平成19年度　班会議　（報告会）

厚生労働科学研究費補助金 こころの健康科学研究事業
「補足運動野連続磁気刺激による大脳基底核疾患治療の開発」班

平成18年度　第２回班会議
開催日時　：　平成19年1月27日(土) AM11:00～PM15:00
場　　所　：　はあといん乃木坂　2F 212号室
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班会議内容

１．　班長からのご挨拶
産業医大神経内科　　辻　貞俊
２．　脳外科の先生方へのアンケート結果に関して
東大神経内科　　濱田　雅
３．　磁気刺激治療研究の途中経過
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東大神経内科　　宇川義一
５．　事務連絡

出 席 者　　辻　貞俊　魚住武則　武藤詩子　玉川　聡　梶　龍兒　漆原　良
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清水俊夫　宇川義一　寺尾安生　花島律子　岡部慎吾　濱田　雅
中馬孝容　小森哲夫　工藤里美
20名

VI. 研究成果の発刊に関する一覧表

研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
横地房子	パーキンソン病の側屈姿勢とPisa症候群	山本光利	パーキンソン病；臨床の諸問題	中外医学社	東京	2006	104～110

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Terao Y, Furubayashi T, Okabe S, Mochizuki H, Arai N, Kobayashi S, Ugawa Y	Modifying the cortical processing for motor preparation by repetitive transcaranikal magnetic stimulation	J Cognitive Neurosci	in press		2007
Kitagawa M, Murata J, Uesugi H, Hanajima R, Ugawa Y	Characteristics and distribution of somatosensory evoked potentials in the subthalamic region	J Neurosurg	in press		2007
Hamada M, Hanajima R, Terao Y, Arai N, Furubayashi T, Inomata-Terada S, Yugeta A, Matsumoto H, Shiroata Y, Ugawa Y	Origin of facilitation in repetitive, 1.5 ms interval, paired pulse transcranial magnetic stimulation (rPPS) of the human motor cortex	Clin Neurophysiol	in press		2007
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Chuma T, Farque MR, Ikoma K, Mano Y	Motor learning of hands with auditory cue in patients with Parkinson's disease.	J Neural Transm	113	175-185	2006

Ⅶ. 班 構 成 員 名 簿

補足運動野連続磁気刺激による大脳基底核疾患治療の開発研究班

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Ⅷ.　業績別刷り

レクチャー

運動の発現機構 ——磁気刺激を用いた検討——

魚住 武則

Key words: 磁気刺激、運動系、ミラーニューロン、運動野、44野

【要旨】 大脳には運動出力の中心的な役割を果たす一次運動野以外に多くの高次運動野が存在する。一次運動野の前方に運動前野があり、背側（PMd）と腹側（PMv）の2領域に分けられる。それぞれ脳の他の領域との連絡は異なっているが、前者は感覚情報を統合して動作との連合ならびに動作企画・準備に重要な働きをもつと考えられている。後者は視覚情報による動作の空間的誘導・動作選択に重要であるが、最近ではPMvの前下方（44野）にmirror neuron systemの存在が明らかになった。補足運動野（SMA）は2つに分けられ、前方を前補足運動野、後方を狭義の補足運動野と呼んでいる。運動を直接制御する機能よりも姿勢調節と動作遂行のバランスを保つ補足的役割を持っているものと考えられている。帯状皮質運動野の前方領域は報酬の価値判断に基づいた動作の選択時に活動が高まり、後方領域は一次運動野に近い活動が観察されている。これまでの研究は多くは動物実験であったが、磁気刺激法の発達により、ヒトでも帯状回以外の領域は直接刺激することが可能となり、多くの知見が得られている。さらには基底核ループと小脳ループとの関係も注目されている。

はじめに

随意運動の発現には認知情報を正しく処理する過程、複数の要素的な運動を目的のある1つの運動に組み上げる過程、運動を学習・記憶する過程および訓練により運動を自動化する過程がある。一次運動野は運動出力の中心であるが、運動の発現・制御に重要な高次運動野が多数存在する。さらには随意運動の二大調節系として大脳基底核と小脳がある。経頭蓋的磁気刺激（transcranial magnetic stimulation, TMS）の開発によりヒトにおけるこれらの運動発現機構が少しずつ解明されてきた[1]。最近では磁気刺激装置の発達により連続してTMSをおこなうことも可能となった。このrepetitive TMS(rTMS)により刺激中および刺激後に続く効果が誘発され、ヒトの高次脳機能の分析や神経・精神疾患への治療に応用されるようになった。rTMSは長期増強long-term potentiation（LTP）と長期抑制long term depression（LTD）に類似した現象を誘発することができることがわかってきた[2]。これを利用して中枢神経系の興奮性の変化を生じさせ、刺激部位と離れた部位の変化をとらえる研究が行われている。本稿ではヒトでの随意運動の発現機構を概説し、それにTMSを用いた研究を紹介する。

産業医科大学神経内科

I. 大脳皮質運動関連領野

大脳には運動出力の中心的な役割を果たす一次運動野以外に多くの高次運動野が存在する（図1）。一次運動野の前方に運動前野premotor cortexがあり、背側(dorsal premotor cortex, PMd）と腹側（ventral premotor cortex, PMv）の2領域に分けられる。それぞれ脳の他の領域との連絡は異なっているが、前者は感覚情報を統合して動作との連合ならびに動作企画・準備に重要な働きをもつと考えられている。後者は視覚情報による動作の空間的誘導・動作選択に重要である。補足運動野（supplementary motor area, SMA）は2つに分けられ、前方を前補足運動野、後方を狭義の補足運動野と呼んでいる。運動を直接制御する機能よりも姿勢調節と動作遂行のバランスを保つ補足的役割を持っているものと考えられている。帯状皮質運動野cingulate motor areaの前方領域は

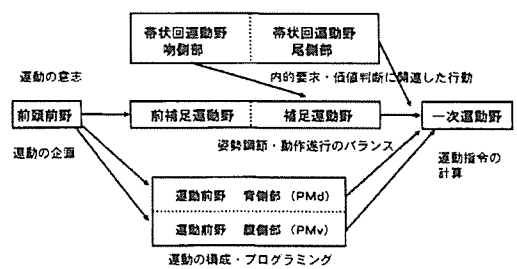

図1. 随意運動情報の流れ

報酬の価値判断に基づいた動作の選択時に活動が高まり、後方領域は一次運動野に近い活動が観察されている[3]。運動前野(PMd)と一次運動野を二連発磁気刺激し両者の結合をみた研究では刺激間隔が2-3 msから始まり、6 msで最大となる一次運動野に対する抑制効果と8-10 ms以降に生じる促通効果が認められている[4]。連続した上肢の運動課題を与え、go signalを出した時点と運動を開始した時点でPMdを磁気刺激し、反応時間と運動時間に対する影響をみた研究では前者の刺激では促通効果、後者では抑制効果が認められた[5]。MEP閾値以下の弱い強度でPMdに1 HzのrTMSを与えると一次運動野に対して抑制効果が認められている[6]。最近rTMSとfunctional MRI (fMRI)を組み合わせた研究も行われるようになってきた。左PMdに3 HzのrTMSを加えた後に生じた有意なBOLD反応をみると右PMd、両側PMv、補足運動野、感覚野、左側頭葉後部、小脳、尾状核に反応が認められた[7]。この手法が進歩すればより詳細なヒトの運動関連領野間の結合が解明されると期待される。ヒトで補足運動野を選択的にしかも正確に磁気刺激することは解剖学的位置の理由から難しいために磁気刺激を用いた研究は進んでいなかったが、ようやく最近補足運動野磁気刺激の影響をみる研究が行われるようになった。反応時間と運動時間に対する補足運動野磁気刺激の影響が検討されており、go signal時に刺激すると運動時間が短縮することが示された[5]。また補足運動野に5 HzのrTMSを与えると一次運動野の興奮性が一過性に高まることがわかった[8]。これらは補足運動野と一次運動野の神経結合を示すと同時に補足運動野磁気刺激の治療的応用の可能性を示すものである。

II. 前頭前野

前頭前野は系統発生学的に最も新しく進化した領域でその機能は不明な点が多い。うつ病の治療として左背外側前頭前野(dorsolateral prefrontal cortex, DLPFC, Brodmann 46野)へのrTMS療法が行われている。運動機能としては記憶された空間的手がかりを思い出している時に活動することから運動記憶の貯蔵に関係していることが推測されている。また前頭前野と後頭頂葉は密接に結合しており、視覚運動課題に関係していることが考えられている。臨床例やrTMSを用いた研究からDLPFCは短期の空間性ワーキングメモリーを調節しており[9]、記憶誘導性サッケード眼運動に関係していること、予期される目標が出現する前に生じるサッケード眼運動の準備にも関係していることが明らかとなった[10]。また左DLPFCに対して経頭蓋的直流電流刺激を行うとワーキングメモリー課題遂行の正確性が増す[11]。それに対して、右DLPFCはright cortical timing networkの重要な領域と考えられており、特に秒単位の時間認知や運動タイミングに関与していることが推測されている。右DLPFCに対してrTMSを与えた研究でもそのことが証明されている[12]。

III. mirror neuronsとBroca野

サルを用いた研究でinferior frontal areaにmirror neuronsの存在が発見されている[14]。これは運動の観察と実行の統合に機能していることが推測されている。ヒトでも同様の機能を有する部位がPET[14]や脳磁図[15]を用いた研究で見出されており、Brodmann 44野に存在することが推測されている。またPMvには前肢の脊髄運動ニューロンに直接投射するニューロンが存在することも見出されている。著者らは健康成人10名を対象とし、8の字コイルで手の一次運動野(M1)と44野を磁気刺激しその反応の差異を検討した[16]。M1での運動閾値をまず計測し、44野刺激強度はその130%とした。44野の刺激部位はCzより11 cm側方3 cm前方の点を中心として手の随意運動に大きく影響する部位とした。この部位は舌のM1の約2-3 cm前方に位置することが多かった。コイルの方向は頭蓋内に流れる渦電流の向きが上方になるようにした。

(1) 44野磁気刺激が与える手の随意運動への影響

両手の第1指と第2指を同時にタッピングさせた状態で44野刺激を行うと瞬間的に両手の指タッピングが停止し、その後にタッピングの振幅が一時的に増大する現象が認められた。一部の被験者では両手のタッピングのリズムが乱れ両手の動きが同期しない場合もみられた。渦巻き描画の途中に44野刺激すると瞬間的な筋収縮による影響のみのM1刺激と比較して明らかに持続の長い影響が認められた。被験者の感想は「思うように動かなくなる」「動作がストップする」「動作が遅くなる」「どう動かしてよいのか瞬間的にわからなくなる」というものであった。

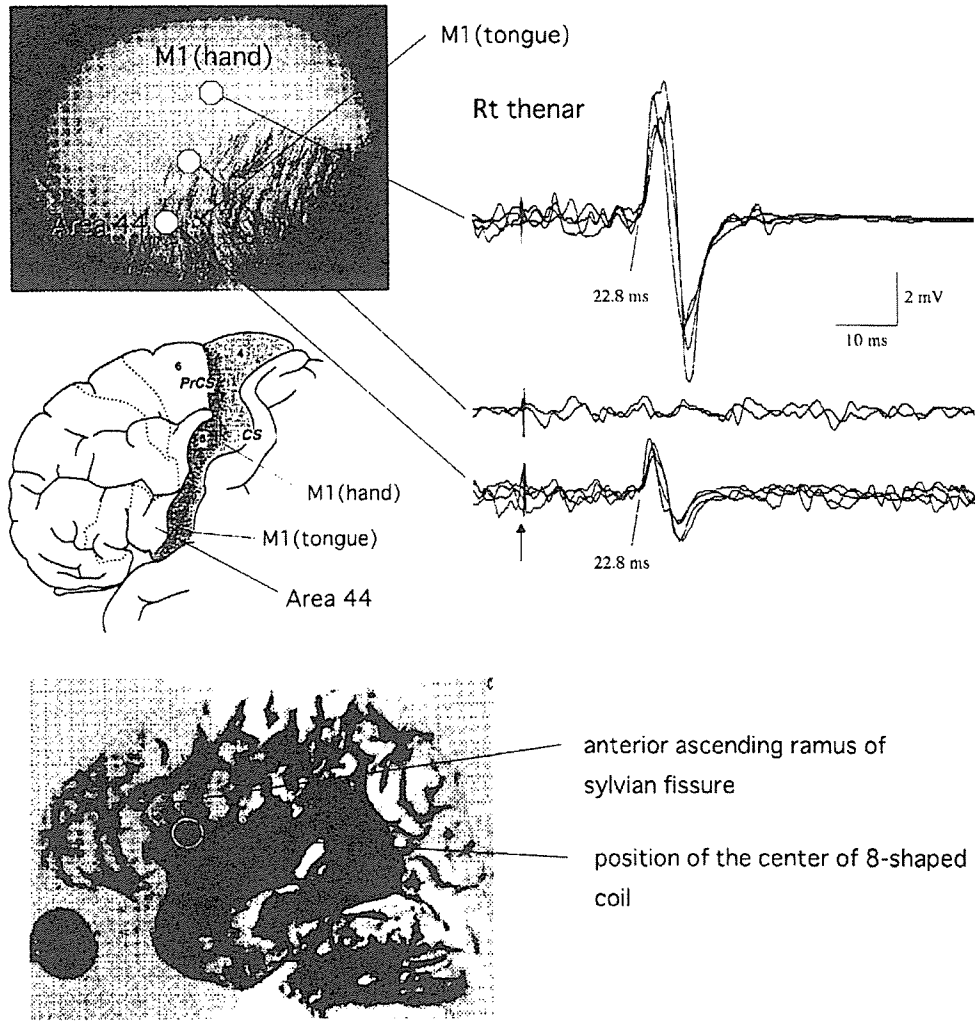

図 2. 44 野磁気刺激により生じる運動誘発電位（文献 16 から引用）

(2) 44 野磁気刺激による MEP の記録（図 2）

44 野刺激により MEP が誘発されるが、安静時では正常人でも誘発されにくい例が多いので中等度の随意収縮時に記録することが必要であった。M1 刺激による MEP と比較して閾値が高く、低振幅であるが潜時はほぼ同じであり、cortical silent period は明らかに短かった。一部の例では刺激と同側の手にも MEP が誘発され、対側の MEP との潜時差は約 7-8 ms であり、脳梁を介した反応と考えられた。

(3) 44 野磁気刺激が背景 EMG 活動に与える影響（図 3）

両手を持続的筋収縮および指タッピングさせた状態で 44 野を磁気刺激したところ背景 EMG 活動に対して強い影響が認められた。持続的筋収縮への影響は MEP 出現後に抑制がかかり、さらに EMG 活動が増幅し、再び抑制されるパターンが認められた。それらの影響は約 200 ms 間続き、M1 刺激とは異なって刺激と同側の筋にも対側の筋と同等の影響が認められた。同側の筋への影響は対側筋へのものと比べて 5–10 ms 遅れていた。指タッピングの直前に刺激した場合は運動による筋活動電位が低振幅となり、持続も延長し、タッピングのリズムに影響を与えた。

(4) ヒトで mirror neurons が存在するのか

44 野を磁気刺激する方法を用い、mirror neurons の機

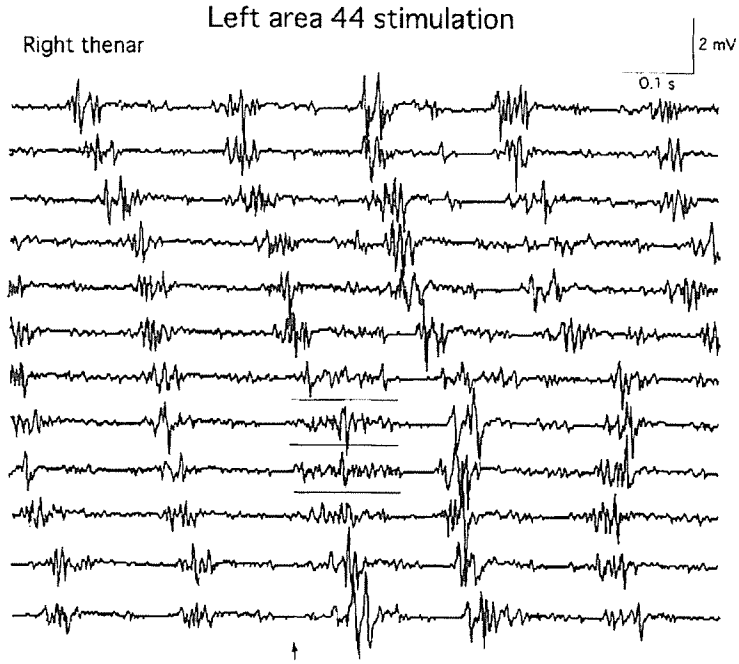

図3　随意収縮時の背景筋電図に対する44野磁気刺激の影響（文献16から頒用）

能を検討した。健康成人6名を対象とし、母指球筋から以下の5つの条件下での44野刺激によるMEPを記録した。(a) 等張性持続筋収縮 (b) 意味のない指タッピング運動 (c) 持続性収縮を保ちながら手指の動きを観察する (d) 数字を示す手指の動作を観察しながら模倣 (e) 数字を発声しながら同時に手でその数を示すジェスチャーを行う。持続筋収縮時と比較して後者2つの条件下ではMEP振幅の明らかな振幅増大が認められた。この結果は同部位が手の模倣および発語に関連した手指の動作に関与していることを示していると考えられた。

(5)　発語障害者における44野および舌M1刺激によるMEP

発語障害患者における中心前回下部・弁蓋部の機能を電気生理学的に評価するために、舌M1と野を磁気刺激し、舌筋、手よりMEPを記録した。失語症、発語失行、高度の発語遅延などを呈する12例を対象とした。発語失行例では44野MEPは正常である例が多く、口・顔面失行や構音障害を伴う例では舌MEPの異常が認められた。発語遅延例では44野MEPが異常であった。

IV.　基底核ループと小脳ループ

サルを用いた研究により、大脳皮質―基底核ループには4つの構造があることが明らかになってきた[17]。運動ループ、前頭前野系ループ、辺縁系ループ、眼球運動ループである。そのうちの運動ループは一次運動野、大脳皮質運動関連領野と主に被殻を結ぶもので運動の遂行に関係する。大脳皮質から受け取った情報は3つの基底核神経回路で調節されている。ハイパー直接路は大脳皮質から興奮性入力を受けた視床下核ニューロン出力核のGABA作動性ニューロンに単シナプス性に最も短時間に投射する経路である。これによりまず視床―大脳皮質投射ニューロンが広く抑制される。直接路はGABAとサブスタンスPを持つ線条体ニューロンが出力核に単シナプス性に投射する経路で基底核出力を減少させ（脱抑制）随意運動に必要な標的ニューロンが活動する。最後に働くのが間接路であり、GABAとエンケファリンを持つ線条体ニューロンが多シナプス性に淡蒼球外節のGABA作動性ニューロンと視床下核のグルタミン作動性ニューロンを介して主力核に投射し、標的ニューロンの活動は再び抑制される。このように基底核には意図した運動以外の競合する運動を抑制する働き（周辺抑制：surround inhibition）があり、時間的空間的に運動をコントロールしている[18]（図4）。surround inhibitionの異常によりジストニア、舞踏病などの不随意運

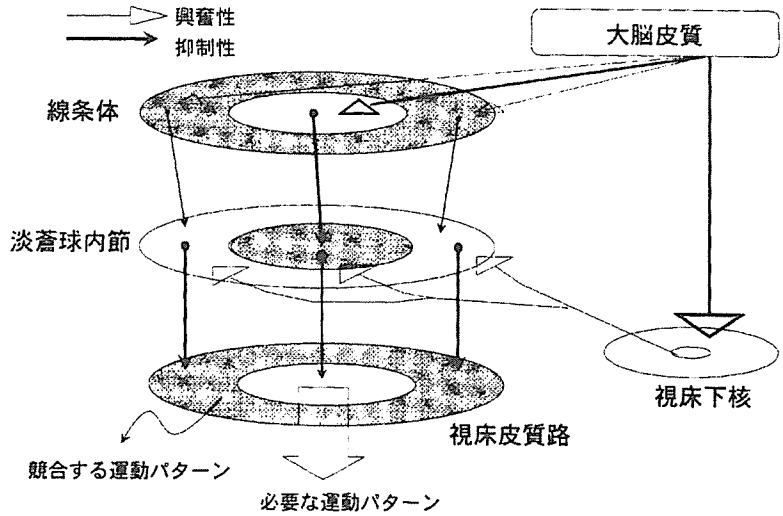

図 4. 周辺抑制の概念（文献 18 から改変引用）

動を説明しようとする考えが提唱されている[19]。この考えに基づいて手の局所性ジストニアを対象として TMS を用いた研究が行われており、示指の屈曲時に MEP を記録すると小指球筋の MEP は正常では抑制されるがジストニア患者では増強し、surround inhibition の障害を示唆する結果が示されている[20]。しかしジストニアの発生機序が全て surround inhibition の障害で説明できるかまだ不明な点もあり今後の解明が待たれる。

小脳皮質への入力線維（前庭系からの入力、脊髄からの入力、橋核からの入力）は小脳皮質と小脳核に終止する。小脳の領域は入力により区分され、前庭小脳、脊髄小脳、橋小脳に分けられる。小脳の出力ニューロンはプルキンエ細胞であり、情報処理された入力情報はこの軸索を介して同側の小脳核へ送られる。前庭小脳は平衡機能、姿勢、眼球運動の調節に関わる。脊髄小脳は脳幹網様体、前庭神経核に出力し、姿勢・歩行・注視に関わる。橋小脳は大脳皮質からの入力を受け、四肢の随意運動調節系に関わる。ヒトでこれらの出入力の機能を生理学的に評価することは非常に難しい。その理由として、小脳の電気活動を頭皮上から記録することは困難であることと、電気刺激あるいは磁気刺激を用いてヒト小脳を刺激してもその刺激効果をみる方法がまだ確立していないことがあげられる。

基底核ループの障害による代表的疾患パーキンソン病と小脳ループの障害による小脳失調症の臨床的特徴は内的情報・外的情報の処理、筋緊張、測定障害、振戦をみても相反するものである。磁気刺激法を用いて一次運動野の興奮性・抑制性機能を検討すると、パーキンソン病と小脳失調症とでは全く逆の現象が認められる。前者では CSP は短縮し、過度の促通効果が認められるが、後者は弱い刺激強度では正常者よりも CSP 持続時間は延長し、抑制機能の亢進が推測されている。パーキンソン病では学習した運動を自動的に遂行することがうまくできない、同時に 2 つの異なった運動ができない、外的刺激・きっかけにより運動障害が改善する特徴がある。最近、パーキンソン病における小脳機能の研究が進められてきている。fMRI を用いた研究では自動運動の遂行時には両側小脳の活動が正常者より亢進しており、小脳の代償的働きと推測されている[21]。一方で視床下核刺激により、刺激と同側の小脳の過興奮が減少することから小脳の過興奮が振戦、固縮、無動に関与している可能性も推測されている[22]。おそらく基底核障害では小脳は代償的働きを持つ反面、そのバランスによってはかえって随意運動障害を生じてしまう可能性がある[23]。それに対して小脳失調症において基底核機能がどのように変化しているのか全くわかっていない。

V. motor overflow

motor overflow とは随意運動の遂行に伴って生じる不随意運動であり、主に associated movement（協同筋との

無関係の同側あるいは対側の筋に生じる不随意運動）と mirror movements (MMs：I 側肢の随意運動に鏡像して、対側肢にも意図しない運動が生じる現象）があげられる[24]。MMs は 10 歳以下の小児にみられ、成長とともに減少・消失する。成人でも複雑で必死に行う様な動作時にわずかに認められることがあるが、それが顕著であれば病的と考えられる。先天性と後天性があり、後者の原因としては脳卒中片麻痺後、パーキンソン病、補足運動野病変などが知られている。motor overflow の発生機序を解明することはすなわち正常ヒトでの運動下行路の同側性支配あるいは両側性支配の機能解析につながる。MMs の発症機序を考えるためには発生学的に非交叉性皮質脊髄路（錐体路）を理解する必要がある。ヒトの錐体路非交叉の存在を確認するには同側性運動誘発電位 (MEP) を記録することが最もよい。正常成人でも一側の運動野を磁気刺激すると同側の上肢近位筋から極めて低振幅の同側性 MEP が記録されることがある[25]。これは非交叉性錐体路を介した反応と推測されている。ヒトの運動野には同側の筋に投射する領域が対側筋の支配領域に近接して存在することが知られている。しかしながら、上肢遠位筋から同側性 MEP を誘発するのは正常成人では困難であるが、小児期やいくつかの病態で同側性 MEP が記録されることがあり、それぞれ異なった出現機序が考えられる。

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High-Frequency Repetitive Transcranial Magnetic Stimulation Improves Refractory Depression by Influencing Catecholamine and Brain-Derived Neurotrophic Factors

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Original Paper

Introduction: Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive and easily tolerated method of altering cortical physiology. To date, numerous open and sham controlled clinical trials have explored the antidepressant potential of rTMS. In the present study, we investigated clinical trials of high-frequency rTMS (20 Hz) for treatment of refractory depression, and also examined the effect of rTMS on plasma levels of catecholamine metabolites and brain-derived neurotropic factor (BDNF). **Methods:** Twenty-six depressed inpatients who met the DSM-IV criteria for major depressive disorder and had failed to respond to treatment with at least two antidepressant drugs given at adequate doses (above 150 mg/day in an equivalent dose of imipramine) and durations (at least 4 weeks for each drug) were enrolled in this study. Eleven were males, 15 females. The ages of the subjects ranged from 19 to 78 years old (mean ± SD = 52.9 ± 17.8). All patients were administered left prefrontal 20 Hz rTMS at 80% MT (total 800 pulses a day) over ten daily sessions. The plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) were analyzed by high-performance liquid chromatography. The plasma levels of BDNF were also measured with the sandwich ELISA method. **Results:** The mean 17-item Hamilton Rating Scale for Depression (Ham-D) score of 20.5 ± 5.2 before rTMS was significantly decreased to 15.6 ± 7.3 after rTMS. Nine of 26 patients (35%) demonstrated some improvement (Ham-D ≥ 25%) by rTMS. The levels of plasma MHPG, but not those of HVA, were significantly reduced after rTMS treatment, and a negative correlation was observed between the change in plasma MHPG levels and the change in scores of agitation. In addition, the plasma levels of BDNF were significantly increased by 23% in responders and partial responders, but not in nonresponders, after rTMS treatment, and a trend for association was found between the changes in Ham-D scores and changes in plasma BDNF levels in all patients after rTMS treatment. **Conclusion:** These results suggest that rTMS treatment brings about some improvement in refractory depression, especially for symptoms such as agitation, by influencing MHPG and BDNF, which is in accordance with previous reports showing that BDNF was increased by various antidepressants treatments.

Introduction

Depression has a lifetime prevalence estimated to be between 1.5–19% [48], and depression, like anxiety disorder, is one of the most common of all psychiatric disorders. Despite the administration of many kinds of antidepressants and various kinds of psychotherapy, treatment failures occur because of the delayed onset of efficacy or because of intolerable side effects. Unlike psychotherapy or drug treatment, electroconvulsive therapy (ECT) has a short onset latency and has been used for patients with serious or treatment-resistant depression [8]. However, it requires anesthetic agents and seizure induction, and can cause

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times cause memory impairment [32]. Repetitive transcranial magnetic stimulation (rTMS) has the advantages of not requiring anesthesia and of not inducing seizures and the associated memory loss. In addition, it allows better control of stimulus frequency and location [4]. rTMS is a neurologic and psychiatric research tool that has gained attention in recent years for its potential application as a treatment not only for neurological disorders, but also for psychiatric disorders such as depression, schizophrenia, and obsessive-compulsive disorder [18]. The initial application of high-frequency (20 Hz) rTMS over the left prefrontal cortex in depressed patients resulted in promising findings [12,20]. Subsequent sham-controlled studies of daily left prefrontal high-frequency rTMS have demonstrated significant clinical improvement in depressed patients [7,16]. In general, high-frequency (3–20 Hz) rTMS is considered to increase cortical excitability and metabolism, whereas low-frequency (≤ 1 Hz) stimulation does the opposite [18]. Current data support the antidepressant effects of excitatory stimulation to the left prefrontal cortex. According to the review of Gershon et al. [21], 41% of 139 patients treated with high-frequency rTMS to the left prefrontal cortex achieved either a 50% decrease in their Hamilton Rating Scale for Depression (Ham-D) scores or a final score under 8. More recently, rTMS has been studied mainly to assess its putative therapeutic effects in the treatment of refractory depression [31]. Although the mechanism through which rTMS may exert therapeutic effects in depression has also been studied in a number of animal models by investigating plasticity and changes in cortical activity [13], it remains to be fully elucidated.

The original catecholamine hypothesis of depression postulated that depression is characterized by a deficiency of functional noradrenaline. Recently, this hypothesis has been tempered and modified to place more emphasis on possible disturbances in the regulation of the catecholaminergic systems as a phase affective/arousal system. A dysregulated noradrenergic system may contribute significantly to the vegetative and anxiety-related symptoms of depression [3,44]. Previously, we proposed that depression might be dichotomized into two groups using plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of noradrenaline [52]. Recently, many animal studies and several human studies have demonstrated that brain-derived neurotrophic factor (BDNF) plays important roles in the pathophysiology of depression. Several lines of evidence indicate that the expression of BDNF might be a downstream target of a variety of antidepressant treatments or ECT [1,22,41].

In the present study, we performed a clinical trial of high-frequency rTMS (20 Hz) for treatment of refractory depression. Moreover, we clarified the effects of rTMS on catecholaminergic systems by analyzing the plasma levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA). In addition, we also investigated the effects of rTMS on plasma BDNF levels. We found that rTMS brings some improvement to persons with antidepressant-resistant depression. A surprising result was a reduction in the plasma MHPG levels and an increase in the plasma BDNF levels after rTMS, and these effects were found to be related to the improvement of the depressive symptoms.

Subjects and methods

Twenty-six depressed inpatients at a psychiatric ward in our university hospital who met the DSM-IV criteria for major depressive disorder, and who were significantly symptomatic despite being on medications, or who were unable or unwilling to try additional medications, were enrolled in this study. The MINI International Neuropsychiatric Interview was used to confirm the DSM-IV diagnosis of a major depressive disorder and rule out combined conditions such as anxiety disorder or personality disorder. The subjects had to have failed to respond (below 50% in their Ham-D score) to at least two prior medication trials judged to be of adequate duration (at least 4 weeks for each drug) and with adequate dosages (above 150 mg/day in an equivalent dose of imipramine). These determinations were made in conference between the first author (T.Y.) and the current treating psychiatrist. Eleven were males and 15 females. A score of at least 15 on the 17-item Ham-D was needed for a subject to be admitted to the study. The ages of the subjects ranged from 19 to 78 years (mean ± SD = 52.9 ± 17.8). All patients were physically healthy and none had a history of alcohol and/or drug abuse. Patients with a history of epilepsy, neurosurgery, and cardiac pacemaker implantation were excluded. A Nihon Koden magnetic stimulator AAA-81077 with a figure-eight coil YM-111B was used in this study. All patients were administered left prefrontal 20 Hz rTMS at 80% MT (total 800 pulses a day) over ten daily sessions. The left prefrontal cortex area was considered to be 5 cm in front of the left motor cortex area of the abductor pollicis brevis muscle. Each patient was assessed to determine his or her motor magnetic threshold at rest only before treatment. The motor threshold was defined as the lowest stimulus intensity capable of producing motor evoked potentials (MEP) of ≥ 50 μV in the relaxed abductor pollicis brevis muscle in at least 5 to 10 consecutive trials. The motor evoked potentials were collected by electromyographic devices (Neuropack, Nihon Koden). Eighty percent of the individual patient motor threshold was then administered on the left dorsolateral prefrontal cortex within a two-week period. Each patient was evaluated every week during the rTMS treatment by one experienced psychiatrist (T.Y.) using the Ham-D. The psychiatrist assessing the Ham-D was blind to the results of the test of plasma levels of catecholamine metabolites and BDNF. All patients continued to receive their preexisting antidepressants or mood stabilizers at the same dosages from 3 weeks before to 3 weeks after the rTMS treatment. We defined the patients with a 50% or more decrease in their Ham-D score as responders, the patients with a 25–49% decrease in their Ham-D score as partial responders, and the remaining patients as nonresponders comparing the score of Ham-D at the two points, just before the first rTMS treatment and two weeks after the last rTMS treatment.

All blood samples were taken at 7:00 am before breakfast (at least 12 hours after the last medication) just before and two weeks after the rTMS treatment. Fifteen milliliters of venous blood was drawn from the patient in the supine position, after the patient had been lying at rest overnight. The plasma samples were quickly separated in a centrifuge (2000 g, 10 min, 4 C) and stored at −80 C until assay. The plasma HVA levels were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) according to the method of

Yeung et al. [50] with slight modification [53]. In short, each cyano-bonded solid-phase extraction cartridge was preconditioned with methanol and then glass-distilled water. To each cartridge were added 0.3 mL of plasma sample or standard, and 0.1 mL of working internal standard solution (5 ng of 5-hydroxyindolecarboxylic acid in 0.01 M KH_2PO_4, pH 7.2). The samples were deproteinized with 1 mL of acetonitrile. After mixing by vortex and centrifugation (1760 g, 4 C for 10 min), an aliquot (5 μL) of supernatant was allowed to pass through the cartridge slowly under a mild vacuum (15 mmHg). The cartridge was washed with 0.2 mL of distilled water, extracted with 1 mL of ethyl acetate, and then an aliquot was evaporated to dryness under nitrogen gas. After dissolving in the mobile phase (200 μL), a 10 μL portion of this solution was injected into the HPLC. The plasma MHPG levels were analyzed according to the method of Minegishi and Ishizaki [34]. In brief, plasma was separated by centrifugation at 2000 g, 10 min at 4 C. Extraction was performed under a vacuum using Bond-Elut columns prepacked with 100 mg of C18-bonded silica (40 μm) in a 1 mL capacity disposable syringe. The columns, which were inserted into a vacuum chamber connected to an aspirator, were prepared by washing with 1 mL of methanol followed by 1 mL of water. After the addition of 50 μL of a solution of vanillyl alcohol (internal standard equivalent to 5 ng/mL) to 1 mL of plasma, the samples were applied to and passed through the columns, followed by 0.75 mL of water to rinse off both residual samples and easily eluted hydrophilic compounds. The adsorbed materials were eluted with 200 μL of methanol to a 0.1 M phosphate buffer (pH 4.8) mixture (40 : 60, v/v). A 20 μL portion of this solution was injected into the HPLC. The plasma BDNF levels were measured using a BDNF Emax Immunoassay Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. In short, 96-well microplates were coated with anti-BDNF monoclonal antibody and incubated at 4 C for 18 hours. The plates were incubated in a blocking buffer for 1 hour at room temperature. The samples diluted with assay buffer by 100-times and BDNF standards were kept at room temperature under conditions of horizontal shaking for 2 hours, followed by washing with the appropriate washing buffer. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 2 hours and washed with the washing buffer. The plates were then incubated with anti-IgY antibody conjugated to horseradish peroxidase for 1 hour at room temperature, and incubated in peroxidase substrate and tetramethylbenzidine solution to induce a color reaction. The reaction was stopped with 1 mol/L hydrochloric acid. The absorbance at 450 nm was measured with an Emax automated microplate reader. Measurements were performed in duplicate. The standard curve was linear from 5 to 5000 pg/mL, and the detection limit was 10 pg/mL. Cross-reactivity to related neurotrophins (NT-3, NT-4, NGF) was less than 3%. Intra- and interassay coefficients of variation were 5 and 7%, respectively. The recovery rate of the exogenous added BDNF in the measured plasma samples was more than 95%.

This study was approved by the ethics committee of the University of Occupational and Environmental Health, and written informed consent was obtained from all participants.

Statistical analysis

The plasma levels of MHPG, HVA, and BDNF before and after rTMS were compared using the Wilcoxon signed-rank test. Spearman's rank correlation coefficient was calculated to investigate the relationship between the changes in the plasma levels of MHPG, HVA, and BDNF and the HAM-D improvement rates. The Mann–Whitney's U test was used to compare the response group and the non-response group with respect to the pretreatment plasma levels of MHPG, HVA, and BDNF, and age. The chi-square test was performed to compare the response group and the nonresponse group with respect to gender. All data were expressed as mean ± standard deviation, and a P-value below 0.05 was considered significant.

Results

All patients were treated with preexisting antidepressants or mood stabilizers for at least 4 weeks or more as follows : 7 patients with milnacipran, 5 patients with paroxetine, 5 patients with sulpiride, 5 patients with lithium carbonate, 5 patients with fluvoxamine, 4 patients with mianserin, 2 patients with amoxapine, 2 patients with imipramine, 2 patients with trazodone, 1 patient with sodium valproate, 1 patient with clomipramine, and 1 patient with maprotiline (Table **1**). The patients had been given prior treatments with 2–6 (mean ± SD = 3.1 ± 1.3) antidepressants or mood stabilizers in adequate dosages and for an adequate duration. The number of prior episodes the patients had suffered ranged from 2 to 6 (mean ± SD = 2.3 ± 2.0), and the mean duration of the current episode ranged from 4–36 months (mean ± SD = 16.2 ± 15.9). Next, rTMS was performed (in addition to the antidepressants or mood stabilizers). The mean Ham-D score of 20.5 ± 5.2 before treatment was significantly decreased to 15.6 ± 7.3 at 2 weeks after rTMS treatment. Five of 26 patients showed an improvement of 50% or higher on Ham-D scores (responders), 4 patients showed an improvement of 25–49% on Ham-D scores (partial responders), and the remaining 17 patients demonstrated an improvement of below 25% (nonresponders). Finally, 7 of 26 patients (27%) achieved remission (Ham-D score < 8).

Table **1** Medications used and the number of patients treated

Antidepressants/Mood stabilizers	*Number of Patients*
milnacipran	7
paroxetine	5
sulpiride	5
lithium carbonate	5
fluvoxamine	5
mianserin	4
amoxapine	2
imipramine	2
trazodone	2
sodium valproate	1
clomipramine	1
maprotiline	1

Some patients are treated with more than two drugs.

No statistically significant differences were observed between responders plus partial responders and nonresponders with respect to sex, age, and the number of patients who had only one depressive episode (first depressive episode) or Ham-D score before rTMS treatment and plasma levels of MHPG and BDNF before rTMS treatment (Tables **2**, **3**). There was no significant correlation between the plasma levels of BDNF, MHPG, or HVA and age or body weight. No significant difference was observed between males and females with respect to the plasma levels of BDNF, MHPG, or HVA (data not shown). No associations were found between changes in the total score of Ham-D and changes in the plasma MHPG or HVA levels. However, on the individual items of the Ham-D, a negative correlation was observed between the changes in the plasma MHPG levels and the changes in the scores of agitation on the Ham-D before and 2 weeks after rTMS (Table **4**).

The plasma levels of MHPG were significantly reduced 2 weeks after rTMS treatment (before: 8.27 ± 5.98 ng/mL, after: 5.69 ± 4.70 ng/mL) (Fig. **1A**). However, there was no significant change in the plasma levels of HVA between before and 2 weeks after the rTMS treatment in all patients (before: 6.71 ± 5.49 ng/mL, after: 5.46 ± 5.17 ng/mL) (Fig. **1B**). The plasma levels of HVA in responders were significantly higher than those in nonresponders (Fig. **2**), though no correlations between the changes in plasma HVA and the changes in the scores of each item of Ham-D were observed. There was a trend for increasing plasma BDNF levels in all patients 2 weeks after rTMS treatment (before; 2.53 ± 2.01 ng/mL, after; 3.11 ± 2.00 ng/mL; $P < 0.1$). In particular, the plasma BDNF levels were increased 2 weeks after rTMS treatment in the responders and partial responders (before; 2.35 ± 1.47 ng/mL, after; 3.87 ± 2.13 ng/mL; $P < 0.05$), while, no significant increase was observed in nonresponders (before; 2.46 ± 2.16 ng/mL, after; 2.60 ± 1.84 ng/mL; n.s.), and there were no differences between the two groups in plasma BDNF levels before rTMS treatment (Fig. **3**). Finally, a trend for association was found between changes in Ham-D scores and changes in plasma BDNF levels in all patients before and 2 weeks after rTMS treatment ($\rho = 0.340$, $P < 0.1$) (Fig. **4**). No correlation was found between stimulation intensity and changes in the plasma levels of MHPG, HVA, and BDNF or changes in the Ham-D score (data not shown).

Table **4** Correlation between the changes in plasma MHPG levels and the changes in scores on each item of Ham-D

Items	*ρ Values*	*P Values*
Depressive mood	-0.02	0.76
Feeling of guilt	0.4	0.23
Suicide	0.14	0.84
Insomnia (early/middle/late)	0.17	0.5
Work and Interest	0.04	0.83
Retardation	0.06	0.6
Agitation	-0.08	0.03*
Anxiety (psychic)	-0.1	0.47
Anxiety (somatic)	0.13	0.96
Somatic symptoms (gastrointestinal)	0.03	0.39
Somatic symptoms (general)	0.07	0.28
Genital symptoms	0.21	0.68
Hypochondriasis	0.2	0.53
Loss of weight	0.18	0.73
Insight	0.29	0.67

* $P < 0.05$ significant correlation.

Discussion

The main findings of the present study are that nine out of twenty-six treatment-resistant patients (35%) at least partially responded to rTMS treatment, and plasma levels of MHPG were significantly decreased in all patients after rTMS treatment. A negative correlation was observed between the change in plasma MHPG levels and the change in the Ham-D scores with regard to agitation. In addition, the plasma BDNF levels were increased in the responders and partial responders by 23%, but not in the nonresponders after rTMS treatment, and a trend for association was found between the changes in the Ham-D score and BDNF

Table **2** Characteristics of the patients

Subgroups	*Sex* *Male*	*Female*	*Age (mean ± SD)*	*Number of patients with first episode*	*Baseline Ham-D (mean ± SD)*
Responders (n = 9)	5	4	48.9 ± 20.8	3	21.0 ± 5.5
Nonresponders (n = 17)	6	11	54.1 ± 16.5	9	19.6 ± 4.9

Ham-D: 17-item Hamilton Rating Scale for Depression.

Table **3** Plasma catecholamine metabolites before and after rTMS

Subgroups	*pMHPG (ng/mL)* *Before*	*After*	*pHVA (ng/mL)* *Before*	*After*	*BDNF (ng/mL)*	
Responders + Partial responders	8.0 ± 3.7	*6.3 ± 5.1	9.2 ± 4.7	9.8 ± 6.5	2.35 ± 1.47	*3.87 ± 2.13
Nonresponders	8.4 ± 7.0	5.3 ± 4.6	5.4 ± 5.6	3.1 ± 2.1	2.61 ± 2.26	2.76 ± 1.90

* $P < 0.05$ before vs. after rTMS.

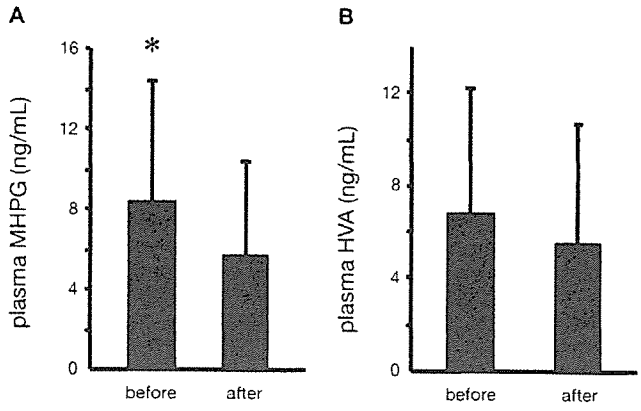

Fig. 1 (A) Plasma levels of MHPG before and after rTMS. Data are presented as the means ± SD. (B) Plasma levels of HVA before and after rTMS. Data are presented as the means ± SD. Plasma levels of MHPG and HVA were analyzed at one experiment in duplicate.

levels before and 2 weeks after rTMS treatment in all patients. No adverse side effects such as memory impairments or seizures were reported during or after rTMS treatment. To date, the clinical efficacy of rTMS treatment for depression has been well demonstrated by performing sham controlled studies [7,10,16,19]. In contrast, Couturier [9] recently reported from six small, but generally well-designed studies, that rapid-rate rTMS was no more efficacious than sham therapy in treating adults with a major depressive episode. Our result, in which only 35% of patients showed some improvement (Ham-D ≥ 25%) is in line with the results of the last meta-analysis performed by Couturier [9]. Recently, rTMS has been proposed and subsequently researched as a putative therapeutic approach for refractory major depression. In addition, Rumi et al. [40] demonstrated that rTMS is effective in accelerating the onset and augmenting the therapeutic response to amitriptyline for severe depressed patients by performing a double blind placebo-controlled study. In general, however, the response rates in rTMS for treatment-resistant depression remain low [17,20,38]. Psychotic symptoms have been reported to be a negative predictor for rTMS [21]. However, Fitzgerald et al. [14] performed a double-blind, placebo-controlled study in treatment-refractory depression and found that treatment for at least 4 weeks is necessary for clinically meaningful benefits to be achieved. The response rates in the present study were relatively low compared with those of other studies. In the present study, 4 out of 26 patients had experienced psychotic symptoms during the depressive phase. The response rates in patients with and those without psychotic features were 50 and 32%, respectively, indicating that the response rate in patients with psychotic features was not significantly different from that in those without psychotic features. One of the reasons why the response rate was lower in this study than in previous studies may have been the length of the treatment period; two weeks are not enough time to achieve an adequate response, as mentioned by Fitzgerald et al. [14].

With regard to the actions of rTMS on catecholamine systems, Keck et al. [27] reported that acute rTMS (20 Hz) of frontal brain regions leads to alterations in the mesolimbic and mesostriatal release patterns of dopamine *in vivo*. On the other hand, Ben-Shachar et al. [5] demonstrated that the dopamine content in the frontal cortex of TMS-treated rats was reduced by 26%, while the contents in the striatum and hippocampus were increased by 25 and 18%, respectively. It can be speculated that the rTMS-mediated dopamine release evident in preclinical trials may provide the underlying mechanism for both the poorer response of patients with psychotic depression and newly occurring psychotic symptoms during rTMS treatment. However, Ben-Shachar et al. [6] demonstrated that brain tissue monoamine levels were unchanged after 10 days of treatment with rTMS. In the present study, the plasma HVA levels were not changed between before and after rTMS treatment, and the plasma HVA levels in responders were significantly higher than those in nonresponders, though no correlations were found between the changes in plasma HVA and the changes in the total Ham-D score or individual items in the Ham-D. These results do not resolve the controversy over the effects of rTMS on the dopaminergic system. However, the plasma MHPG levels were significantly decreased after rTMS treatment, and were associated with the improvement of agitation in depressed patients. In a previous study [52], we considered that depressed patients might be dichotomized into two groups, one characterized by anxiety and/or the perception of powerlessness with high plasma MHPG levels, and another by psychomotor retardation with low plasma MHPG levels. We also found correlations between scores of agitation/anxiety in Ham-D and plasma levels of MHPG in 87 patients with major depressive disorder, indicating that depressed patients who revealed predominantly agitation/anxiety were characterized by higher plasma MHPG levels [52]. Eschweiler et al. [11] proposed that anxiety is a positive predictor for a successful clinical outcome after rTMS. Fitzgerald et al. [14] also reported that baseline psychomotor agitation predicted a successful response to rTMS. Taken together, these findings suggest that rTMS might be especially effective for depressed patients characterized by agitation/anxiety with high plasma MHPG levels. In fact, Fleischmann et al. [15] demonstrated an effect of rTMS on noradrenergic neurons, suggesting that chronic rTMS treatment directly downregulates β-adrenoceptors. In addition, Kole et al. [28] reported that chronic rTMS treatment also downregulates 5-HT_{1A} and 5-HT_{2A} receptors in the frontal cortex, which might indirectly influence the noradrenergic neurons, given that there are well-known interactions between serotonergic and noradrenergic neurons in the re-

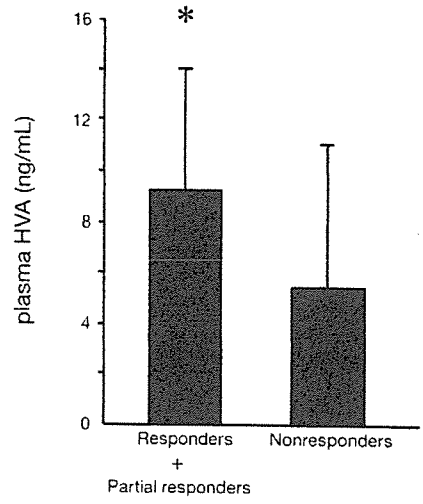

Fig. 2 Plasma levels of HVA before rTMS. Data are presented as the means ± SD. Patients with a 50% or more decrease in their Ham-D score are classified as responders, the patients with a 25 – 49% decrease in their Ham-D score as partial responders, and the remaining patients as nonresponders. * $P < 0.01$, compared with nonresponders. Plasma levels of HVA were analyzed at one experiment in duplicate.

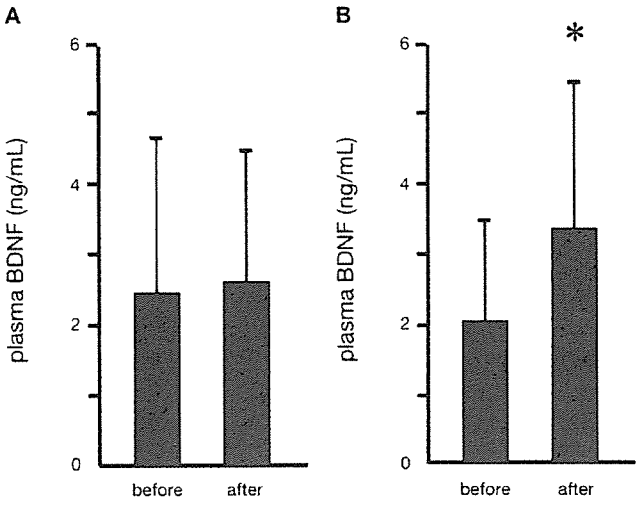

Fig. 3 (A)Plasma levels of BDNF before and 2 weeks after rTMS in non-responders. (B) Plasma levels of BDNF before and 2 weeks after rTMS in responders plus partial responders. Data are presented as the means ± SD. * $P < 0.05$, compared with before rTMS. Plasma levels of BDNF were assayed at one experiment in duplicate.

gion [46]. However, the exact mechanism by which rTMS treatment alters plasma MHPG remains unknown. As respective indicators of central noradrenergic and dopaminergic neuron activity, plasma MHPG and HVA should be used with caution, due to the fact that they only partially reflect activity in the brain. It has been hypothesized that only one third of the plasma MHPG and 30–50% of the plasma HVA are derived from the brain [52]. However, measuring plasma levels of catecholamine metabolites has the advantage of allowing more frequent sampling intervals than would be possible with urinary measures; in addition, the plasma measurement procedure is more convenient and comfortable for patients than the CSF measurement procedure. Thus, we have used plasma measures in a previous study [36,43,47,51].

We also found that rTMS treatment increased the plasma BDNF levels in responders plus partial responders, but not in nonresponders. In addition, a trend of association was found between the changes in the Ham-D scores and those in the plasma BDNF levels in all patients. These results suggest that the improvement of depressive symptoms by rTMS is in part related to the increase of plasma BDNF. Muller et al. [35] reported that rTMS increased BDNF mRNA and BDNF protein in the hippocampus as well as in the parietal and periform cortex. However, Jacobsen and Mønk [23] performed parallel measurements of BDNF mRNA and protein expression in the frontal cortex and hippocampus of the rat after chronic treatment with ECT, lithium, desipramine, or escitalopram. In their results, ECT increased BDNF mRNA and protein in the hippocampus and BDNF protein in the frontal cortex. Desipramine, a tricyclic antidepressant moderately increased BDNF mRNA in the dentate gyrus but did not change BDNF protein in either region. Escitalopram, a selective serotonin reuptake inhibitor did not affect BDNF mRNA, but decreased BDNF protein in the frontal cortex and hippocampus. Lithium increased the BDNF protein expression in the hippocampus and frontal cortex, but overall decreased BDNF mRNA. Taken together, it is difficult to conclude that the increased expression of BDNF mRNA and protein is a common action of antidepressant drug treatment and ECT. Karege et al. [24] reported that the serum BDNF levels were significantly decreased in antidepressant-free depressed patients, and that the serum BDNF levels were negatively correlated with the Montgomery-Åsberg Depression Rating Scale. Shimizu et al. [42] also demonstrated that serum BDNF was significantly lower in an antidepressant-naïve group than in either a treated or in a control group, and that there was a significant negative correlation between serum BDNF and Ham-D scores in all patients. Furthermore, they reported preliminary findings that decreased serum BDNF levels in antidepressant-naïve patients recovered to normal levels in association with lower Ham-D scores after treatment with antidepressant medication. Moreover, Lang et al. [29] demonstrated that decreased serum BDNF levels were observed in healthy volunteers with neuroticism, and depression-related personality traits. The authors also speculated that BDNF levels have some influence on central serotonergic activity. Angelucci et al. [2] reported that daily low-frequency (1 Hz) rTMS motor cortex stimulation for 8 days is associated with a progressive reduction of the BDNF plasma levels in healthy subjects, but has no effect on the BDNF plasma levels in amyotrophic lateral sclerosis patients. On the other hand, high frequency (20 Hz) rTMS demonstrated a transient decrease in plasma BDNF levels. The authors speculated that this effect was due to the loss of motor cortex pyramidal cells. Although BDNF is highly concentrated in the brain, it is also present in the plasma and serum. The source of circulating BDNF remains unknown. Platelets, brain neurons, and vascular endothelial cells are considered candidate sources. Previously, it was reported that BDNF could cross the blood-brain barrier [37], and that BDNF levels in the brain and serum underwent similar changes during the maturation and aging process in rats [25], indicating that plasma BDNF levels might in part reflect the BDNF levels in the brain. In contrast to this, Radka et al. [39] reported that the BDNF detected in human plasma was derived from platelet degranulation, and

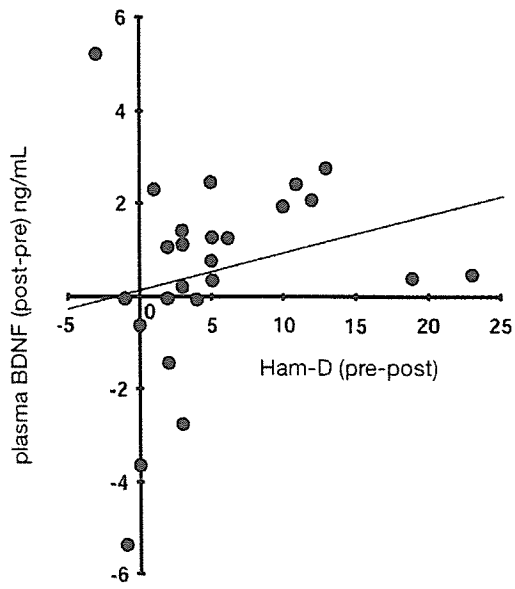

Fig. 4 Changes in plasma levels of BDNF and Ham-D scores. pre: before rTMS, post: 2 weeks after rTMS. Data are presented as the means ± SD. Plasma levels of BDNF were assayed at one experiment in duplicate