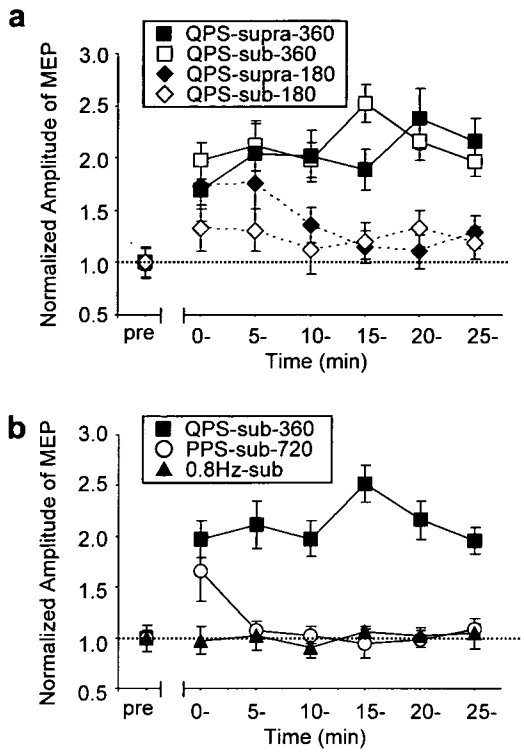

Fig. 4. (a) Effect of the intensity and the number of trains of QPS on aftereffects. Time courses of normalized amplitudes of cortical MEPs (mean ± SE) after four conditioning: QPS-supra-360 (filled squares, $n = 12$), QPS-sub-360 (squares, $n = 12$), QPS-supra-180 (filled diamonds, $n = 7$), and QPS-sub-180 (diamonds, $n = 5$). After both QPS-supra-360 and QPS-sub-360 conditioning, cortical MEPs were facilitated for at least 30 min. Significant effects of the number of trains ($p < 0.01$) and time ($p < 0.001$) were found, as well as a significant interaction between the number of trains and time ($p < 0.005$) using three-way repeated ANOVA. Cortical MEPs after both QPS-sub-360 and QPS-supra-360 were significantly facilitated for 30 min post conditioning (post hoc analysis using Bonferroni's method: each period, $p < 0.05$). After QPS-supra-180, cortical MEPs were facilitated significantly for only 10 min (post hoc analysis using Bonferroni's method: 0–5 min, $p < 0.05$; 5–10 min, $p < 0.05$; 10–30 min, $p > 0.05$). No significant cortical MEP changes were found after QPS-sub-180 (one-factor ANOVA: effect of time, $p > 0.05$). (b) Comparison between QPS and PPS with the same total number of pulses. Time courses of the normalized amplitudes of cortical MEPs (mean ± SE) after three conditioning: QPS-sub-360 (filled squares, $n = 12$), PPS-sub-720 (circles, $n = 5$), and 0.8 Hz-sub (filled triangles, $n = 5$). Even with the same total number of pulses (1440 pulses), only QPS-sub-360 induced long-lasting MEP facilitation after conditioning (two-way repeated ANOVA: conditioning × time, $p < 0.05$; effect of conditioning, $p < 0.005$; effect of time, $p > 0.05$; post hoc analysis, difference between QPS-sub-360 and PPS-sub-720 ($p < 0.05$), or 0.8 Hz-sub-30 ($p < 0.01$)). Cortical MEPs after PPS-sub-720 were facilitated only for 5 min (post hoc analysis using Bonferroni's method: 0–5 min, $p < 0.05$; 5–30 min, $p > 0.5$). No significant cortical MEP changes were found after 0.8 Hz-sub (one-factor ANOVA: effect of time, $p > 0.05$).

3.3. Experiment 3: Comparison between QPS and PPS with the same total number of pulses

The effects of the total number of pulses should be examined when we compare the aftereffects of QPS with those of PPS. Indeed, the time course of normalized amplitude of cortical MEPs after QPS-supra-180 (180 trains, total 720 pulses; Fig. 4a) seemed to be similar to that after PPS-supra-360 (360 trains, total 720 pulses; Fig. 3b) (two-way repeated ANOVA: effect of time, $p < 0.001$; effect of conditioning, $p > 0.05$; conditioning × time interaction, $p > 0.05$). Hence, QPS, PPS or 0.8 Hz conditioning with 1440 pulses in all were performed. Even with the same total number of conditioning pulses, cortical MEPs were significantly facilitated for only 5 min after PPS-sub-720 (Fig. 4b) (two-way repeated ANOVA: effect of conditioning, $F[2,19] = 8.153$, $p = 0.003$; effect of time, $F[6,114] = 2.160$, $p = 0.080$; conditioning × time interaction, $F[8.177, 77.677] = 2.648$, $p = 0.012$; post hoc analysis: QPS-sub-360 vs. PPS-sub-720, $p = 0.020$; QPS-sub-360 vs. 0.8 Hz-sub, $p = 0.008$). No significant MEP changes were found after 0.8 Hz sub (Fig. 4b).

3.4. Experiment 4: Duration of QPS aftereffects

The duration of the cortical MEP amplitude enhancement was studied by obtaining MEPs for 90 min following QPS-sub-360 (Fig. 5). Cortical MEPs were facilitated significantly for 75 min, and returned to the baseline level at 90 min (effect of time, $F[6,36] = 6.964$, $p < 0.001$). Cortical MEPs after QPS-supra-360 also returned to the baseline level at 90 min ($n = 3$, data not shown).

3.5. Experiment 5: Effects of QPS on brain-stem MEPs

The QPS-supra-360 did not affect the brain-stem MEPs for 30-min post conditioning (Fig. 6a). Fig. 6b shows the effects of QPS-supra-360 on cortical and brain-stem MEPs obtained in a randomized order. Only cortical MEPs were facilitated significantly, although brain-stem MEPs remained unchanged (Fig. 6b) (two-way repeated ANOVA: effect of stimulation pattern, $F[1,2] = 9.944$, $p = 0.088$; effect of time, $F[1,2] = 54.954$, $p = 0.018$; time × stimulation pattern interaction, $F[1,2] = 87.116$, $p = 0.011$; post hoc analysis: cortical MEPs after

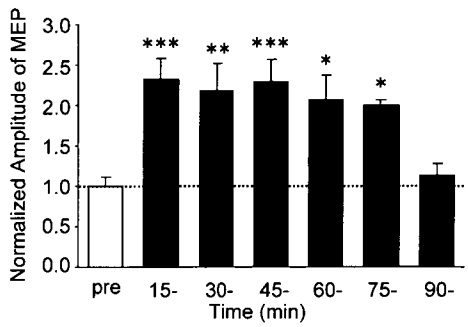

Fig. 5. Duration of QPS aftereffect. Time courses of normalized amplitudes of cortical MEPs (mean ± SE) after QPS-sub-360 ($n = 7$). The period of measurement was extended to 90 min after the conditioning to study when the effects of QPS-sub-360 returned to the baseline level. Cortical MEPs were facilitated for 75 min post conditioning and returned to the baseline level at 90 min post conditioning (one-factor ANOVA: effect of time, $p < 0.001$). Asterisks $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, by post hoc analysis using Bonferroni's method.

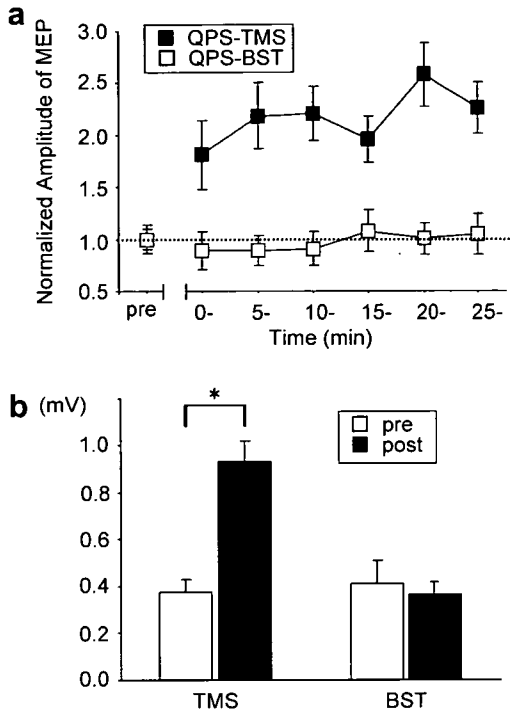

Fig. 6. Effects of QPS on brain-stem MEPs. (a) Time courses of normalized amplitudes of brain-stem MEPs (mean ± SE) after QPS-supra-360 (squares, $n = 5$). Normalized amplitude of brain-stem MEPs did not change significantly for 30 min after conditioning (one-factor ANOVA: effect of time, $p > 0.05$). For comparison, cortical MEPs after QPS-supra-360 (filled squares) are also depicted. (b) Cortical and brain-stem MEPs obtained before (pre, bars) and after (post, filled bars) QPS-supra-360. The responses were sampled in a pseudorandom order to avoid the effect of anticipation of the uncomfortable brain-stem stimulation ($n = 3$). The bars show absolute amplitudes of MEPs (mV, mean ± SE). Only cortical MEPs (i.e., MEPs to single-pulse TMS) were facilitated, whereas brain-stem MEPs (i.e., MEPs to BST electrical stimulation) remained unchanged (two-way repeated ANOVA: effect of time, $p < 0.05$; effect of stimulation pattern, $p > 0.05$; stimulation pattern × time, $p < 0.05$). Asterisks, $p < 0.05$, by post hoc paired t-test.

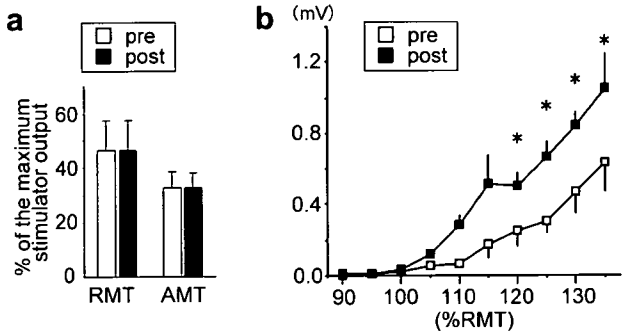

Fig. 7. Effects of QPS on motor threshold and recruitment curves. (a) RMT and AMT before (pre, bars) and after (post, filled bars) QPS-supra-360 ($n = 5$). The bars show the mean ± SD threshold as a percentage of the maximum stimulator output (MSO). No changes in RMT and AMT were found after QPS conditioning (RMT pre: 46.6 ± 11.1%, RMT post: 46.4 ± 11.2%, $p > 0.05$; AMT pre: 32.8 ± 5.9%, AMT post: 32.6 ± 5.7%, $p > 0.5$, by paired t-test). (b) Recruitment curves before (pre, squares) and after (post, filled squares) QPS-supra-360 ($n = 5$). The abscissa shows the stimulus intensity relative to the motor threshold. The ordinate shows absolute peak-to-peak MEP amplitudes (mV, mean ± SE). The recruitment curve after QPS-supra-360 became steeper than that before (two-way repeated ANOVA: intensity × time (pre and post), $p < 0.05$; effect of time, $p < 0.005$; effect of intensity, $p < 0.01$). Asterisks, $p < 0.05$, by post hoc analysis using Bonferroni's method.

QPS-supra-360, $t = -8.888$, $p = 0.012$; brain-stem MEPs, $t = 4.009$, $p = 0.057$).

3.6. Experiment 6: Effects of QPS on motor threshold, recruitment curves and somatotopy

3.6.1. Motor threshold and recruitment curve

The MTs (RMT and AMT) did not change significantly after QPS-supra-360 (Fig. 7a). However, the recruitment curve after QPS-supra-360 became steeper (Fig. 7b) (two-way repeated ANOVA: effect of time, $F[1,4] = 87.481$, $p = 0.001$; effect of intensity, $F[1.355, 5.421] = 19.324$, $p = 0.005$; intensity × time interaction, $F[1.328, 5.311] = 11.376$, $p = 0.015$).

3.6.2. Cortical MEPs from the FCU muscle

The QPS-supra-360 showed topographically specific modulation of the motor cortex (Fig. 8). Paired t-tests showed a significant facilitation of cortical MEPs from FDI muscle ($t = -4.492$, $p = 0.011$), although no significant changes were evoked in cortical MEPs from FCU muscle ($t = -1.119$, $p = 0.326$).

3.7. Experiment 7: Safety study

Post-TMS EMG activity was observed in two of three studied subjects (Fig. 9), but SE was not observed during either QPS-supra-360 or QPS-sham-360 in any subject. The occurrence rate of post-TMS EMG activity during QPS-supra-360 was not different from that during QPS-sham-360 (Table 1).

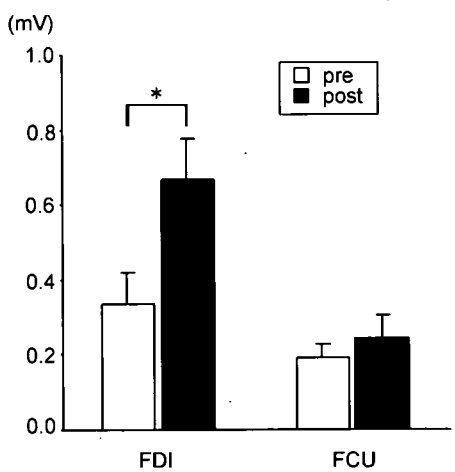

Fig. 8. Effects of QPS on somatotopy. Absolute amplitudes of cortical MEPs recorded from FDI and FCU muscles before (pre, bars) and after (post, filled bars) QPS-supra-360 applied over the *hot spot* for FDI ($n = 5$). Each bar shows the MEP amplitude (mV, mean ± SE). Only cortical MEPs from FDI muscle were facilitated after QPS conditioning. Asterisks, $p < 0.05$, by paired t-test.

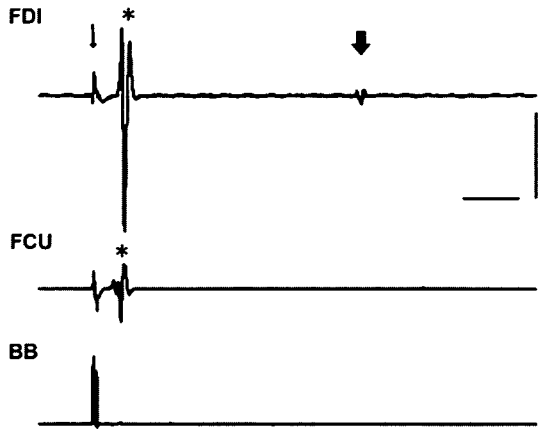

Fig. 9. Representative example of post-TMS EMG activity. Surface EMGs were recorded simultaneously from the right first dorsal interosseus (FDI), flexor calpi ulnaris (FCU) and biceps brachii (BB) muscles during QPS-supra-360. They are single EMGs from three muscles. Post-TMS EMG activity (arrow) was observed following a QPS train. The thin arrow indicates an artifact of stimulation. Asterisks, MEP elicited by one train of four magnetic pulses. Calibration bars, 50 ms, 0.5 mV.

Table 1
Rate of post-TMS EMG activity during QPS-supra-360 and QPS-sham-360

Subject	Post-TMS EMG activities/trains	
	QPS-supra-360	QPS-sham-360
A	8/360	18/360
B	0/360	0/360
C	7/360	8/360

4. Discussion

4.1. Comparison between QPS and PPS

The main finding of this study was that a long-lasting cortical MEP facilitation was induced by QPS. Results showed that the total number of pulses or the number of trains was unable to account for the disparity of duration of aftereffects between QPS and PPS. Therefore, the number of pulses per train might be critical for determining the duration of aftereffects, similar to the well-known results of animal experiments (Nakao et al., 2004).

The effects on MEPs during conditioning differed between QPS and PPS (Fig. 2), although MEPs to single-pulse TMS immediately after each conditioning were similarly facilitated (Fig. 3b). More specifically, we observed no marked facilitation of MEPs to paired TMS during PPS-supra-360, but MEPs to single-pulse TMS were enhanced immediately after conditioning. In addition, MEPs to QPS at the end of QPS-supra-360 were more enhanced than MEPs to single-pulse TMS immediately after conditioning. A possible explanation for this discrepancy in MEP size between the last part of conditioning and the first part of aftereffects is that different populations of descending volleys contribute to MEP generation in single-pulse, paired-pulse, and quadro-pulse TMS. At the end of both conditioning types, the motor cortical excitability might be similarly enhanced because the MEPs obtained immediately after the QPS and PPS conditioning were almost equivalent. However, the MEPs collected at the end of conditioning were different in size between the two conditioning types because one was MEP to paired-pulse TMS and the other to quadro-pulse TMS. In any case, a similar amount of facilitation was induced by both QPS and PPS when we evaluated motor cortical excitability, as indexed by MEP amplitude to single-pulse TMS, whereas it continued longer after QPS than PPS. These results support the hypothesis that the number of pulses per train plays an important role in determining the duration of the aftereffects and not so for the degree of size enhancement. Another implication from results of the present investigation is that single-pulse TMS should be used for correctly probing motor cortical excitability.

4.2. Possible origin of QPS aftereffects

In fact, QPS did not enhance the brain-stem MEPs. This finding strongly suggests that cortical MEP facilitation after QPS occurs at the motor cortex. Several possible explanations exist for cortical MEP facilitation during and after QPS.

First, excitability changes of the postsynaptic neuronal membrane of corticospinal neurons might occur after QPS (Woody et al., 1991; Aou et al., 1992). Several precedent pharmacological studies have proposed that motor thresholds reflect postsynaptic neuronal membrane excitability and a part of synaptic efficacy within the motor cortex (Mavroudakis et al., 1994, 1997; Inghilleri et al., 1996; Ziemann et al., 1996a,b; Chen et al., 1997a; Di Lazzaro et al., 2003). Because motor thresholds (both AMT and RMT) were unchanged after QPS (Fig. 7a), the neuronal membrane excitability changes might not have contributed to cortical MEP facilitation after QPS.

Second, an enhancement of cortico-cortical synaptic efficacy within the motor cortex might explain the aftereffects of QPS. Although motor thresholds were unchanged, QPS made the recruitment curves steeper (Fig. 7b). These results are probably attributable to the induction of an enhanced synaptic efficacy, which contributes much to the generation of MEPs, but little to the threshold determination (threshold depends on synapses for small sized MEPs). The enhancement of synaptic efficacy might occur either at the synapses between the corticospinal neurons and interneurons or between interneurons within the motor cortex. Whichever synaptic efficacy enhancement occurs, the spatial distribution of excitable elements can change, and more motor cortical neurons might be activated by stimuli at the same intensity, which in turn would cause a steeper recruitment curve.

Third, the aftereffect might be explained by a decrease of synaptic efficacy at the inhibitory interneurons. For example, rTMS, such as TBS, elicited a mixture of facilitatory and inhibitory effects on synaptic transmission (Huang

et al., 2005). Similarly, the magnitude of aftereffects of QPS presumably depends on the balance between inhibition and facilitation.

Fourth, a recent study examined the direct recording of descending volleys before, during, and after PPS at I-wave periodicity in a single subject (Di Lazzaro et al., 2007). The authors concluded that the facilitation produced by PPS at I-wave periodicity might involve circuits different from those involved in I-wave generation (Di Lazzaro et al., 2007). A similar mechanism might underlie the facilitatory aftereffects by QPS.

Despite the lack of any experimental evidence, the properties shown here might provide some clues for speculation on the mechanism of action of QPS-induced plasticity.

4.3. Properties and possible mechanisms for QPS aftereffects

Sustained and long-lasting modulation of MEP amplitudes has been proposed to reflect LTP-like plasticity of the human motor cortex by several rTMS interventions (Chen et al., 1997b; Ziemann et al., 1998; Stefan et al., 2000; Wolters et al., 2003; Huang et al., 2005; Quartarone et al., 2006). The long duration of the aftereffects of QPS (longer than 60 min) is comparable to the durations described in those studies.

According to previous knowledge of synaptic plasticity, one characteristic of LTP is cooperativity, which implies the existence of a threshold for LTP induction, depending on the stimulus intensity, the total number of pulses, and the pattern of tetanic stimulation (Malenka, 1991; Trepel and Racine, 1998). We found a threshold of QPS train numbers for inducing long-lasting motor cortical enhancement. More specifically, 360 trains of QPS induced a long-lasting MEP facilitation, although 180 trains of QPS did not.

Another characteristic of LTP is input specificity (Bliss and Collingridge, 1993), which means that LTP occurs only along the pathways activated by the conditioning stimulation: not along other pathways. We confirmed that QPS applied to the left-hand motor area for FDI induced facilitation of the right FDI, but not FCU.

These properties of QPS (the duration of facilitation, *cooperativity* and *input specificity*) led us to conjecture that QPS induces LTP or closely related phenomena in the human motor cortex. However, a limitation of our investigation is that we showed no other basic properties of LTP (i.e., *N*-methyl-D-aspartate (NMDA) dependency and associativity; Bliss and Collingridge, 1993). Notwithstanding, the present results provide at least some clues indicating the mechanism of action of rTMS. Further studies are necessary to clarify the mechanism of plasticity induction by QPS.

4.4. Safety issues

No subject reported any adverse effect during or after an intervention. Moreover, the spread of excitation to proximal muscles was not observed. The occurrence rate of post-TMS EMG activity during QPS-supra-360 was not different from that during QPS-sham-360. In many cases, it is difficult to distinguish post-TMS EMG activity from poor muscle relaxation (Chen et al., 1997b). Consequently, occurrence of post-TMS EMG activity during QPS-sham-360 might be a false positive indicating some voluntary activities. The present interpretation of these as after discharges is a kind of conservative approach we adopted for safety monitoring. These findings provide evidence that QPS can safely induce motor cortical plasticity. However, in the safety guideline of rTMS (Wassermann, 1998), a safe number of pulses at high-frequency stimulation (1–25 Hz) were determined based on the stimulus intensity normalized to RMT. Consequently, the establishment of new safety guidelines for complex rTMS protocols such as QPS, PPS (Thickbroom et al., 2006), or TBS (Huang et al., 2005) awaits further investigation. Although we never observed SE or any significant increment of after discharges during QPS, adequate EMG monitoring is necessary to recognize early signs of serious side effects during future monophasic rTMS studies.

4.5. Conclusion

We conclude that QPS can induce long-lasting, topographically specific enhancement of motor cortical excitability safely, which might be a consequence of the induction of LTP or closely related phenomena in the human motor cortex. Although we cannot define mechanisms of the QPS aftereffects, the present results provide useful information for further studies addressing the mechanism of plasticity induction by QPS.

Acknowledgements

Part of this work was supported by Research Project Grants-in-Aid for Scientific Research Nos. 17590865 (R.H.), 18590928 (Y.T.), and 16500194 (Y.U.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, grants for the Research Committee on rTMS treatment of movement disorders from the Ministry of Health, Labour and Welfare of Japan (17231401), the Research Committee on Dystonia, the Ministry of Health, Labour and Welfare of Japan, and a grant from the Committee of the Study of Human Exposure to EMF, Ministry of Internal Affairs and Communications.

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原　著　臨床神経生理学　35(6)：473—478，2007

反復経頭蓋磁気刺激の位相に関する生理的研究
—体性感覚誘発電位を用いて—

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要旨　本研究は磁気刺激の位相の違いが体性感覚誘発電位（SEP）に与える影響について検討することを目的とした。運動前野に0.2 Hzの反復経頭蓋磁気刺激（rTMS）を単相と二相の異なる位相およびshamコイルを用いる条件で与えた前後で正中神経刺激によるSEPを記録し，各条件でのrTMSによるSEPの変動について解析した。その結果，sham刺激以外の磁気刺激後には，緩徐な陰性電位変動が生じる傾向に加え，特に単相性の磁気刺激ではF3とF4におけるN30成分の振幅を増大させ，二相性の磁気刺激ではF4におけるN30成分の振幅のみを増大させた。これは単相性パルスによるrTMSのほうが二相性パルスによるrTMSよりも広範な影響を及ぼした結果と考えられ，運動前野に対するrTMSの位相の違いはSEPに対して異なる影響をもたらすことが示された。またこの違いがrTMSによる治療効果の違いを生じさせていることが推察された。

目　的

反復経頭蓋磁気刺激（rTMS）は中枢神経系に持続的な興奮性の変化を誘発する。rTMSによりもたらされる影響は刺激の頻度に依存して異なり，1 Hz以下で抑制性の効果[4,21)]をもたらし，5 Hz以上で興奮性の効果[13,14)]をもたらすと考えられている。このような効果を利用してrTMSの治療応用を目指した研究が進められているが一貫した結果は得られていない。運動野や運動前野の過活動が報告されている書痙に対しても，近年，低頻度のrTMSによる治療が試みられている[12,17)]。Muraseら[12)]は単相性パルスのrTMSを0.2 Hzで250回，補足運動野，運動前野，運動野に対して行い，運動前野への刺激でのみ治療効果が認められたと報告している。また二相性パルスによる1 HzのrTMSを使用した研究では，運動前野に対する1,800回の刺激では効果がなく[18)]，運動野に1,200回の刺激を与えた場合には治療効果があることが報告されている[17)]。このように研究者により刺激部位や位相，頻度の組合せにより効果が異なり，どのパラメーターがどのように影響していたのかについての詳細な検討は行われていない。

書痙では感覚運動連関機能の異常が指摘されていることから前述のrTMSによる治療メカニズムを検討するうえでrTMSによるSEPへの影響を調べた研究が手がかりになると考えられる。Muraseら[12)]と同様の刺激方法である単相性パルスによる0.2 HzのrTMSを健常者の補足運動野，運動前野，運動野に与えSEPへの影響を検討した結果，運動前野への刺激でのみ正中神経刺激によるSEPのN30成分が変化することが報告されている[22)]。また二相性パルスによる1 HzのrTMSを用いて同じ正中神経刺激のSEPに対する影響を調べた研究では，運動前野に対する刺激ではSEPは変化せず，運動野への刺激では変化することが報告されている[7)]。これらの書痙患者および健常者を対象として行われた研究で用いられたrTMSの設定には，0.2 Hzと1 Hzという頻度の違い，運動野と運動前野という刺激部位の違いとともに単相と二相という位相の違いがある。このような位相による刺

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受付日：2007年4月26日
採択日：2007年7月17日

激効果の違いについて，同一頻度の単相性パルスと二相性パルスのrTMSによる運動誘発電位(MEP)の振幅への影響の違いが検討されているが，単相性パルスによるrTMSのほうが刺激の効率が高いと報告されている[2,19]。しかし，これらの研究の多くは運動野を対象としており，運動前野での報告はみられない。またSEPに対する影響も調べられていない。そこで本研究では，書痙治療としてのrTMSの最適な刺激条件について検討することを目的とし，運動前野へのrTMSの位相の違いがSEPに与える影響について検討を行った。

実験方法

1．対　象

被験者は右利き健康成人男性8名(31.4±8.7歳)とした。実験に先立ち，被験者に実験の内容を十分説明し同意を得た。

2．実験手続き

被験者に与えるrTMSとして単相性パルスと二相性パルスによる刺激を行う条件と，さらにshamコイルを使って刺激する条件の3条件を設定し，それぞれの条件によるrTMSを行う前後で正中神経刺激によるSEPを記録した。なお各条件は1週間以上あけて行い，施行順は被験者ごとに無作為に設定した。磁気刺激は安全性ガイドラインに従って設計し[23]，研究の実施については徳島大学倫理委員会によって承認された手続きに基づき行った。

3．SEP

被験者はシールドルーム内の安楽椅子に座り，実験中開眼安静状態を維持してもらった。右手根部での正中神経への電気刺激によりSEPを誘発した。電気刺激は刺激強度を右短母指外転筋の運動閾値の直上とし，持続時間0.2 ms，刺激頻度1 Hzで表面電極により与えた。刺激電極は陽極を末梢側，陰極を中枢側に配した。記録電極は国際式10-20法によるF3，F4，およびC3，C4の2 cm後方にあたるC3'，C4'に設置し基準電極には両耳朶連結を使用した。眼球運動の記録のために左の眼角の2 cm上方と2 cm下方に電極を設置した。すべての電極は銀-塩化銀電極を用いた。サンプリングレートは10 kHzとし，記録帯域周波数は脳波用電極を1～5,000 Hz，眼球運動用電極は0.5～1,000 Hzとした(日本光電製MEB-2200)。脳波は電気刺激後0～100 ms後までの区間について記録し，オフラインでアーチファクトを除外したうえで200回以上加算した波形を解析に用いた。

4．rTMS

書痙への治療効果を報告しているMurase ら[12]の刺激方法に従い運動前野に対して0.2 Hzの頻度で250回の磁気刺激を行った。右の第一背側骨間筋から導出されるMEPが最大となる場所をhot spotとし，その2 cm前方，1 cm内側を運動前野とした。刺激強度はhot spotにおける安静時運動域値（RMT）の85%とした。RMTは50 μVのMEPが5/10回以上記録できる刺激装置の出力とした。単相性パルスによる刺激条件（mono条件）はMagstim 200 stimulatorに，二相性パルスによる刺激条件（bi条件）はMagstim rapid stimulatorに直径8.7 cmの8字コイル（Magstim社）を接続し刺激を行った。Sham刺激条件（sham条件）はMagstim 200 stimulatorにshamコイルを接続し刺激を行った。Shamコイルから放出された磁力は1回の刺激につき0.44 teslaであった。実験を通じて開眼安静状態を維持させた。

5．解　析

全被験者で同定された成分を解析対象とした。F3からはP14，P22，N30，N60の4成分，F4からはP14，P22，N30の3成分，C3'からはP14，N20，P26，N34，P45の5成分，C4'からはP14の1成分が導出された(**Fig. 1**)。上記の全成分について解析を行った。基線算出区間を刺激後2～6 msとして，各成分のpeak潜時とbase-to-peak振幅を計測した。刺激条件(mono条件，bi条件，sham条件)と時間(rTMS前，rTMS後)の相互作用について検討するために反復二元配置分散分析を行い，有意差のみられた成分について，刺激前後の変化をpaired t検定により確認した。いずれの解析においても有意水準は5%以下とした。

結　果

SEPの各成分におけるpeak潜時を解析した結果，すべての条件でrTMS前後での有意な変化は認められなかった。各成分の振幅について成分ごとに条件と時間を繰り返し要因とする反復二元配置分散分析を行った結果，F3のN30成分($F=7.482$，$p<0.01$)とF4のN30成分($F=6.131$，$p<0.05$)に相互作用が認められた。これらの成分において，条件ごとにrTMS前後の振幅の変化についてpaired t検定を行った結果，F3のN30成分ではmono条件でのみrTMS後に振幅が有意に増大していた($t=-7.114$，$p<0.001$)

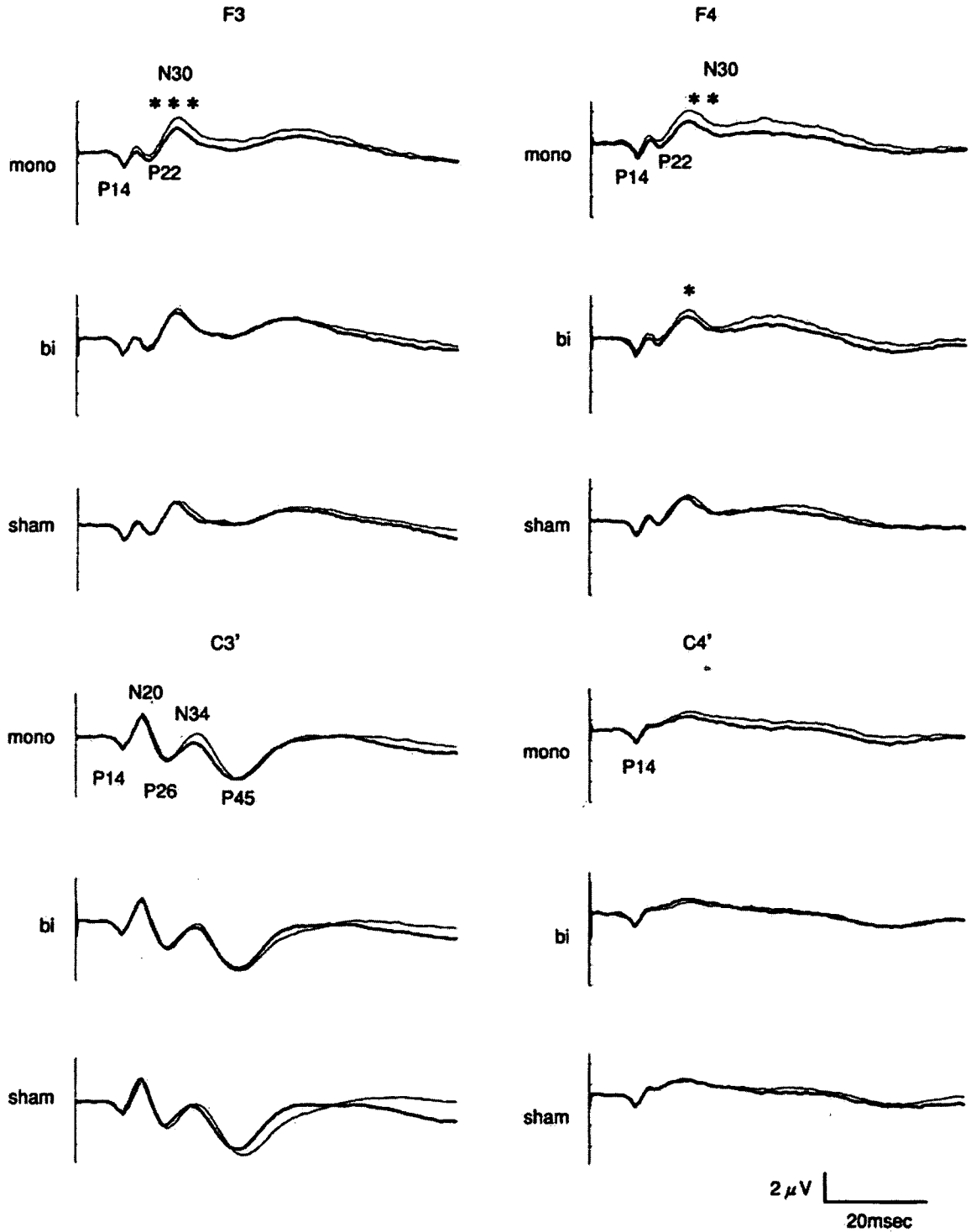

Fig. 1 Grand-averaged SEP waveforms are recorded from the electrodes at F3, C3', F4 and C4' before (thick wave) and after (thin wave) monophasic, biphasic and sham rTMS
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, mono : monophasic condition, bi : biphasic condition

が，bi 条件，sham 条件では変化はみられなかった (Fig. 2 a)。F4 の N30 成分では，mono 条件 ($t=-4.681$, $p<0.01$) と bi 条件 ($t=-3.177$, $p<0.05$) で有意に振幅が増大していたが，sham 条件では変化は認められなかった (Fig. 2 b)。

考 察

本研究では，rTMS の位相の違いによる影響を検討するために SEP を記録した。その結果，mono 条件では rTMS 後の SEP の振幅の増大が F3 と F4 の両方から記録された N30 成分でみられたのに対して，bi 条件では F4 の N30 成分のみにみられ，位相による

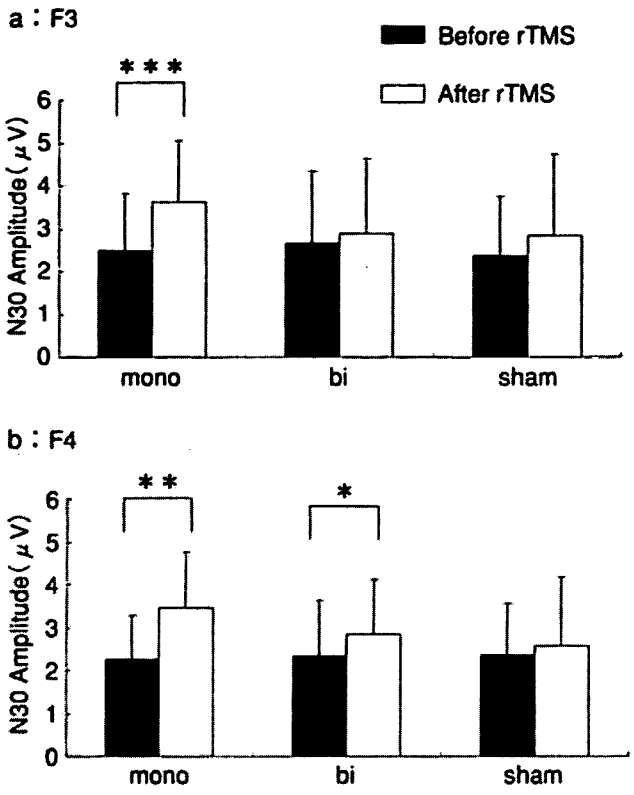

Fig. 2 Comparison of amplitudes (±S. D.) of N 30 components between before (black bar) and after (white bar) rTMS
At F3, the amplitude of N30 component increased after only monophasic rTMS (a). At F4, the increases of N30 amplitudes were shown after not only monophasic but biphasic rTMS (b).
*$p<0.05$, **$p<0.01$, ***$p<0.001$, mono : monophasic condition, bi : biphasic condition

rTMSの刺激効果に違いがみられた。ただし，これらの振幅変動の背景には，緩徐な陰性電位変動の影響も考えられた。

mono条件での記録では，F3，F4，C4'から記録された波形においてP14成分後からN60成分にかけて，rTMS後の緩徐な陰性電位変動がみられた。この変動は，P14後からみられること，広範にわたっていることから，N18成分もしくはそれを含む持続性電位の変動ではないかと考えられる。Sonooら[20]によれば，N18は脳幹の楔状側核由来とされている。運動野へのrTMSが脳幹梗塞後の慢性疼痛を悪化させたという報告[10]があることからも，同じように視床との連絡がある運動前野へのrTMSが視床を介して脳幹へ影響を及ぼした可能性は否定できない。

しかし，一方で，同じmono条件のC3'から記録された波形やbi条件でのF4以外の電極からの記録については，持続性の電位変動はみられず，N18の変動を相殺するような要因の存在も考えられるが，本実験で得られた結果からの考察は困難であり，この問題の解決に対して，よりN18を明確に記録することのできる頭部外基準電極を用いた研究が今後期待される。

いずれにしてもN30成分の振幅の増大はこのような緩徐性電位変動に付加する形で観察され，特にrTMSの同側から記録されたSEPは，運動前野に対して単相性パルスによるrTMSを与えたUrushiharaら[22]の報告と同様に，F3のN30成分の増大がみられたが，bi条件ではどの成分にも変化が認められなかった。このことから運動前野に対するrTMSによるSEPへの影響としてmono条件がbi条件よりも強い効果をもつことが推測される。運動野にrTMSを与え単相性パルスと二相性パルスの刺激効果を比較した先行研究[2,19]では単相性パルスによるrTMSのほうが強力であると報告されている。この理由として単相性パルスでは刺激により発生する過電流が一方向に流れるのに対し，二相性パルスによる刺激では双方向に流れ刺激効果を打ち消してしまうために効率が悪くなることがあげられている。本実験でみられたSEPに対する影響の違いもこのことが関係していたと考えられる。振幅に変化がみられたF3から記録されるN30成分の起源について，一次感覚野（3b野，1野）を起源とする接線方向の発生源の陰性成分[1]や3b野と4野からの合成成分[6,8]，補足運動野[3]などが報告されている。F3のN30成分が一次感覚野に起源をもつ成分の鏡像であるとすれば，C3'から記録される成分も同様に変化するはずであるが，今回の結果においてC3'ではどの成分も変化をしていなかったことから本研究で用いた単相性パルスによるrTMSは前頭葉を起源とする成分により強く影響している可能性が示唆された。

rTMSの対側で記録したSEPでは，mono条件とbi条件ともにF4から導出されるN30成分の振幅が増大していた。F4で記録されるN30成分の発生源はF3のN30と同一起源の遠隔電場電位であると考えられ，mono条件でみられたF4のN30成分の振幅の増加はF3でのN30成分の増加を反映するものであるだろう。bi条件では，F4でのみN30成分の増大がみられたが，F3のN30成分増大のメカニズムで検討したように運動前野へのrTMSは前頭葉の発生源により強く影響し，これらの発生源の活動の総和としてのdipoleの向きを変化させていた可能性が考えられる。

その一方，一次体性感覚野の発生源での変化がない，もしくは軽微であったとすればF3，C3'，C4'では振幅が変化せずF4のみで変化が表れることになる。このdipoleの変化がbi条件でのF3とF4の違いを生じさせていたと推測され，mono条件の結果とbi条件での結果の違いは，dipoleの変化の大きさが異なったためであるとも考えられる。いずれにしてもmono条件とbi条件はSEPに異なる影響を及ぼし，F3のN30成分に強く影響を与えたのはmono条件であった。

上肢ジストニア患者を対象とした先行研究では，SEPのfrontal componentは振幅が増加しており，病態生理学的に非一次運動野（PMC）の活動が亢進しているのを反映するものと考察されている[15]。治療効果を期待する磁気刺激によって健常者のN30成分が増大した今回の結果は一見矛盾した結果のようにもみえる。しかし，患者を対象とした研究とは異なり，健常者のN30成分は運動想起[5]や運動準備状態[16]など非一次運動野（PMC）の活動亢進時に振幅が減少することが報告されており，磁気刺激後に健常者でのN30成分の振幅が増大することは同部位の活動性を低下させた結果とも考えられる。事実，健常者にみられる運動準備状態でのN30成分の振幅減衰（pre-movement gating）[16]は，書痙患者においては認められない[11]。これらから，本実験において単相性パルスが二相性パルスよりもN30成分をより大きく変化させたという結果は，単相性パルスがより強い抑制性効果をもつことを示唆し，単相性パルスによるrTMSでは治療効果があり二相性パルスによるrTMSでは効果がなかったという先行研究[18]とも矛盾しない。ただし，書痙患者の運動前野ではrTMSに対する反応性そのものが健常者と異なることも考えられ，書痙患者においてSEPがどのように変動するのかについては，今後実際に患者を対象とした比較研究を行う必要がある。

0.2 Hzの単相性パルスによるrTMSは，運動前野へ与えればSEPを増大させるが運動野では効果がなく[22]，一方，1 Hzの二相性パルスによるrTMSは運動野への刺激では効果を示すが運動前野では効果がないことが報告されている[7]。これらのことからも本実験での結果はすべての皮質領域に対して単相性パルスによるrTMSが二相性パルスによるrTMSよりも適していることを支持するものではないが，少なくとも運動前野に対しては単相性パルスが二相性パルスよりも高い効果を示すようである。ただし，Gerschlagerら[9]は二相性の磁気刺激を1 Hzの頻度で運動前野に対して1,500回与えることによりMEPの振幅が減少すると報告している。これは二相性パルスによる刺激が運動前野に対して全く影響を与えることができないのではなく十分な刺激回数を確保すれば影響を与えることができる可能性を示す結果であると考えられ，今後刺激回数についての検討も必要であろう。本研究の結果，もし刺激回数を追加することにより二相性パルスでの刺激効果が得られたとしても，より少ない回数，低い頻度で刺激効果を与えられるという安全性の利点から，治療応用においてはやはり単相性パルスによるrTMSがより有用となる可能性が示唆された。

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The difference of the somatosensory evoked potential modulation between monophasic and biphasic repetitive transcranial magnetic stimulation over premotor cortex

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To investigate the differences of therapeutic effects between phases of magnetic stimulation on the patients with writer's cramp, we compared the changes of somatosensory evoked potential (SEP) by monophasic with biphasic repetitive transcranial magnetic stimulation (rTMS) in 8 healthy volunteers. With monophasic, biphasic or sham stimulation, 250 magnetic stimuli were provided over the left premotor cortex at 0.2 Hz. Stimulation intensity was 85% of the individual resting motor threshold. Median SEP was evoked by the electrical stimulation at right wrist, and were recorded at F3, F4, C3' and C4' (International 10-20 system) immediately before and after the application of rTMS. The amplitudes of N 30 components increased at both F3 and F4 after monophasic rTMS, while only at F4 after biphasic rTMS. Monophasic rTMS over premotor cortex could be more useful as a treatment for writer's cramp than biphasic rTMS.

Key Words : somatosensory evoked potential, repetitive transcranial magnetic stimulation, monophasic, biphasic

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Effect of repetitive transcranial magnetic stimulation applied over the premotor cortex on somatosensory-evoked potentials and regional cerebral blood flow

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Received 18 September 2005; revised 25 November 2005; accepted 15 December 2005
Available online 8 February 2006

Somatosensory-evoked potentials (SEPs) are attenuated by movement. This phenomenon of 'gating' reflects sensorimotor integration for motor control. The frontal N30 component after median nerve stimulation was shown to be reduced in amplitude prior to hand movement. To investigate the mechanism of this sensory gating, we recorded median SEPs immediately before and after application of monophasic very low-frequency (0.2 Hz) repetitive transcranial magnetic stimulation (rTMS) of 250 stimuli over motor cortex (MC), premotor cortex (PMC), or supplementary motor area (SMA) in 9 healthy volunteers. The stimulus intensity for MC or PMC was set 85% of the resting motor threshold for the hand muscle, and that for SMA was at the active motor threshold for the leg muscle. SEPs showed significant increases in amplitudes of the frontal N30 component after PMC stimulation, but not after SMA or MC stimulation. Low-frequency (1 Hz) biphasic stimulation over PMC showed no significant N30 changes in 6 out of 9 subjects tested, indicating the effect being specific for 0.2 Hz monophasic stimulation. To examine the functional anatomy of the N30 change, single photon emission computed tomography was performed immediately before and after monophasic 0.2 Hz rTMS over PMC in all the 9 subjects. Regional cerebral blood flow showed significant increases mainly in PMC and prefrontal cortex, indicating the involvement of these cortical areas in sensory input gating for motor control.

Keywords: Somatosensory-evoked potential; Repetitive transcranial magnetic stimulation; Single photon emission computed tomography; Sensorimotor integration; Gating; N30

Introduction

Somatosensory-evoked potentials (SEPs) have been used to explore the central mechanism of sensory input processing. SEP amplitudes are attenuated during voluntary (Papakostopoulos et al., 1975; Cohen and Starr, 1987) and passive (Brooke et al., 1996) movement or under mental simulation of movement (Cheron and Borenstein, 1992; Rossi et al., 2002). This attenuation is referred to as "gating". SEPs are also gated before movement (Starr and Cohen, 1985; Shimazu et al., 1999; Asanuma et al., 2003), and clarification of its precise mechanism should help understand the sensorimotor integration in motor control of normal subjects and patients with basal ganglia disorders (Murase et al., 2000).

Transcranial magnetic stimulation (TMS) is a useful tool for studying the excitability and conductivity of the entire motor pathway from the cortex to the target muscle or the connectivity of the cerebral cortex. Recently, repetitive TMS (rTMS) has been used to apply a series of stimuli to a specific cortical area (Siebner and Rothwell, 2003; Murase et al., 2005). This can lead to long-lasting aftereffects on the excitability not only in the area itself, but also those areas that are functionally linked to it (Munchau et al., 2002). Because of its inhibitory effect on cortical excitability, low-frequency rTMS (<1 Hz) has been used for treating disorders related to brain hyperexcitability (Siebner et al., 1999; Hoffman and Cavus, 2002; Murase et al., 2005), whereas high-frequency rTMS (>5 Hz) exerts an excitatory influence on the cortex.

Non-primary motor areas may have an important role in sensorimotor integration for motor control because of their closer link to basal ganglia than the primary motor cortex. Although several studies have reported the effects of rTMS on SEPs (Enomoto et al., 2001; Tsuji and Rothwell, 2002; Satow et al., 2003; Ragert et al., 2004), only a few investigations have explored

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.neuroimage.2005.12.027

the effect of rTMS applied over non-primary motor areas (Siebner et al., 2003; Murase et al., 2005). In this study, using the clinically effective stimulation parameters in writer's cramp (Murase et al., 2005), we recorded SEPs immediately before and after application of monophasic very low-frequency (0.2 Hz) rTMS over the primary and non-primary motor cortices of normal subjects to investigate the role of these areas on processing sensory input. In rTMS over PMC, we also recorded SEPs immediately before and after biphasic low-frequency (1 Hz) rTMS to investigate the frequency or phase specificity of the rTMS aftereffects on median SEPs. In addition, we recorded single photon emission computed tomography (SPECT) and compared regional cerebral blood flow (rCBF) images immediately before and after monophasic 0.2 Hz rTMS over PMC to investigate changes in cortical blood flow associated with those in SEPs.

Methods

Subjects

Nine healthy right-handed subjects (all men aged 30.2 ± 8.8 years) participated in this study. All subjects gave their informed consent for the study, which was approved by the Ethics Committee of the University of Tokushima, School of Medicine. The subjects were free from neurological and psychiatric diseases.

Experimental design

SEPs were recorded immediately before and after application of monophasic rTMS; 250 pulse trains were delivered at 0.2 Hz over the right-hand motor area (MC), the premotor area (PMC), or the supplementary motor area (SMA) in 9 subjects. To compare the effects of monophasic 0.2 Hz rTMS to the standard low-frequency rTMS used in many previous studies, we also recorded SEPs immediately before and after application of biphasic 1 Hz rTMS (250 pulse trains) over PMC in 6 of 9 subjects. The sessions were performed on separate days in a counterbalanced order at intervals of at least 1 week. In addition, we evaluated the effect of premotor monophasic very low-frequency rTMS on cortical blood flow using SPECT in the same 9 subjects who had SEP studies on separate days at least 1 week apart from the SEP recording session. In the present study, parameters of rTMS were in accordance with the international safety guidelines (Wassermann, 1998).

Recording and analysis of SEPs

In an electrically and auditory shielded room, the subjects sat comfortably on a reclining chair with their feet on the foot-rest and the neck supported by a U-shaped pillow to avoid head movement. SEPs were obtained by applying a 0.2-ms square electrical pulse at 1 Hz to the median nerve at the right wrist through a pair of surface electrodes. The intensity was adjusted just above the thumb twitch threshold. SEPs were recorded with silver chloride disk surface electrodes at F3 and C3′ (2 cm posterior to C3), according to the International 10–20 system. The linked earlobe electrodes served as the reference. The impedance of these electrodes was kept below 3 kΩ. The electrooculogram (EOG) was also recorded with a pair of silver chloride disk electrodes at 2 cm above and 2 cm below the right outer canthus. Signals from scalp electrodes and the EOG were amplified and acquired at a sampling rate of 10 kHz and filtered at 1–5000 Hz and 0.5–1000 Hz respectively (MEB2200 amplifier; Nihon Koden, Tokyo, Japan). All signals were recorded for 100 ms after the onset of median nerve stimulation and stored on a personal computer for off-line analysis. We collected at least 150 artifact-free sweeps and then averaged them off-line. To ensure SEP reproducibility, electrodes were left attached at the initial positions without being connected to the amplifier throughout the application of rTMS. Our preliminary studies confirmed that no current injury or electrode heating occurred after this procedure.

We identified 5 components at C3′, an initial positive peak with a latency of 10–16 ms (P14), a following negative large peak (N20), a second positive peak (P26), a second negative peak (N34) and a third positive peak (P45). For recordings from F3, 3 components following P14 were analyzed: a positive peak of 15–25 ms (P22) and two negative peaks (N30 and N60). We measured the base-to-peak amplitudes and the peak latencies of these components. The baseline was defined as the segment between 2 and 6 ms after stimulation.

In 2 of 9 subjects, we recorded median SEPs before and after application of monophasic 0.2 Hz rTMS over PMC with 62 scalp electrodes (a Ag/AgCl surface electrode cap system, Quickcap; Neuromedical Supplies Inc., El Paso, Texas, USA) to obtain SEP topographical mapping on the subject's real head model. Electrical stimulation was applied to the right median nerve as mentioned above. Signals were sampled at 5000 Hz and filtered at 1–1000 Hz (SynAmp amplifier and Scan software; Neuroscan Inc., El Paso, Texas, USA). We performed two recording sessions before and after application of rTMS, and about 200 sweeps were recorded in a single session. The data were stored in a personal computer and grand-averaged over 200 artifact-free sweeps for off-line analysis. With these waveforms, topographical maps of N30 components were calculated on a reconstructed realistic head model from their MRI images (Curry software; Neuroscan Inc., El Paso, Texas, USA).

rTMS

We used monophasic rTMS at 0.2 Hz over three sites (MC, PMC, SMA) by the same procedure in the previous clinical study with writer's cramp (Murase et al., 2005) and biphasic rTMS at 1 Hz only over PMC.

For stimulating MC on the left, a figure-of-eight stimulation coil (outside diameter of one half-coil, 8.7 cm) connected to a Magstim 200 stimulator (2.2 T at the coil surface when connected to the Magstim 200; Magstim Co. Ltd., OHR Wales, UK) was placed over the area 2 cm anterior and 3.5 cm lateral to Cz (International 10–20 System). The intensity of stimulation was increased from 30% of the maximum output of the stimulator in 5% steps until an MEP became just visible. The coil was then moved in 0.5-cm steps in all four directions, medially, laterally, posteriorly and anteriorly, until the maximum MEP was found on the right first dorsal interosseous muscle (hot spot). The stimuli were applied over the 'hot spot' with the figure-of-eight coil at a stimulus intensity set at 85% of the resting motor threshold (RMT).

The stimulation site for PMC was determined 2 cm anterior and 1 cm medial to the hot spot over the left hemisphere (Schluter et al., 1998). This was estimated from the dorsal premotor cortex established in a PET study (Fink et al., 1997). Stimuli were applied with the figure-of-eight coil, and the stimulus intensity was set at 85% of RMT for MC. We used the same coil and stimulator in MC session for monophasic 0.2 Hz stimulation. For biphasic 1 Hz

stimulation session, we used a figure-of-eight coil connected to Magstim rapid stimulator (Magstim Co. Ltd., OHR Wales, UK) to estimate RMT for MC and to deliver rTMS over PMC at 85% RMT using the same procedure as in monophasic stimulation.

The site for stimulating SMA was 2 cm anterior to the leg motor area (Muri et al., 1994; Fink et al., 1997). A double-cone coil (Magstim Company Limited, OHR Wales, UK; outside diameter of one half-coil, 12.5 cm; angle of two surfaces, 95°) connected to Magstim 200 stimulator (1.4 T at the coil surface when connected to Magstim 200) was used. We searched for the leg motor area during active contraction of the leg muscles. Subjects were asked to continuously contract the right tibialis anterior muscle with a constant force of approximately 50% of the maximum EMG output, which was fed back to the subjects by sound. The double-cone coil was then placed 2 cm anterior to the Cz. The stimulus intensity was increased from 20% of the maximum output in 5% steps until an MEP larger than 200 μV became just visible. The coil was then moved in 0.5-cm steps posteriorly or anteriorly and, if necessary, also medially or laterally, until the point of the maximum MEP was reached (the leg motor area). We then determined the active motor threshold (AMT), which was defined as the lowest stimulus intensity at which 5 out of 10 consecutive stimuli elicited a reliable MEP larger than 200 μV. We applied rTMS over SMA on the sagittal midline (Muri et al., 1994; Fink et al., 1997; Cunnington et al., 1996) with the active motor threshold intensity for the leg motor area. In the leg motor area, RMT is often not attainable in some subjects. Since AMT in the hand motor area is approximately 85% of RMT (Murase et al., 2005; Rounis et al., 2005), we chose the AMT for the leg muscle for stimulation of SMA.

In all conditions, the stimulation coil was held by hand. The coil position was marked on the head clearly with red ink to ensure accurate repositioning of the coil and was monitored continuously throughout the experiment. To confirm the anatomical position of the coil with regard to the subject's own cortical areas, we reproduced the anatomical TMS coil positions on the realistic cortex model-reconstructed MRI images using an image-guided TMS system (Brainsight: Magstim Co. Ltd., Carmarthenshire, Wales, UK) on 1 of 9 subjects (Fig. 1). In all of three conditions, the coils were found at appropriate anatomical positions.

SPECT

The perfusion SPECT images were measured before and after application of rTMS over PMC. Each subject received an injection of 555 MBq of 99mTc-ethylcysteinate dimer (ECD) via an intravenous line in order to avoid pain. Data acquisition was started 5 min after injection and performed with a double-head gamma camera (E.CAM Signature; SIEMENS, USA) with a total acquisition time of 7 min. Then, the subjects were given monophasic 0.2 Hz rTMS (250 pulses) over PMC. As soon as the application of rTMS finished, they received an injection of 99mTc-ECD and underwent SPECT studies again. During this session, the subjects lay supine on the scanning bed and were instructed not to move. The head of each subject was immobilized using a head holder.

Data were reconstructed so that images were converted into DICOM format after transfer to a workstation. All images were reconverted into ANALYZE format for statistical parametric mapping analysis and underwent normalization onto the template and smoothing using easy Z score imaging system (eZIS, version 2.0.0, developed by Matsuda H. of National Center of Neurology and Psychiatry, Mizumura S. of Nihon Medical University, Souma S. and Takemura N. of Daiichi Radioisotope Laboratory; Matsuda et al., 2004; Kanetaka et al., 2004). The differences in adjusted rCBF between before and after rTMS were determined by a voxel-by-voxel paired t test setting at height threshold (P = 0.001), uncorrected for independent multiple comparison. These differences were considered significant if they survived a correction for multiple comparisons with cluster level at P = 0.001 with the

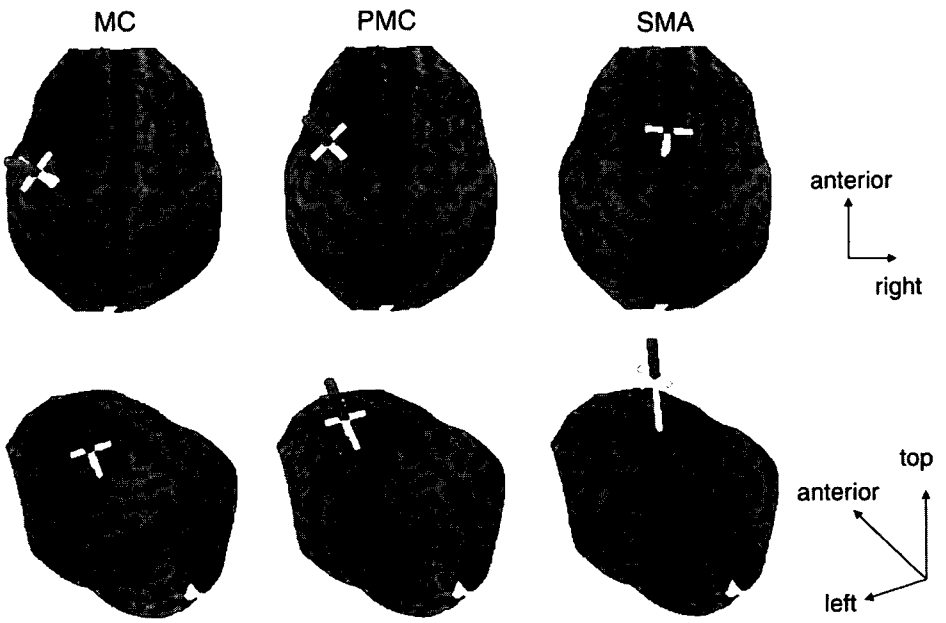

Fig. 1. TMS coil positions on realistic cortex model reconstructed MRI images from top (upper row) and left-sided back view (lower row). The cross-points of the yellow bars indicate the centers of the figure-of-eight or double-cone coils. The yellow bars indicate the sagittal and horizontal center lines of the coils to represent the horizontal plane of the coils. The red bars show the directions of the vertical axis through the center of each coil. The yellow lines as extended red lines are placed anatomically on each target area.

statistical parametric mapping software (SPM for windows, version 1.01, programmed by Sergey Pakhomov and Nick Tsygankov). The parametric maps were generated to determine significant regions of only increased activity because no regions reached significant decreases in activity. Then, the coordinates that reached the significance level in a MNI standard brain model were transformed into the Talairach and Tournoux coordinates (Talairach and Tournoux, 1988).

Statistical analysis of SEPs

We compared the aftereffects of rTMS between stimulation sites using the data from all 9 subjects who had SPECT studies. To evaluate the changes after rTMS, we subtracted SEP data before rTMS from those after rTMS for each measured component. These differences were analyzed by two-way repeated measures ANOVA using two factors: COMPONENT (latencies and amplitudes of each component) and SITE (stimulation sites at MC, PMC and SMA). When statistical significance is reached, we performed one-way repeated measures ANOVA using SITE as a factor to examine the stimulation site specific effects of rTMS. Post hoc comparison was carried out using Scheffe's F test.

Using the data from 6 of 9 subjects who participated in biphasic 1 Hz rTMS over PMC session, we compared the aftereffects of monophasic 0.2 Hz to biphasic 1 Hz rTMS over PMC. The differences between before and after rTMS were analyzed by two-way repeated measures ANOVA using two factors: COMPONENT and FREQUENCY (stimulation frequencies at 0.2 Hz and 1 Hz). When statistical significance is reached, paired t test was carried out for the examination of the stimulation frequency-specific effects and for the amplitudes of SEP components before and after rTMS. All data were analyzed with standard statistical software (Statview; SAS Institute, Cary, USA).

Results

SEPs

Fig. 2 shows the grand-averaged waveforms from 9 subjects. Because of the variations of latencies across the subjects, grand-averaged waveforms were constructed by adjusting the time to coincide the P14 peaks of each average. At both electrodes, the subcortical far-field P14 component was the first activity detected in all subjects. Table 1 shows the peak latencies and amplitudes of each component and their differences before and after application of monophasic 0.2 Hz rTMS over three cortical areas (MC, PMC, SMA).

The changes of *latencies* of median SEP components following rTMS showed no differences in two-way repeated measures ANOVA; SITE × COMPONENT ($F[16,128] = 0.930$, $P = 0.537$). In the *amplitudes* of SEP components, SITE × COMPONENT interaction was significant ($F[16,128] = 2.108$, $P = 0.012$), and one-way repeated measures ANOVA for these components using the factor of SITE (MC versus PMC versus SMA) was carried out. The factor of SITE was significant on two frontal negative components (N30: $F[2,16] = 10.257$, $P = 0.014$, N60: $F[2,16] = 3.765$, $P = 0.046$). Post hoc analysis was performed for these components. For N30 component, the amplitude change after application of monophasic 0.2 Hz rTMS over PMC was

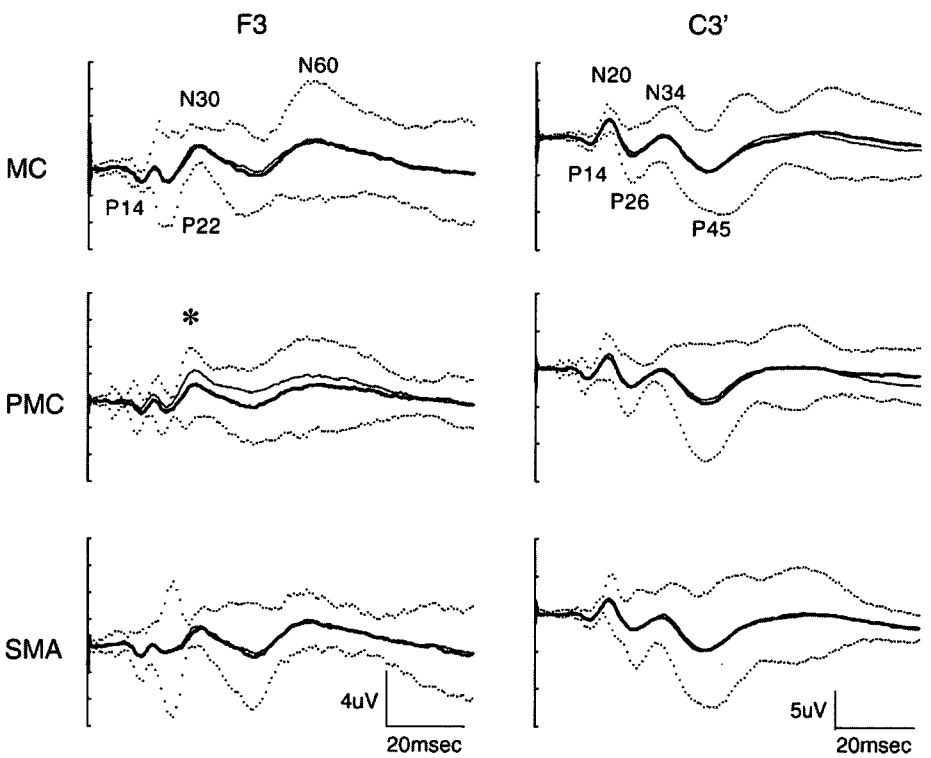

Fig. 2. Grand-averaged SEP waveforms from F3 (left column) and C3′ (right column) before (thick wave) and after (thin wave) application of monophasic 0.2 Hz rTMS over each stimulated site, MC, PMC and SMA. After application of rTMS over MC or SMA, no major changes occurred in SEPs, whereas significant increase in amplitude of N30 component (asterisk) was observed after application of rTMS over PMC. Dotted lines show 95% confidence interval of each SEP waveform recorded before rTMS.

Table 1
Peak latencies (a) and amplitudes (b) of each SEP component before and after application of rTMS over MC, PMC or SMA

(a) Latency (ms)

	F3											
	P14			P22			N30			N60		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
MC	13.7 ± 0.76	13.7 ± 0.97	0.0 ± 0.40	20.0 ± 1.37	20.2 ± 1.48	0.1 ± 0.36	28.6 ± 2.70	29.1 ± 2.69	0.5 ± 0.54	59.3 ± 3.45	58.6 ± 3.96	−0.8 ± 2.12
PMC	13.8 ± 0.90	13.5 ± 0.82	−0.3 ± 0.37	20.0 ± 1.23	20.0 ± 1.01	−0.0 ± 0.85	28.4 ± 1.98	28.4 ± 2.76	−0.0 ± 1.94	58.3 ± 4.95	57.5 ± 3.88	−0.8 ± 2.50
SMA	13.7 ± 0.82	13.9 ± 0.84	0.2 ± 0.32	20.1 ± 1.64	20.6 ± 1.66	0.5 ± 0.82	29.3 ± 3.27	28.9 ± 3.40	−0.4 ± 1.69	56.0 ± 3.45	56.7 ± 3.25	0.7 ± 3.11

	C3′														
	P14			N20			P26			N34			P45		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
MC	13.8 ± 0.76	13.7 ± 0.99	−0.1 ± 0.43	18.9 ± 0.86	18.9 ± 1.29	−0.1 ± 0.51	24.6 ± 1.22	25.0 ± 1.16	0.4 ± 0.74	33.2 ± 1.31	33.3 ± 1.32	0.1 ± 1.30	45.0 ± 3.01	44.4 ± 2.74	−0.6 ± 1.49
PMC	13.8 ± 0.85	13.7 ± 0.90	−0.1 ± 0.27	19.1 ± 0.91	19.1 ± 0.77	0.1 ± 0.20	24.9 ± 1.47	24.6 ± 1.24	−0.3 ± 0.63	32.3 ± 2.00	32.5 ± 1.70	0.2 ± 1.59	43.7 ± 2.01	43.8 ± 1.84	0.1 ± 1.28
SMA	13.8 ± 0.77	14.2 ± 0.77	0.4 ± 0.40	19.1 ± 0.88	19.1 ± 0.78	0.1 ± 0.32	24.8 ± 1.46	24.9 ± 1.32	0.1 ± 0.60	32.4 ± 1.58	32.9 ± 1.81	0.5 ± 1.32	44.2 ± 2.17	44.6 ± 2.00	0.4 ± 1.41

(b) Amplitude (μV)

	F3											
	P14			P22			N30			N60		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
MC	1.17 ± 0.27	1.21 ± 0.45	0.05 ± 0.44	1.57 ± 1.67	1.18 ± 1.30	−0.40 ± 0.70	−2.14 ± 0.63	−2.11 ± 0.87	−0.03 ± 0.51	−2.58 ± 1.65	−2.60 ± 1.77	0.02 ± 0.67
PMC	1.30 ± 0.39	1.21 ± 0.55	−0.09 ± 0.36	1.13 ± 0.87	0.89 ± 0.63	−0.24 ± 0.66	−1.64 ± 1.31	−2.52 ± 1.53	**0.88 ± 0.55**	−1.82 ± 1.42	−2.65 ± 1.34	0.83 ± 0.51
SMA	1.22 ± 0.69	1.15 ± 0.66	−0.07 ± 0.40	1.32 ± 1.75	1.44 ± 1.96	0.12 ± 0.57	−1.75 ± 0.58	−1.95 ± 0.63	0.20 ± 0.32	−2.56 ± 2.28	−2.27 ± 1.09	−0.29 ± 1.11

	C3′														
	P14			N20			P26			N34			P45		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
MC	1.17 ± 0.51	1.07 ± 0.44	−0.10 ± 0.41	−2.76 ± 1.24	−2.79 ± 1.13	0.03 ± 0.64	2.88 ± 2.25	2.70 ± 1.55	−0.18 ± 0.58	−0.58 ± 1.80	−0.88 ± 1.98	0.30 ± 0.61	4.94 ± 2.37	4.53 ± 3.15	−0.41 ± 1.52
PMC	1.33 ± 0.39	1.21 ± 0.38	−0.12 ± 0.12	−2.37 ± 0.80	−2.62 ± 0.90	0.25 ± 0.44	2.89 ± 1.84	2.96 ± 2.28	0.08 ± 0.74	−0.29 ± 0.84	−0.58 ± 1.28	0.28 ± 0.74	5.24 ± 3.18	4.78 ± 2.90	−0.46 ± 0.99
SMA	1.07 ± 0.43	1.07 ± 0.37	0.01 ± 0.35	−2.29 ± 0.89	−2.42 ± 0.94	0.13 ± 0.44	2.82 ± 2.16	2.83 ± 2.00	0.01 ± 0.73	−0.05 ± 1.64	−0.29 ± 1.53	0.34 ± 0.61	5.02 ± 3.61	4.88 ± 3.32	−0.14 ± 0.56

Values are expressed as mean ± standard deviation. Δ indicates subtraction of values before from those after rTMS. Bold figures show the significantly increased value after rTMS over PMC than MC or SMA.

significantly larger than that after MC stimulation ($P = 0.021$) or SMA stimulation ($P = 0.014$). But, N60 amplitude change after application of monophasic 0.2 Hz rTMS over PMC was not different from after MC stimulation ($P = 0.201$) and SMA stimulation ($P = 0.053$).

In addition, to confirm whether this PMC rTMS aftereffect on median SEP is specific in very low-frequency (monophasic 0.2 Hz) rTMS or not, we compared the aftereffects of monophasic 0.2 Hz rTMS over PMC on median SEP to biphasic 1 Hz rTMS in 6 of 9 subjects (Fig. 3 and Table 2). The changes of *latencies* of median SEP components following rTMS at both frequencies showed no differences in two-way repeated measures ANOVA: FREQUENCY × COMPONENT ($F[8,40] = 0.281$, $P = 0.969$). In the *amplitudes* of SEP components, FREQUENCY × COMPONENT interaction was significant ($F[8,40] = 3.740$, $P = 0.024$). The significant differences between the aftereffects of monophasic 0.2 Hz and biphasic 1 Hz rTMS were found for frontal N30 ($P = 0.022$) and N60 ($P = 0.012$) components and parietal N34 ($P = 0.020$) component by paired t test. However, the amplitude differences before and after rTMS were not significant for the parietal N34 ($P = 0.27$) in contrast with frontal N30 ($P = 0.002$) and N60 ($P = 0.029$). Therefore, the changes of these frontal components after monophasic 0.2 Hz stimulation were significantly larger than those after biphasic 1 Hz stimulation, and the differences were significant in frontal N30 and N60 only after monophasic 0.2 Hz stimulation.

We also recorded median SEPs using a 62-electrode cap system in 2 subjects. Topographical mapping at N30 latency demonstrated frontal N30 enhancement after application of monophasic 0.2 Hz rTMS over PMC, whereas parietal positive components were unchanged (Fig. 4), suggesting that SEP changes by rTMS occurred mainly at the frontal radial component rather than the tangential component as discussed below.

SPECT

The analyses of SPECT images revealed significant increases of cerebral blood flow in several regions after application of monophasic 0.2 Hz rTMS over PMC, whereas there were no regions with significant decreases. Fig. 5 shows areas with significant changes in cerebral blood flow after rTMS, and Table 3 shows their exact coordinates. These areas showing significant increases in blood flow included the regions, left middle frontal gyrus and precentral gyrus, under and near the magnetic coil (Fig. 5). These gyri correspond to areas 9 and 6 in Brodmann cytoarchitectural map of the human brain and include PMC and prefrontal cortex. An additional region showing blood flow increase was the cingulate gyrus.

Discussion

In the present study, we compared median SEPs before and after application of monophasic very low-frequency subthreshold rTMS over the primary and non-primary motor cortices. Application of monophasic 0.2 Hz rTMS over PMC, but not over MC or SMA, significantly increased the amplitude of frontal N30 component, but not of the parietal counterpart, and this effect was not seen after biphasic 1 Hz rTMS over PMC. This change was associated with increased rCBF in PMC and prefrontal cortex, as confirmed using SPECT imaging analysis. Using the same stimulation parameters as those in this study, we have shown that subthreshold monophasic very low-frequency rTMS over PMC, but not over MC or SMA, significantly improved symptoms of writer's cramp (Murase et al., 2005). The present findings corroborate these clinical effects specifically seen after PMC stimulation.

SEP waveforms before rTMS were slightly different among sessions of stimulating over MC, PMC and SMA (Fig. 2) or stimulation over PMC at monophasic 0.2 Hz and biphasic 1 Hz (Fig. 3) performed at least 1 week apart. This is probably due to the technical difficulty of reproducing the same recording electrode position. The comparison of waveforms before and after rTMS, the main analysis in this study, was free from this problem because the electrodes were kept attached to the scalp during the sessions. Although the coil positions were confirmed anatomically, it is conceivable that stimulation over PMC may have spread to MC or

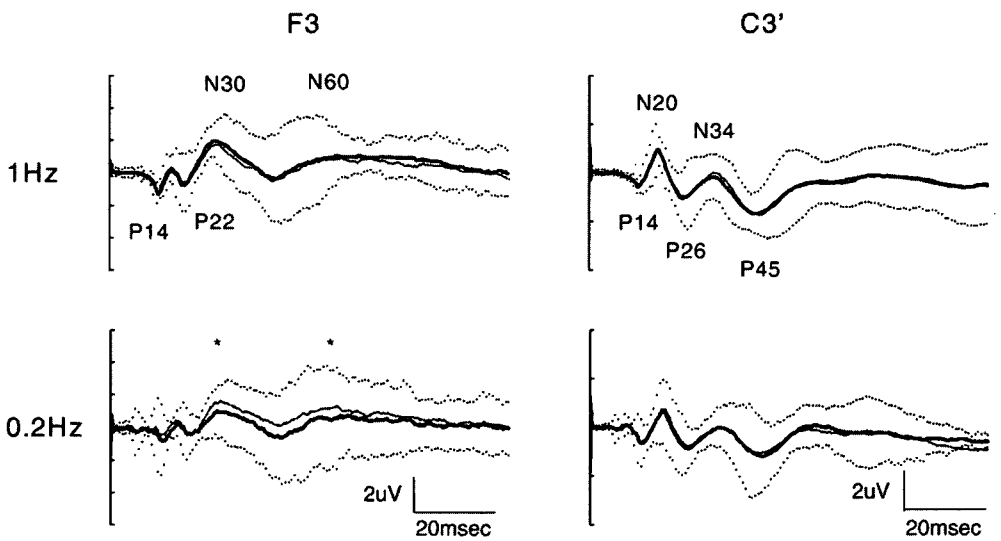

Fig. 3. Grand-averaged SEP waveforms from F3 (left column) and C3′ (right column) before (thick wave) and after (thin wave) application of rTMS over PMC at each stimulation frequency, biphasic 1 Hz and monophasic 0.2 Hz in 6 of 9 subjects. Asterisks show that the components showed larger aftereffects of monophasic 0.2 Hz than biphasic 1 Hz rTMS over PMC. Dotted lines show 95% confidence interval of each SEP waveform recorded before rTMS.

Table 2
Peak latencies (a) and amplitudes (b) of each SEP component before and after application of rTMS over PMC at monophasic 0.2 Hz or biphasic 1 Hz

(a) Latency (ms)

	F3														
	P14			P22			N30			N60					
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ			
0.2 Hz	13.4 ± 0.85	13.1 ± 0.81	−0.3 ± 0.32	19.7 ± 1.20	19.6 ± 0.66	−0.1 ± 1.09	28.4 ± 1.89	27.3 ± 2.32	−1.0 ± 1.62	57.3 ± 4.87	56.2 ± 3.40	−1.0 ± 3.07			
1 Hz	13.3 ± 0.86	13.5 ± 1.12	0.2 ± 0.74	20.0 ± 0.67	20.2 ± 1.22	0.2 ± 1.47	27.9 ± 1.99	27.4 ± 1.36	−0.6 ± 1.52	61.4 ± 7.55	61.8 ± 8.03	0.4 ± 4.32			
	C3′														
	P14			N20			P26			N34			P45		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
0.2 Hz	13.5 ± 0.90	13.4 ± 0.89	−0.2 ± 0.20	18.7 ± 0.70	18.8 ± 0.65	0.1 ± 0.18	24.9 ± 0.78	24.8 ± 0.90	−0.1 ± 0.46	32.7 ± 1.84	32.4 ± 1.57	−0.3 ± 1.79	43.1 ± 2.09	43.2 ± 1.97	0.1 ± 0.99
1 Hz	13.4 ± 1.01	13.9 ± 0.95	0.5 ± 0.65	18.7 ± 0.71	18.8 ± 0.73	0.1 ± 0.10	24.4 ± 1.39	24.6 ± 1.04	0.2 ± 0.40	31.5 ± 2.48	32.1 ± 2.36	0.6 ± 1.29	42.9 ± 2.79	43.7 ± 2.63	0.8 ± 0.91

(b) Amplitude (μV)

	F3														
	P14			P22			N30			N60					
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ			
0.2 Hz	1.31 ± 0.42	1.11 ± 0.24	−0.20 ± 0.26	0.74 ± 0.59	0.72 ± 0.65	−0.01 ± 0.19	−1.49 ± 0.79	−2.09 ± 0.69	**0.61 ± 0.24**	−1.32 ± 1.06	−2.11 ± 0.68	**0.78 ± 0.63**			
1 Hz	1.37 ± 0.35	1.45 ± 0.27	0.08 ± 0.38	0.84 ± 0.89	1.01 ± 0.88	0.17 ± 0.28	−2.19 ± 0.62	−2.02 ± 0.89	−0.18 ± 0.44	−1.80 ± 0.73	−1.53 ± 0.65	−0.27 ± 0.38			
	C3′														
	P14			N20			P26			N34			P45		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
0.2 Hz	1.41 ± 0.13	1.27 ± 0.15	−0.15 ± 0.12	−2.51 ± 0.49	−2.55 ± 0.78	0.04 ± 0.35	2.57 ± 1.56	2.50 ± 1.53	−0.07 ± 0.49	−0.19 ± 0.98	−0.23 ± 1.55	0.04 ± 0.85	4.06 ± 1.12	3.76 ± 1.52	−0.31 ± 1.02
1 Hz	1.51 ± 0.38	1.40 ± 0.10	−0.10 ± 0.44	−2.71 ± 1.06	−2.87 ± 0.96	0.16 ± 0.25	2.84 ± 1.62	2.90 ± 1.82	0.05 ± 0.86	−0.23 ± 1.16	0.14 ± 1.70	−0.36 ± 0.72	4.74 ± 1.14	4.83 ± 1.87	0.09 ± 0.95

Values are expressed as mean ± standard deviation. Δ indicates subtraction of values before from those after rTMS. Bold figures show the significantly increased value after monophasic 0.2 Hz than biphasic 1 Hz rTMS over PMC.

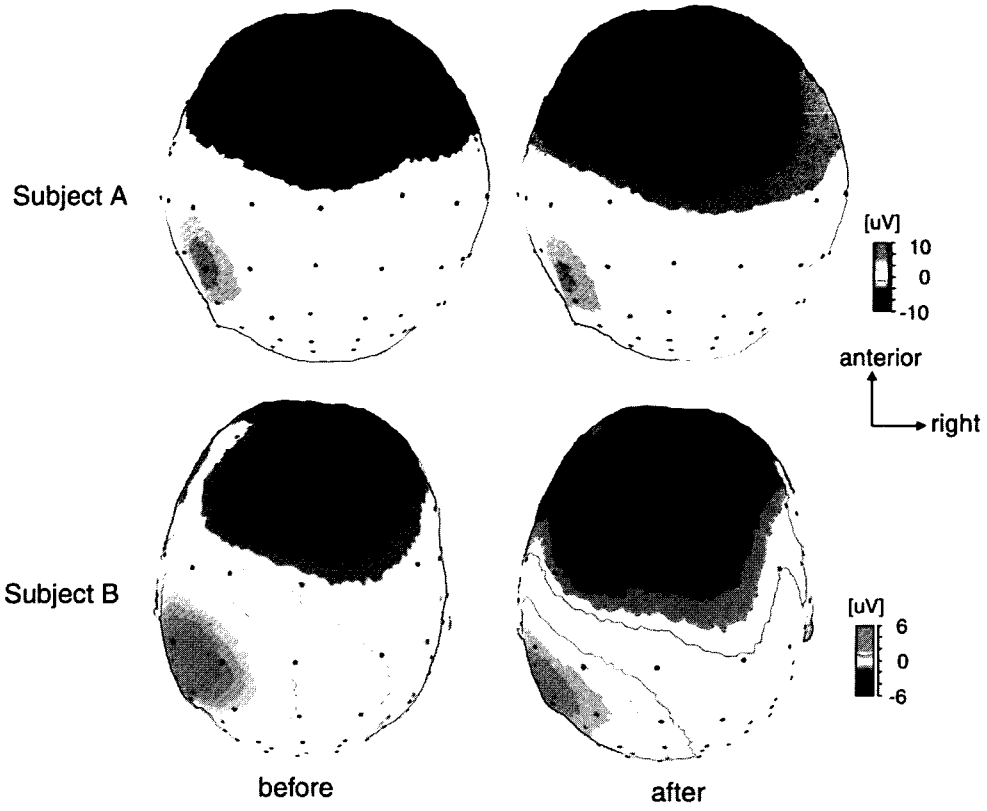

Fig. 4. Topographical SEP mapping using 62-electrode recording in 2 subjects on their realistic heads shape at N30 latency from before (left) to after (right) the application of monophasic 0.2 Hz rTMS over PMC. The amplitude distribution was practically unchanged, whereas the amplitude of the frontal component was clearly increased. Blue–red color scale illustrates the amplitudes, and maximum positivity is coded as red and maximum negativity as blue.

SMA because the extent of the field stimulated by the coil was not exactly defined. If so, SEPs should have changed after stimulation of not only PMC but also MC or SMA. The lack of these changes indicates that amplitude increases of frontal N30 component were elicited by the effects of monophasic 0.2 Hz rTMS over PMC or the more rostral sites such as the prefrontal cortex. Previous studies have reported changes in SEPs following rTMS over MC (Enomoto et al., 2001) or somatosensory cortex (Ragert et al., 2004), whereas no changes were found after rTMS over PMC (Enomoto et al., 2001; Satow et al., 2003). The discrepancy may be due to different stimulation parameters: Enomoto et al. (2001) applied rTMS over PMC at a lower intensity (1.1 times the active motor threshold) and a smaller number (200) of stimuli than those used in this study. Satow et al. (2003) used an intensity (90% RMT) similar to this study and a larger number (900) of stimuli than this study. The most striking difference was the frequency and the total duration of stimulation: these studies used higher frequencies (1 or 0.9 Hz) and shorter total durations (200 and 1000 s) than the present study (0.2 Hz, 1250 s). It is noteworthy that very low-frequency stimulation (0.2 Hz) used in this study and a recent study on dystonia (Murase et al., 2005) produced lasting effects on SEPs, rCBF and clinical symptoms. The previous studies using similar frequencies as a single but not repetitive TMS could be reanalyzed with the view that 0.2 Hz stimulation is repetitive. Finally, it should be noted that the TMS pulses in the present experiments were monophasic rather than the usual biphasic pulses that are employed in higher frequency rTMS. As noted by Tings et al. (2005), biphasic pulses produce effects that may be a combination of effects from two monopolar pulses of different directions. Since these may well be different and even cancel each other, monophasic rTMS appears to be more efficient in generating aftereffects than the usual biphasic rTMS. This may be another reason why the results in the present experiments differ from (and in some respects are more powerful than) those of higher frequency biphasic rTMS. The lack of changes after 1 Hz biphasic stimulation, as shown in the present study, warrants further studies determining whether the frequency or the phase or both are responsible for the cortical effect. If the effect of rTMS over motor cortex on SEPs is orientation selective, it may also account for the absence of effect in the present experiments.

After the application of monophasic very low-frequency rTMS over PMC, rCBF increases were observed most significantly in the left PMC and the prefrontal cortex, the regions under the coil. Although previous study reported decreases of rCBF after low-frequency (1 Hz) rTMS over PMC (Siebner et al., 2003), another study reported increases under the coil (Speer et al., 2003) during the same frequency rTMS over MC or no changes at stimulation site after the same frequency rTMS over MC in healthy subject with artificial pain (Tamura et al., 2004). After less than 1 Hz frequency (0.25 Hz) rTMS at Cz for 2 weeks, rCBF of the stimulus area increased in depressed patients (Peschina et al., 2001). From these studies, although a major hypothesis has been that low-frequency rTMS results in inhibitory physiological changes, imaging studies have yielded inconsistent results. It must, however, be emphasized that increases in inhibitory interneuronal activities could increase rCBF while decreasing cortical excitability. In support of this hypothesis, cortical inhibitory mechanisms as tested with paired-pulse TMS are associated with increase in rCBF, as has

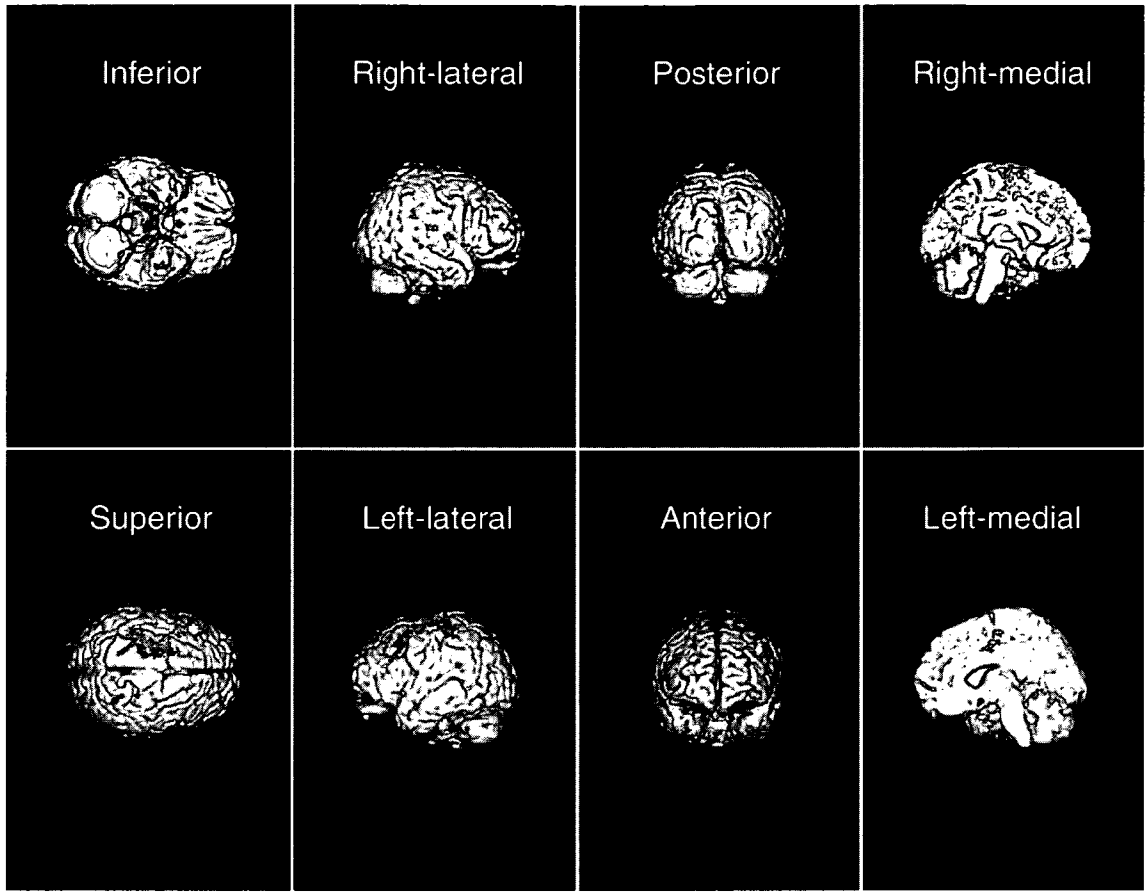

Fig. 5. Parametric statistical rendering maps showing areas of increased blood flow after application of monophasic 0.2 Hz rTMS over PMC compared with before. Regions of increase in the left frontal lobe, including Brodmann areas 9 and 6, correspond to prefrontal cortex and PMC and the right cingulate gyrus (see also Table 2).

been shown in previous study (Strafella and Paus, 2001). Similar rCBF increase was also described using different rTMS frequencies exerting inhibitory and excitatory effect on the cortex (Rounis et al., 2005). Changes in rCBF were also seen in the left middle frontal cortex and right cingulate gyrus, distant from the stimulated site. These areas are connected anatomically and functionally to PMC. Basal ganglia could modulate activities of these areas via PMC, which is the thalamo-cortical projection.

The previous studies showed that the frontal N30 component decreases in amplitude prior to hand movement (Starr and Cohen, 1985; Shimazu et al., 1999) or during motor imagery (Cheron and Borenstein, 1992; Rossini et al., 1997). Although similar gating has also been reported in the parietal P26 components (Starr and Cohen, 1985; Shimazu et al., 1999), the changes of SEPs in this study were observed only in frontal N30 component, not in parietal counterpart (P26 component) after monophasic 0.2 Hz rTMS over PMC. These frontal and parietal components are composed of tangential and radial dipoles. Allison et al. (1991) concluded that N30 is generated in area 3b for its tangential component and in area 1 for its radial component. Other studies have suggested the precentral radial generators of frontal N30 component, especially on SMA (Desmedt and Bourguet, 1985; Cheron and Borenstein, 1992; Mima et al., 1999). If monophasic 0.2 Hz rTMS over PMC was affected on the tangential and/or postcentral radial dipole, the parietal component should also change with the frontal component. In the present study, however, topographic changes in SEP after application of monophasic 0.2 Hz rTMS over PMC were observed only in the frontal component (Fig. 4). Although performed in only two subjects, this study suggested that monophasic 0.2 Hz rTMS over PMC hardly affected the postcentral generator of the tangential and radial component and implies that the increase of frontal N30 component is due to an action on precentral generators

Table 3
Areas of rCBF that increased by rTMS over PMC

Brain region	MNI coordinates			Z-score
	x	y	z	
Left middle frontal gyrus (Brodmann Area 9)	−38	28	38	4.90
Left precentral gyrus (Brodmann area 6)	−16	2	66	4.83
Right limbic lobe cingulate gyrus (Brodmann area 24)	18	−2	42	4.80

The panel shows the MNI coordinates and Z scores of maximal peaks of regions where cerebral blood flow was significantly increased after application of monophasic 0.2 Hz rTMS over PMC compared with before (see also Fig. 5).