of the N20-component from C3′–Fz was 17.85 ± 0.87 in the present study.

Experiment 2: time courses of changes in SEPs and HFOs after PAS over the motor cortex

Figure 4 reveals the comparison between time courses of changes in SEPs (Fig. 4b) and HFOs (Fig. 4a) after PAS_{25} and PAS_{10} over the motor cortex. One-way, repeated-measures ANOVA revealed that the late and total HFOs and the P25–N33 amplitude recorded from C3′–Fz changed significantly following PAS_{25} (late HFOs: $F_{(3,21)} = 24.075$, $P = 0.002$; total HFOs: $F_{(3,21)} = 16.852$, $P = 0.010$; P25–N33: $F_{(3,21)} = 6.916$, $P = 0.046$), whereas the early HFOs and the P14–N20 and N20–P25 amplitudes did not (Fig. 4). Post hoc analysis revealed that the amplitude of the late HFOs and P25–N33 increased significantly for 60 min from just after PAS_{25} relative to the pre-PAS_{25} values (late HFOs: 0–20 min, $P = 0.039$; 20–40 min, $P = 0.002$; 40–60 min, $P = 0.022$; P25–N33: 0–20 min, $P = 0.033$; 20–40 min, $P = 0.001$; 40–60 min, $P = 0.003$). The amplitude of the

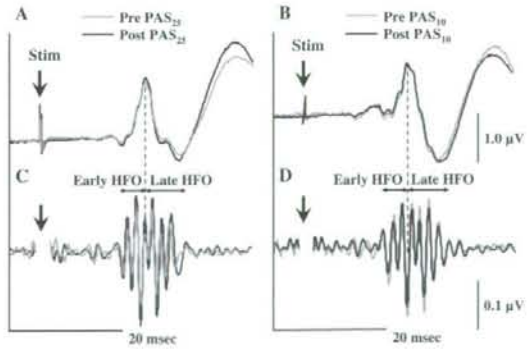

Fig. 2 Typical SEPs (*upper traces*) and HFOs (*lower traces*) from C3′–Fz were obtained in a subject before and after paired-associative stimulation (*PAS*). Waveforms before and after PAS were superimposed. **a**, **b** The N20–P25 and P25–N33 amplitudes increased/decreased significantly after $PAS_{25/10}$. **c**, **d** HFO amplitudes were enlarged/reduced significantly after $PAS_{25/10}$

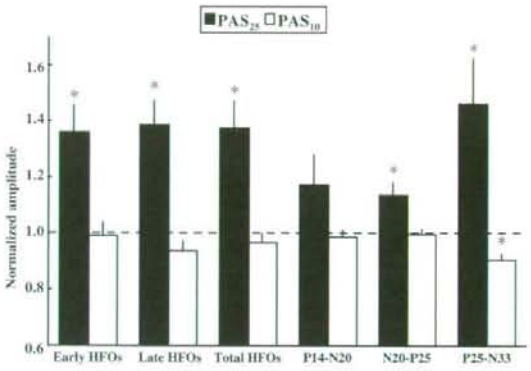

Fig. 3 The effect of PAS on SEP and HFO amplitudes. The size ratios (just after PAS/pre PAS) of the components of the HFOs (early, late, and total HFOs) and SEPs (P14–N20, N20–P25, and P25–N33) from C3′–Fz are shown. The HFO amplitudes and the N20–P25 and P25–N33 amplitudes recorded from C3′–Fz increased significantly following PAS_{25}, whereas the P25–N33 amplitude from C3′–Fz decreased following PAS_{10}. *$P < 0.05$ by a one-way analysis of variance (ANOVA) with Time (before PAS vs. just after PAS) as a within-subject factor. *Error bar* standard error of the mean

total HFOs increased for only 20–40 min after PAS_{25} relative to the pre-PAS_{25} value (0–20 min, $P = 0.068$; 20–40 min, $P = 0.012$; 40–60 min, $P = 0.118$). One-way, repeated-measures ANOVA revealed that the HFOs and the N20–P25 and P25–N33 amplitudes from C3′–Fz decreased significantly after PAS_{10} (early HFOs: $F_{(3,18)} = 6.619$, $P = 0.049$; late HFOs: $F_{(3,18)} = 11.795$, $P = 0.019$; total HFOs: $F_{(3,18)} = 24.499$, $P = 0.005$; N20–P25: $F_{(3,18)} = 9.311$, $P = 0.001$; P25–N33: $F_{(3,18)} = 55.533$, $P = 0.001$). As shown in Fig. 4, post hoc analysis revealed that the N20–P25 amplitude decreased significantly from 20 min after PAS_{10} relative to the pre-PAS_{10} value (0–20 min, $P = 0.951$; 20–40 min, $P = 0.003$; 40–60 min, $P = 0.002$). The P25–N33 amplitude decreased from just after to 40 minutes after PAS_{10} relative to the pre-PAS_{10} value (0–20 min, $P = 0.003$; 20–40 min, $P = 0.019$; 40–60 min, $P = 0.681$). The HFOs were reduced notably from just after PAS_{10} relative to the pre-PAS_{10} values (early HFOs: 0–20 min, $P = 0.040$; 20–40 min, $P = 0.053$; 40–60 min, $P = 0.078$; late HFOs: 0–20 min, $P = 0.459$; 20–40 min, $P = 0.014$; 40–60 min, $P = 0.180$; total HFOs: 0–20 min, $P < 0.001$; 20–40 min, $P < 0.001$; 40–60 min, $P < 0.001$). There was no significant change in the SEPs and HFOs from C4′–Fz and in the SEPs from F3–A2 after both PAS sessions.

Experiment 3: comparison of the effects of PAS over the motor cortex with those of PAS over the somatosensory cortex

Figure 5a, b reveals the comparison between changes in SEPs and HFOs after $PAS_{25/10}$ over the motor cortex and after $PAS_{25/10}$ over the somatosensory cortex. With the PAS_{25} intervention, two-way, ANOVA of mixed design revealed that the HFO and SEP amplitudes recorded from C3′–Fz displayed statistically significant Time × Stimulation Site interactions (late HFOs: $F_{(1,20)} = 6.328$, $P = 0.021$; total HFOs: $F_{(1,20)} = 5.975$, $P = 0.024$; N20–P25: $F_{(1,20)} = 6.807$, $P = 0.017$; P25–N33: $F_{(1,20)} = 4.748$, $P = 0.041$) and main effects of Stimulation Site (early HFOs: $F_{(1,20)} = 6.188$, $P = 0.022$; late HFOs: $F_{(1,20)} = 7.323$, $P = 0.014$; total HFOs: $F_{(1,20)} = 7.384$, $P = 0.013$), as shown in Fig. 5. These results revealed that the effects of PAS_{25} depended on the stimulation site. Separate one-way, repeated-measures ANOVAs revealed that the HFOs and the N20–P25 and P25–N33 amplitudes increased significantly after PAS_{25} over the motor cortex (Experiment 1), whereas there were no significant changes after PAS_{25} over the somatosensory cortex relative to the pre-PAS_{25} values (early HFOs: $F_{(1,6)} = 0.006$, $P = 0.939$; late HFOs: $F_{(1,6)} = 0.098$, $P = 0.764$; total HFOs: $F_{(1,6)} = 0.017$, $P = 0.899$; N20–P25: $F_{(1,6)} = 3.628$, $P = 0.105$; P25–N33: $F_{(1,6)} = 1.826$, $P = 0.225$). With the PAS_{10} intervention, the P25-N33 amplitude from C3′–Fz displayed a statistically significant

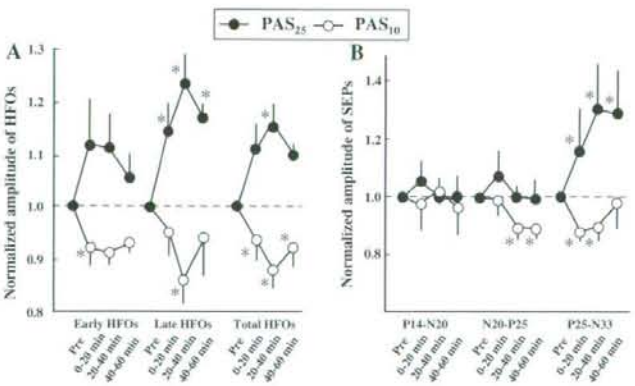

Fig. 4 Time courses of changes in SEPs and HFOs after PAS. The size ratios (post PAS/pre PAS) of **a** components of HFOs and **b** SEPs from C3′–Fz are shown. The late HFO and the P25–N33 amplitudes increased significantly after PAS_{25} and lasted for 60 min. The N20–P25 and P25–N33 amplitudes decreased notably for more than 30 min after PAS_{10}. The HFOs also decreased after PAS_{10} and lasted for 60 min

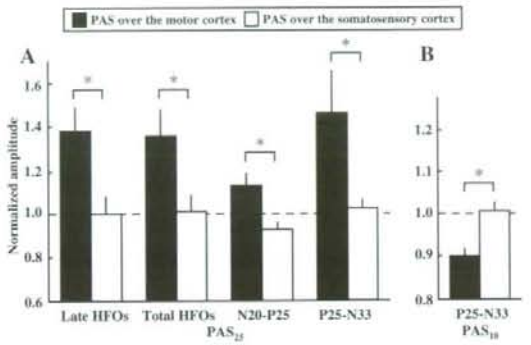

Fig. 5 **a**, **b** Comparison of the effects of PAS over the motor cortex with those of PAS over the somatosensory cortex. The size ratios (post PAS/pre PAS) of components of HFOs and SEPs from C3′–Fz are shown. SEPs and HFOs after PAS over the motor cortex changed significantly, whereas there was no change after PAS over the somatosensory cortex

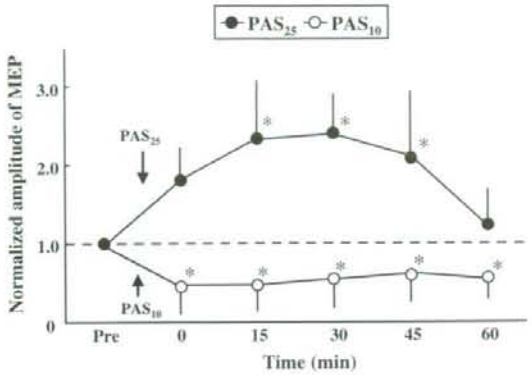

Fig. 6 Effects of PAS on motor-evoked potentials (*MEPs*). The size ratios (post PAS/pre PAS) of the MEPs are shown. The MEPs were enlarged/reduced significantly following $PAS_{25/10}$, and these effects lasted for more than 30 min

Time × Stimulation Site difference ($F_{(1,20)} = 4.938$, $P = 0.038$), as shown in Fig. 5. These results revealed that the effects of PAS_{10} depended on the stimulation site. Separate one-way, repeated-measures ANOVAs revealed that the P25–N33 amplitude decreased notably after PAS_{10} over the motor cortex (Experiment 1), but there was no significant change after PAS_{10} over the somatosensory cortex relative to the pre-PAS_{10} value ($F_{(1,6)} = 0.001$, $P = 0.993$). There were no statistically significant main or interaction effects on the SEPs and HFOs from C4′–Fz.

Experiment 4: the effect of PAS over the motor cortex on motor cortical excitability

Figure 6 reveals the comparison between time courses of changes in MEPs after PAS_{25} and PAS_{10} over the motor cortex. There was a significant pattern of change in the MEP amplitude following the PAS intervention ($F_{(5,60)} = 5.905$, $P = 0.014$) with factors of Time × Intervention (Fig. 6). Post hoc analysis showed that a significant increment in the MEP amplitudes continued from 15 to 45 min after PAS_{25} relative to the pre-PAS_{25} values (0 min, $P = 0.094$; 15 min, $P = 0.006$; 30 min, $P = 0.005$; 45 min, $P = 0.027$; 60 min, $P = 0.982$), and a significant decrement in the MEP amplitudes following PAS_{10} lasted for 60 min relative to the pre-PAS_{10} values (0 min, $P = 0.002$; 15 min, $P = 0.002$; 30 min, $P = 0.010$; 45 min, $P = 0.015$; 60 min, $P = 0.018$).

Discussion

This study revealed that PAS over the motor cortex changed the cortical excitability in a distant site, the somatosensory cortex. The cortical components of SEPs from C3′–Fz increased significantly after PAS_{25}, whereas changes in the cortical SEPs were reversed after PAS_{10}. The HFOs from C3′–Fz were enlarged significantly after PAS_{25}, whereas the HFOs decreased notably after PAS_{10}. Since these changes lasted more than 30 min, it was revealed that the LTP/LTD-like phenomena occurred on the SEPs and HFOs, respectively. There were no effects on the SEPs and HFOs following PAS over the somatosensory cortex.

Changes in SEPs and MEPs after PAS

The PAS-induced increment/decrement of cortical excitability was distinctly timing dependent (Stefan et al. 2002; Wolters et al. 2003, 2005). In the classical model of associative, Hebbian plasticity, synaptic transmission is strengthened when a presynaptic neuron is activated before activation of the postsynaptic neuron, whereas the opposite effect is induced when the sequence of events is reversed. Afferent inputs elicited by median nerve stimulation reach the somatosensory cortex at the latency of the N20 component of the SEPs (Allison et al. 1989). In humans, a peripheral somatosensory signal generally reaches the motor cortex 4 ms after the arrival of the signal in the somatosensory cortex (Goldring et al. 1970). Since the latency of the N20-component of the SEPs was 17.85 ± 0.87 ms in the present study, the events triggered by TMS also followed the events elicited by median nerve stimulation in the motor cortex at PAS_{25} (PAS_{25}; ISI = 25 ms > N20-latency + 4 ms), whereas the sequence of events was reversed at PAS_{10} (PAS_{10}; ISI = 10 ms < N20-latency + 4 ms). Therefore, these results indicated that the LTP/LTD-like phenomena were produced in the motor cortex with $PAS_{25/10}$ over the motor cortex.

The N20–P25 and P25–N33 amplitudes changed after PAS over the motor cortex in the present SEP study. The origin of each SEP component is different. P14 is probably generated from a subcortical structure, the caudal medial lemniscus (Noel et al. 1996), and N20 is thought to be generated from the somatosensory cortex in the posterior lip of the central fissure (Nuwer et al. 1994). The later potentials (P25, N33, and later potentials) are usually assumed to represent the arrival of the sensory volley at the somatosensory cortex (Tsuji and Rothwell 2002). A previous study revealed that low-frequency repetitive TMS (rTMS) over the motor cortex suppressed the N20–P25 amplitude without any changes in the $N20_{onset}$–$N20_{peak}$ amplitudes. The authors speculated that this suppression occurred in the somatosensory cortex, because the N20 component reflects an activation of the somatosensory cortex by thalamocortical fibers (Enomoto et al. 2001). In the present study, not the P14–N20, but the N20–P25 and P25–N33 amplitudes changed after intervention. Therefore, we speculated that PAS over the motor cortex did not affect subcortical pathways, but did affect the later processes, including the somatosensory cortex. These results are similar to those of previous studies (Enomoto et al. 2001; Tsuji and Rothwell 2002).

The cortical SEP components changed significantly following $PAS_{25/10}$ over the motor cortex, and these effects lasted for more than 30 min, as in our MEP study, whereas $PAS_{25/10}$ over the somatosensory cortex produced no significant SEP changes. We offer the following two reasons for this discrepancy. One reason is the ISIs of PAS. A previous SEP study suggested that PAS over the somatosensory cortex caused the LTP/LTD-like phenomena in the somatosensory cortex, but the ISIs of PAS over the somatosensory cortex were shorter by 6.8 ms than those over the motor cortex. Therefore, $PAS_{25/10}$ over the somatosensory cortex could not produce such phenomena in the somatosensory cortex (Wolters et al. 2005). In the present study, no significant SEP changes were obtained for the same reason. Another reason is that a difference in the sensitivity to TMS effects between the motor and somatosensory cortices might exist. Low-frequency rTMS over the motor cortex suppressed the cortical SEP for a long time via the cortico-cortical connections between the motor and somatosensory cortices, whereas low-frequency rTMS directly over the somatosensory cortex had a slight facilitatory effect of SEP, and this effect lasted a few minutes (Enomoto et al. 2001). In a continuous theta burst stimulation (cTBS) study, SEP changes following cTBS over the somatosensory cortex were shorter than MEP and SEP changes following cTBS over the motor cortex. The authors speculated that this discrepancy was caused by a threshold difference for TMS effects between the motor and the somatosensory cortices (Ishikawa et al. 2007). Because the stimulus paradigm in the present study was different from those in the previous studies, we could not compare our results directly to the previous results. However, TMS effects over the somatosensory cortex are weaker than those over the motor cortex. We suppose that the threshold for TMS effects over the somatosensory cortex may be higher than that over the motor cortex. Consequently, we speculated that the LTP/LTD-like phenomena in the motor cortex induced by $PAS_{25/10}$ over the motor cortex were not caused in the somatosensory cortex directly, but might spread to the somatosensory cortex, since the dense cortico-cortical connections exist between the motor and somatosensory cortices, and the same LTP/LTD-like effects appeared in the somatosensory cortex. These SEP results are in line with previous MEP results (Stefan et al. 2002; Wolters et al. 2003).

PAS over the motor cortex did not affect the frontal P22–N30 component from F3–A2 in the present study. This result revealed that the changes in SEPs from C3′–Fz were not contaminated by the frontal P22–N30 component. A previous study reported that the frontal P22–N30 amplitude increased just after a paradigm similar to our PAS_{25} paradigm: rTMS over the motor cortex paired with a preceding repetitive motor point stimulation (0.1 Hz, ISI of 25 ms), but the effect did not continue for 10 min (Tsuji and Rothwell 2002). The discrepancy could reflect methodological differences. There were different methods of SEP recording in the two studies: in Tsuji and Rothwell's study, 256 stimuli were delivered at 1 Hz; in our study, 5,000 stimuli at about 4–5 Hz were given for about 20 min. Even if the frontal P22–N30 amplitude changed, as in Tsuji and Rothwell's study, the effect would be too short to record in the present study. Therefore, the changes in the SEPs from C3′–Fz after PAS did not affect the frontal component in the present study.

HFOs changes after PAS

In spite of extensive research concerning HFOs superimposed on the N20 primary cortical responses, the origin of HFOs still remains controversial. One hypothesis is that HFOs are generated from GABAergic inhibitory interneurons (Hashimoto et al. 1996). Based on the reciprocal relationship between HFOs and N20 representing an ensemble of EPSPs of the glutamatergic pyramidal neurons in the 3b area, HFOs were postulated to represent a localized activity of GABAergic inhibitory interneurons in layer 4 of area 3b (Curio 2000; Hashimoto et al. 1996, 1999). Although the following hypothesis may be speculative at present, we propose that the HFO changes that occur after PAS reflect the activities of GABAergic inhibitory interneurons.

The close interactions between the glutamatergic pyramidal neurons and the GABAergic inhibitory interneurons

in the cortex have been well established (Porter et al. 2001; Sun et al. 2006a, b). The existence of constant inhibitory regulation of the activity of large pyramidal neurons by the surrounding inhibitory neurons in the sensorimotor cortex was demonstrated (Storozhuk et al. 2003). In the central nucleus of the inferior colliculus, the major structure of the central auditory system, glutamatergic excitation can be modulated by presynaptic $GABA_B$ receptors. This mechanism might serve to prevent overstimulation by feedback inhibition from GABAergic inhibitory interneurons or to maintain an appropriate balance of excitation and inhibition for the neural representation of auditory signals (Sun et al. 2006a, b). There are a number of $GABA_B$ receptors in the presynaptic and postsynaptic membranes of glutamatergic pyramidal neurons, and these receptors modulate glutamatergic neurotransmission in the rat hippocampus (Kulik et al. 2003). GABAergic inhibitory interneurons regulate glutamatergic pyramidal neurons not only by exposing them to the neurotransmitters directly, but also by modulating glutamatergic neurotransmission through $GABA_B$ receptors. The LTP/LTD-like phenomena after PAS are considered to be mediated by the NMDA receptors on postsynaptic glutamatergic neurons (Stefan et al. 2002; Wolters et al. 2003), and numerous NMDA receptors are distributed throughout the brain, including the sensorimotor cortices (Scheperjans et al. 2005). When glutamatergic neurons are facilitated, GABAergic inhibitory interneurons might also be activated by the enhanced glutamatergic neurons, which in turn modulate glutamatergic excitation. Conversely, if glutamatergic neurons are regulated, GABAergic inhibitory interneurons may no longer be modulated. In our study, the HFOs as well as the N20–P25 and P25–N33 amplitudes increased significantly after PAS_{25}. After PAS_{10}, the HFOs and the N20–P25 and P25–N33 amplitudes decreased notably. Because glutamatergic neurons were facilitated by NMDA receptor activation, and GABAergic inhibitory interneurons might be activated secondarily after PAS_{25}, we speculate that the increased N20–P25 and P25–N33 amplitudes and the increased HFO amplitudes reflect the increased activity of pyramidal cells and inhibitory interneurons, respectively. On the other hand, the N20–P25 and P25–N33 amplitudes and the HFO amplitudes decreased notably after PAS_{10}, probably reflecting a down-regulation of glutamatergic neurons and GABAergic inhibitory interneurons. Since a previous animal experiment revealed that a $GABA_A$ antagonist did not affect the HFO activity (Jones and Barth 2002), we speculated that changes in HFOs following PAS over the motor cortex might be produced through $GABA_B$ receptors.

In conclusion, not only the MEP but also the cortical SEPs and HFOs increased/decreased, and these effects continued for more than 30 min after PAS over the motor cortex. These results revealed that the LTP/LTD-like phenomena in the motor cortex may spread to a distant site, the somatosensory cortex. Enhanced HFOs after PAS_{25} over the motor cortex may reflect the activation of GABAergic inhibitory interneurons for the purpose of regulating pyramidal neurons in the somatosensory cortex. Because there is no need to modulate deactivated pyramidal neurons after PAS_{10} over the motor cortex, the HFO activity may be reduced.

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High-frequency oscillations change in parallel with short-interval intracortical inhibition after theta burst magnetic stimulation

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Abstract

Objective: Theta burst transcranial magnetic stimulation (TBS) causes changes in motor cortical excitability. In the present study, somatosensory-evoked potentials (SEPs) and high-frequency oscillations (HFOs) were recorded before and after TBS over the motor cortex to examine how TBS influenced the somatosensory cortex.
Methods: SEPs following electric median nerve stimulation were recorded, and amplitudes for the P14, N20, P25, and N33 components were measured and analyzed. HFOs were separated by 400–800 Hz band-pass filtering, and root-mean-square amplitudes were calculated from onset to offset. SEPs and HFOs were measured before and after application of either intermittent or continuous TBS (iTBS/cTBS; 600 total pulses at 80% active motor threshold) over the motor cortex. Motor-evoked potentials (MEPs) and short-interval intracortical inhibition (SICI) of the first dorsal interosseous muscle were examined before and after TBS.
Results: MEPs, SICI, and HFO amplitudes were increased and decreased significantly after iTBS and cTBS, respectively. Wide-band SEPs did not change significantly after TBS.
Conclusions: TBS changed the cortical excitability of the sensorimotor cortices. Changes in HFOs after TBS were parallel to those in SICI.
Significance: The mechanisms of changes in HFOs after TBS may be the same as those in SICI.

Keywords: High-frequency oscillations; Short-interval intracortical inhibition; Theta burst stimulation; GABAergic inhibitory interneurons

1. Introduction

Low-amplitude, high-frequency oscillations (HFOs) of 500–800 Hz superimposed on the ascending slope of the N20 primary response following stimulation of the median nerve have attracted increasing attention in the past several years (Curio et al., 1994; Hashimoto et al., 1996). Although studies using electroencephalography (EEG) or magnetoencephalography (MEG) provided a wide range of information about the generators of these high-frequency wavelets, from the thalamus (Eisen et al., 1984; Klostermann et al., 2002) and thalamocortical presynaptic action potentials (Gobbele et al., 1998) to the somatosensory cortex (Curio et al., 1997; Hashimoto et al., 1999; Sakuma and Hashimoto, 1999; Sakuma et al., 1999, 2004; Shimazu et al., 2000), the precise anatomical location of the generator remains unclear. Hashimoto and colleagues hypothesized that HFOs represented a localized activity of the GABAergic inhibitory interneurons in layer 4 of area 3*b*, whereas the N20 component was considered to be generated by excitatory postsynaptic potentials of pyramidal neurons (Hashimoto et al., 1996).

Transcranial magnetic stimulation (TMS) not only is an important noninvasive method for neurophysiological investigation of the corticospinal tract in humans, but also

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is a useful tool for the treatment of neurological and psychiatric disorders. Repetitive TMS (rTMS) has been used for neurophysiological examinations and treatments of neuropsychiatric diseases (Lefaucheur et al., 2004; Pascual-Leone et al., 1994a,b, 1996). The effects of rTMS generally depend on the stimulus frequency; low-frequency (<1 Hz) rTMS decreases cortical excitability (Chen et al., 1997; Gerschlager et al., 2001), while high-frequency (>1 Hz) rTMS can increase cortical excitability (Di Lazzaro et al., 2002; Matsunaga et al., 2005). Recently, a new rTMS protocol, theta burst stimulation (TBS), was introduced. Intermittent or continuous TBS (iTBS/cTBS) over the motor cortex increased/decreased, respectively, both motor-evoked potentials (MEPs) and short-interval intracortical inhibition (SICI) (Huang et al., 2005). cTBS over the occipital cortex increased the phosphene threshold (Franca et al., 2006), and this finding revealed that TBS changed the cortical excitabilities in the sensory system as well as in the motor system.

rTMS affected somatosensory-evoked potentials (SEPs) and HFOs. For example, 1 Hz rTMS over the motor cortex suppressed the N20–P25 and P25–N33 amplitudes (Enomoto et al., 2001). Results of a recent study showed that cTBS over the sensorimotor cortices affected the cortical components of SEPs (Ishikawa et al., 2007). HFOs were increased significantly after 0.5 Hz rTMS over the somatosensory cortex, whereas SEPs were not changed (Ogawa et al., 2004). We hypothesized that TBS over the motor cortex affected the somatosensory cortex, because of the dense cortico-cortical connections between motor and somatosensory cortices (Enomoto et al., 2001). Furthermore, we speculated that HFOs as well as SICI might be affected by TBS, because both HFOs and SICI may reflect the functions of GABAergic inhibitory interneurons (Chen et al., 1998; Hashimoto et al., 1996; Kujirai et al., 1993; Ziemann et al., 1998b; Ziemann, 2004). In the present study, we recorded SEPs and HFOs before and after TBS sessions (iTBS and cTBS) and examined how TBS over the motor cortex influenced the somatosensory cortex.

2. Methods

2.1. Subjects

We studied 28 right-handed healthy volunteers (15 women, 13 men), ages 21–39 years (mean age, 27.1 ± 4.8 years). None had a history of physical or neurological illness. Some subjects took part in more than one experiment. This study was approved by the Human Ethics Committee of Tottori University and was conducted in accordance with the Declaration of Helsinki. All participants gave their informed consent prior to participation.

2.2. TBS protocol

rTMS over the left primary motor cortex was applied using the Magstim Super Rapid magnetic stimulator (Magstim Co., Dyfed, Wales). The magnetic stimulus had a biphasic waveform with a pulse width of 200 μs. The coil with a figure-of-eight and external loop diameters of 80 mm (Magstim Co., Dyfed, Wales) was held tangentially to the skull, with the handle pointing backward and laterally at a 45-deg angle to the sagittal plane. The center of the linear contiguous segment of the coil was placed over the hand area of the left motor cortex. Intensities were expressed as a percentage of the maximum output of the stimulator. The stimulation intensity was defined in relation to the active motor threshold (AMT). The AMT was evaluated as the minimum single pulse intensity required to produce an MEP greater than 200 μV on more than five of 10 trials from the right first dorsal interosseous (FDI) muscle while the subject was maintaining a voluntary contraction of about 20% of maximum using visual feedback.

TBS paradigms were safe in normal subjects and capable of producing consistent, rapid, and controllable electrophysiological and behavioral changes in the function of the human motor system. The pattern of delivery of TBS (continuous vs. intermittent) was crucial in determining the direction of change in synaptic efficacy (Huang et al., 2005). According to the Huang's report (Huang et al., 2005), rTMS was performed using the TBS pattern in which three pulses of stimulation were given at 50 Hz, repeated every 200 ms for a total of 600 pulses delivered over the motor cortex. In the iTBS pattern, a 2-s train of TBS was repeated every 10 s for a total 190 s. In the cTBS pattern, a 40-s train of uninterrupted TBS was given. The stimulus intensity was set at 80% of the AMT.

2.3. Experimental protocols

2.3.1. Experiment 1: the effects of TBS on SEPs and HFOs

Sixteen subjects participated in the SEP experiments. Six subjects participated in the "iTBS over the motor cortex" paradigm, and 6 subjects participated in the "cTBS over the motor cortex" paradigm, with 4 subjects participating in both experiments on different days. These experiments were conducted at least one month apart.

Subjects lay supine on the bed and were instructed to stay awake with their eyes closed and to pay no attention to the stimuli to avoid sleepiness due to monotonous presentation of stimulus (Ogawa et al., 2004). Alertness was monitored by EEG recording. When a subject became drowsy or sleepy, recordings were stopped, and they were re-started when the subject was completely awake after a nap (Mochizuki et al., 2003). Electrical stimuli of 0.2 ms duration were delivered alternately to the bilateral median nerves at the wrists (cathode proximal). The stimulus intensity was adjusted to three times the sensory threshold so as to induce a small muscular twitch in the thenar muscles. The stimuli were delivered at irregular intervals, with interstimulus intervals (ISIs) between 211 and 262 ms. Recording electrodes were placed on C3′ (2 cm posterior to C3), C4′ (2 cm posterior to C4), and Fz of the International