厚生労働科学研究費補助金（こころの健康科学研究事業）
分担研究報告書

退行期～老年期うつ病例の臨床病理学的検討

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研究要旨:
退行期～老年期にうつ状態を初発し、脳に血管性病変を伴ううつ病患者群、脳に血管性病変を伴わないうつ病患者群、正常対照群の３群間で、大脳白質の細動脈血管壁の厚さを比較したところ、血管性病変を伴ううつ病患者群において血管壁の肥厚（細動脈硬化が高度であった。このような細動脈硬化は脳血流を低下させ、これが器質要因となってうつ状態を起こす一因となっていると考えられた。

A. 研究目的

退行期～高齢期のうつ病 (late-life depression: LLD) は若年期のうつ病に較べて、抑うつ気分が軽い一方で、意欲低下や精神運動抑制が前景に出たり、焦燥感が強い、感情失禁や心気的傾向を伴う、難治性であるなどの特徴がある。このような特徴を示す LLD には器質的な背景があり、脳の血管性要因、炎症性反応や内分泌・栄養状態の関与が指摘されている。これまでの研究で、脳に粗大な病変を伴わない LLD では、大脳白質において、細動脈硬化病変ば認められないが、うつ病を伴わない対照群に較べて、ミクログリアの増加や活性化ほかの炎症性反応の亢進が確認されている。本年度は脳に血管性病変を伴う LLD 症例群における大脳白質細動脈病変について検討し、治療のための基礎的データを得ることを目的とした。

B. 研究方法

東京都精神医学総合研究所に保存されている剖検脳のなかから、退行期～老年期発症のうつ病で、脳血管性病変を伴う 6 例 (LLD I 群: 60～86 歳,平均年齢 74.0 歳) 退行期～老年期発症うつ病で、脳血管性病変を伴わない 7 例 (LLD II 群: 59～77 歳, 平均年齢 69.7 歳)、正常対照 7 例 (C 群: 63～79 歳,平均年齢 69.9 歳) を対象とした。前頭前野を検索領域とし、10 μm 厚パラフィン切片を用いて、以下の項目について検討した。

細動脈硬化の程度を知る目的で、白質の細動脈壁厚の測定を行った：各症例について大脳白質に出現する細動脈をすべて光学顕微鏡の対物レンズ 20 倍下で CCD カメラで取り込み、そのなかから正円形に近い５個の細動脈を計測対象に選んだ。選んだ細動脈について、フォトショップを用いて、タブレット上で描画して画像として取り込み、各細動脈の壁の外周 (A) とその面積 (B)、内周 (C) とその面積 (D) を NIH イメージで計測した。各細動脈の平均壁厚 (S) を S＝2(B-D)/A + C で求め、各症例の血管壁の平均値を計算

し、各群間（LLD I, LLD II, C）で比較した。

（倫理面への配慮）

倫理面への配慮であるが、今回、研究に共された剖検脳は厚労省指針のB群試料に相当する。東京都精神医学総合研究所と都立松沢病院の研究倫理委員会で研究承認を得、情報管理者による匿名化操作を経て研究に供した。

C. 研究結果

血管壁の平均の厚さからみた細動脈硬化につては、脳血管性病変を伴わないLLD II群は正常対照のC群に較べて、血管壁がわずかに厚い傾向があったが、全体的には殆ど変わらず、両者の間に有意な差はなかった。これに対して、脳血管性病変を伴うLLD I群の血管壁はLLD II群、C群のいずれに対しても有意に肥厚していた。LLD I群の６症例の脳血管病変は、以下の如くであった。症例１：基底核のラクナ梗塞、外障部梗塞、白質の淡明化、症例２：白質の淡明化、Fahr 病様の血管の類石灰化病変、症例３：基底核のラクナ梗塞、白質の淡明化、症例４：淡蒼球のラクナ梗塞、橋・延髄の小梗塞、症例５：白質の多発性小梗塞、症例６：基底核小梗塞、白質細動脈硬化。このようなラクナ梗塞や大脳白質病変形成の背景には細動脈硬化の存在が指摘されており、今回の結果はこれを証明するものであった。以下に、細動脈硬化の存在と退行期～高齢期に出現するうつ病について考察する。

D. 考察

脳梗塞後には約30%程度の患者がうつ状態を示し、「脳梗塞後うつ病（post-stroke depression）」として知られている。その後、MRIなどの画像研究で、臨床症状を示すには至らない程度の脳梗塞を伴う高齢者にうつ状態患者が多く見出されることから、MRI-defined vascular depressionという病態も知られるようになった。今回の研究は脳に明らかな血管性病変を伴ううつ病患者の検討を通して、その病因的背景を明らかにし、治療の手がかりを得ることを目的とした。脳にラクナ梗塞や白質病変を伴う症例群では、大脳白質の細動脈の壁の肥厚（細動脈硬化）が高度であることが数値として示された。このような細動脈硬化はラクナ梗塞や白質病変を形成する原因であることが知られており、その際に、脳血流低下が起こることが知られている。血管性病変に加えて、このような脳血流低下は精神症状、痴呆、せん妄など、さまざまな症状を引き起こし、その中のひとつがうつ状態であると考えられる。このような病態があった場合に必ずうつ状態が起こるわけではないが、病前の素質や、環境要因と相俟って、うつ状態が引き起こされやすいものと考えられる。一方、脳に粗大な病変がないLLD II群では血管壁の肥厚という形での硬化性病変はみられないが、大脳白質のミクログリアの増加や活性化という形での異常がこれまでの研究で示されている。このような事実は高齢者のうつ病の病因は単一ではないことを示しており、治療への応用として、脳血流の改善や抗炎症剤の使用を試みる価値があると考える。

E. 結論

退行期～老年期にうつ状態を初発し、脳

に血管性病変を伴ううつ病患者群の検討では大脳白質細動脈硬化が高度であることが示された。一方、脳に血管性病変を伴わないうつ病患者群では正常対照群と較べて、血管壁の肥厚に差はみられなかったが、これまでの研究で前頭葉白質の炎症性反応を伴う症例が多いことが分かっている。これらの事実は治療に応用が期待される。

F. 健康危険情報

なし。

G. 研究発表

なし。

1. 論文発表

池田研二:うつ病と帯状回. Clin Neurosci 23: 1298-1300, 2005

2. 学会発表

池田研二, 石津秀樹 秋山治彦: Late-life depression (LLD) の神経病理学的検討. 第27回日本生物学的精神医学会, 平成17年7月6日, 大阪

H. 知的財産権の出願・登録状況(予定を含む。)

1. 特許取得

なし。

2. 実用新案登録

なし。

3. その他

なし。

厚生労働科学研究費補助金（こころの健康科学研究事業）
分担研究報告書

うつ病における認知障害及び認知症への進展に関わる神経生物学的基盤解明のための研究

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研究要旨：感情障害における認知機能および認知症への移行に関する生物学的基盤を解明することを目的とし，抑うつを呈した感情障害患者を対象に病相寛解期に神経心理学的認知機能検査を施行し，様々な臨床情報との関連を調査した．その結果，器質的所見のあるうつ病患者では寛解期の認知機能とうつの重症度や臨床経過に関連性がある可能性が示唆された．これは一部のうつ病と認知機能の関係には脳器質的脆弱性が関与している可能性を示唆しており，今後追跡調査をすることによりうつ病から認知症への移行に関する生物学的基盤を解明する一助となるものと考えられる．

A. 研究目的

以前より認知症のリスクファクターのひとつに抑うつがあることが知られており，臨床的にもうつ病から認知症へ移行する症例を経験することも多い．近年うつ病をはじめとする感情障害患者の認知機能が注目されており，病相が寛解した後でも一部の認知機能が低下しているとの報告もみられている．今回我々はうつ病を中心とする感情障害をとりまく様々な要素（重症度や臨床経過，免疫・内分泌学的所見など）と認知機能との関連性を調査し，感情障害における認知機能障害および認知症への移行に関する生物学的基盤を解明することを目的とした．

B. 研究方法

対象は抑うつを呈し，順天堂越谷病院に入院した患者のうち，DSM-IV において気分障害に分類される疾患の診断基準を満たした 61 例（男性 24 例，女性 37 例，年齢は 25 歳から 73 歳，平均 52.4(S.D 11.9)歳，大うつ病性障害 48 例，双極性障害 10 例，気分変調症 3 例）に対し調査を行った．頭部ＣＴにおいて年齢を考慮しても軽度以上と評価される脳萎縮や血管性の所見，または脳波にて徐波傾向やα波のびまん性出現などの器質的脆弱性を示唆する所見を器質的所見とした．この基準による器質的所見を認めるものが 28 例，認めないものが 24 例，不明が 10 例であった．ただし，明らかな脳梗塞など直接精神症状に影響を与えると考えられる器質的病変を有する症例は除外した．

これらの対象群に対し入院時に cortisol や ACTH などの内分泌学的検査およびNK細胞活性などの免疫学的検査を施行した．入院時より経時的にハミルト

ンのうつ病評価尺度（HAM-D）にて状態評価を行い，HAM-D が 7 点以下となった状態で寛解と評価し，以後 Wisconsin Card Sorting Test （WCST），Stroop Color Word Test（SCWT），Verbal Fluency Test （VFT）を用いて認知機能検査を行った．

これら認知機能検査の結果と大うつ病性障害患者の年齢，教育年数，入院時 HAM-D，病相回数，総病相期間（推定発病時から各うつ病相期間の総和），総治療期間（寛解後の維持療法を含めた治療期間の総和）との関係を器質的所見の有無によって 2 群に分けて検討した．さらに大うつ病性障害と双極性障害との認知機能の違いについて検討した．

（倫理面への配慮）

本研究の実施にあたっては順天堂大学医学部研究等倫理委員会の承認を得た上で，患者には研究の目的・方法・協力の任意性・個人情報の保護などについて十分な説明を行い，文書にて同意を得た．

C. 研究成果

対象を大うつ病性障害 48 例に限定すると．男性 18 例，女性 30 例，平均 59.3（S.D 12.3 歳であった．器質的所見を有するものが 23 例，無いものが 18 例，不明が 7 例であった．器質的所見を有する群は有意に年齢が高かった（$p<0.005$）．また年齢と総病相期間に有意な負の相関がみられた（$p<0.05$）．

WCST では器質的所見を有する患者群において保続的エラーを表す PEM および PEN と年齢に正の相関傾向がみられた（$p=0.06$）．達成カテゴリー数を表す CA は器質的所見を有する患者群においてのみ入院時の HAM-D スコアとの間に有意な負の相関がみられた（$p<0.01$）．また保続的エラーを表す PEM （$p<0.005$）および PEN（$p<0.01$）はやはり器質的所見を有する患者群において入院時 HAM-D スコアと有意な正の相関がみられた．

SCWT では器質的所見のない患者群において課題 1 と課題 2 の時間差と年齢との間に有意な正の相関がみられ（$p<0.01$），誤答数と教育年数との間に有意な負の相関がみられた（$p<0.05$）．一方器質的所見のある患者群で課題 1 と課題 2 の時間差と病相回数（$p<0.05$），および総治療期間（$p<0.005$）との間にそれぞれ有意な正の相関がみられた．

VFT では器質的所見のない患者群において得点と教育年数との間に有意な正の相関がみられた（$p<0.001$）．

大うつ病性障害群と双極性障害群ではいずれの認知機能検査においても有意差はなかった．

D. 考察

WCST における過去の報告では，病相期のうつ病患者は健常者と比較して保続的エラーが優位に多く，WCST の結果とうつ病の重症度との関連性が示唆されている．その背景として，うつ病患者は否定的な認知の構えを適切に転換する柔軟性が障害されるため，反応行動における構えの構造や変更能力を評価する WCST にそれらが反映されるといわれている．本研究では，器質的変化のある患者群において寛解期の保続的エラー数と入院時 HAM-D の得点に正の相関がみられた．このことから器質的所見を認めるうつ病患者では，うつの重症度と寛解期における認知機能

に何らかの関連性があることが示唆された．また，SCWT と VFT では，病相期のうつ病患者は健常者と比較して成績が低下しているとが報告されている．さらにうつ病患者の SCWT の成績低下は寛解期にも認められると報告されており，SCWT で評価される認知機能の低下は，うつ病に対する脆弱性を反映している可能性が示唆された．本研究では，器質的所見のある患者群において寛解期の SCWT の課題と課題２の時間差と病相回数および総治療期間にそれぞれ相関がみられ，器質的所見を認めるうつ病患者では寛解期の認知機能と臨床経過との関連性が示唆された．

これらの結果から器質的変化がうつ病患者の認知機能に影響をあたえ，これによってうつ病相の重症化や経過の長期化が起こりやすくなると考えることもできる．しかし，今回は相関のみをみたため，これらの因果関係は明らかにされず，今後さらなる詳細な調査が必要であると考えられた．また今後は前方向視的追跡調査を行い，再発や認知症への移行に関する生物学的基盤の解明につながる要因を調査する予定である．

E. 結論

今回の結果から器質的所見のある抑うつ患者では寛解期の認知機能とうつの重症度や臨床経過に関連性がある可能性が示唆された．これは一部のうつ病と認知機能の関係には脳器質的脆弱性が関与している可能性を示唆しており，今後追跡調査をすることによりうつ病から認知症への移行に関する生物学的基盤を解明する一助となるものと考えられる．

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

なし

2. 学会発表

中野祥行，馬場元，前嶋仁，石塚卓也，河野るみ子，山崎学，北島明佳，馬場奏子，酒井佳永，鈴木利人，新井平伊
うつ病患者の認知機能に関する研究
第２回　日本うつ病学会総会（2005）東京

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

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
なし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Matsubara T, Funato H, Kobayashi A, Nobumoto M, Watanabe Y.	Reduced glucocorticoid receptor expression in mood disorder patients and first-degree relatives.	Biological Psychiatry			In press
Kumano H, Ida I, Oshima A, Takahashi K, Yuuki N, Amanuma M, Oriuchi N, Endo K, Matsuda H, Mikuni M	Brain metabolic changes associated with predisposition to the onset of major depressive disorder and adjustment disorder in cancer patients. A preliminary PET study.	J Psychiat Res			In press
Kameyama M, Fukuda M, Yamagishi Y, Sato Toshimasa, Uehara T, Ito M, Mikuni, M,	Frontal lobe function in bipolar disorder:A multichannel near-infrared spectroscopy study.	NeuroImage	26	172-184	2006
Kunugi H, Ida I, Owashi T, Kimura M, Inoue Y, Nakagawa S, Yabana T, Urushibara T, Kanai R, Aihara M, Yuuki N, Otsubo T, Oshima A, Kudo K, Inoue T, Kitaichi Y, Shirakawa O, Isogawa K, Nagayama H, Kamijima K, Nanko S, Kanba S, Higuchi T, Mikuni M;	Assessment of the dexamethasone/CRH test as a state-dependent marker for hypothalamic-pituitry-adrenal axis abnormalities in major depressive episode:A multicenter study.	Neuropsychopharmacol	31	212-220	2006
Yuuki N, Ida I, Oshima A, Kumano H, Takahashi K, Fukuda M, Oriuchi N, Endo K, Matsuda H, Mikuni M;	HPA axis normalization, estimated by DEX/CRH test, but less alteration on cerebral glucose metabolism in depressed patients receiving ECT after medication treatment failures.	Acta Psychiat Scand	112	257-265	2005
池田研二	うつ病と帯状回	Clin Neurosci	23	1298-1300	2005
Ito M, Fukuda M, Suto T, Uehara T, Mikuni M.	Increased and decreased cortical reactivities in novelty seeking and persistence: a multichannel near-infrared spectroscopy study in healthy subjects.	Neuropsychobiol	52	45-54	2005

研究成果の刊行物・印刷物

Reduced Glucocorticoid Receptor α Expression in Mood Disorder Patients and First-Degree Relatives

Toshio Matsubara, Hiromasa Funato, Ayumi Kobayashi, Masaaki Nobumoto, and Yoshifumi Watanabe

Background: *Individuals with mood disorders exhibit altered function of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress. The glucocorticoid receptor (GR) plays an important role in the negative feedback regulation of the HPA axis. There are two protein isoforms of GR, GRα and GRβ, which have distinct biological activity. It has not been examined whether GRα messenger RNA (mRNA) and GRβ mRNA expressions are altered in peripheral blood cells of mood disorder patients.*

Methods: *Using quantitative reverse transcription polymerase chain reaction (RT-PCR), GRα mRNA and GRβ mRNA were measured in peripheral blood cells of major depressive disorder patients (depressive* n = *18; remissive* n = *38), bipolar disorder patients (depressive* n = *13; remissive* n = *35), normal control subjects* (n = *31), and first-degree relatives of major depressive* (n = *17) and bipolar* (n = *15) disorder patients.*

Results: *Reduced expression of GRα mRNA was shown in both bipolar and major depressive disorder patients in a current depressive state as well as in remission. First-degree relatives of bipolar disorder patients also showed GRα mRNA reduction. Altered GRβ mRNA expression was not found in mood disorder patients.*

Conclusions: *Our results suggest that reduced GRα mRNA expression might be trait-dependent and associated with the pathophysiology of mood disorders.*

Key Words: Glucocorticoid receptor isoforms, α, β, mood disorder, first-degree relatives of mood disorders, HPA axis, trait-marker, DEX/CRH test

Individuals with mood disorders often exhibit hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, such as increased concentration of plasma cortisol and blunted suppression to dexamethasone as measured by the dexamethasone suppression test and dexamethasone/corticotropin-releasing hormone (DEX/CRH) test (Arborelius et al 1999; Holsboer 2001; Nestler et al 2002; De Kloet 2003). Although the molecular mechanism of aberrant regulation of the HPA axis in mood disorder patients remains unclear, one candidate is the dysfunction of the glucocorticoid receptor (GR), which plays an important role in the negative feedback of the HPA axis and adaptation to stress (Holsboer 2000; Pariante and Miller 2001). Glucocorticoid receptor, a member of the nuclear receptor superfamily proteins, binds glucocorticoids in the cytoplasm and then translocates into the nucleus to work as a transcription factor, resulting in inhibition of secretion and synthesis of both corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH). In addition to the role on the HPA axis, GR expressed broadly throughout the brain is thought to modulate various neural functions such as learning and memory (Karst et al 2000; Lupien et al 2005). In response to stress, GR is associated with stress-induced effects on the brain, including shrinkage of neural dendrites, suppressed neurogenesis, and reduced serotonin metabolism (Lopez et al 1998; McEwen 2000; Sapolsky et al 2000; De Kloet 2003).

There are two protein isoforms of GR, GRα and GRβ, produced by alternative splicing. In contrast to GRα, which exerts glucocorticoid effects, GRβ is not able to bind glucocorticoids and is thought to form a heterodimer with GRα to exert a dominant-negative effect on GRα-mediated transcription (Oakley et al 1999; Vottero and Chrousos 1999). The proposed role for GRβ can explain the finding that enhanced expression of GRβ was associated with glucocorticoid resistance in allergic disease (Bamberger et al 1995; Leung et al 1997; Sousa et al 2000; Webster et al 2001). In addition, GRβ was reported to inhibit apoptosis induced by glucocorticoids in vitro (Strickland et al 2001), although the biological function of GRβ remains controversial (Carlstedt-Duke 1999; Vottero and Chrousos 1999).

Recently, reduced expression of GRα messenger RNA (mRNA) on postmortem brains has been reported in the distinct regions of the cortex and hippocampus of major depressive disorder and bipolar disorder brains (Webster et al 2002; Knable et al 2004; Perlman et al 2004). In line with aberrant expression of GRα mRNA in the brain, lymphocytes of depressed patients showed reduced response to dexamethasone (Wodarz et al 1991, 1992; Calfa et al 2003) and a reduced number of glucocorticoid binding sites (Gormley et al 1985; Whalley et al 1986; Yehuda et al 1993), although the lack of alteration in glucocorticoid binding sites was also reported (Wassef et al 1990; Rupprecht et al 1991). These previous findings strongly support the important role of GR in the pathophysiology of mood disorders. To date, there have been no direct measurements of GRα mRNA and GRβ mRNA on peripheral blood cells of mood disorder patients. Moreover, postmortem brain studies lack information on plasma cortisol concentration and the HPA axis activity assessed by the DEX/CRH test. It is difficult to determine whether the reduced GRα expression is recognized only in the depressive state or continues after recovery from the depressive state, in other words, state-dependent or trait-dependent.

The aim of this study was to evaluate GRα mRNA and GRβ mRNA levels in the peripheral white blood cells of individuals with major depressive disorder and bipolar disorder. Furthermore, to examine whether altered GR mRNA expression is state-dependent or trait-dependent, mood disorder patients in remission and first-degree relatives of mood disorder patients were also assessed.

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Table 1. Summarized Profile of Antidepressant Medication for Longitudinally Followed Subjects with Major Depressive Disorder and Bipolar Disorder

	MDD				BPD			
	Treatment Period (Months)				Treatment Period (Months)			
Anti-depressant Medication	0	2	4	6	0	2	4	6
Tricyclic Antidepressant	3				1			
Tricyclic Antidepressant + SSRI	3	2	2	1				
Tricyclic Antidepressant + SNRI	2	2						1
SSRI	2	7	6	6	4	3	2	1
SNRI	2	2	2	2	2	1	1	
SSRI + SNRI		2		2	1		1	
Tetracyclic Antidepressant	3	3	1		1	4	2	2
No Antidepressant	5	1			4	2	3	4
Total Number	20	19	11	11	13	10	9	8

MDD, major depressive disorder; BPD, bipolar disorder; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin-noradrenaline reuptake inhibitor.

Methods and Materials

Subjects

Major depressive and bipolar disorder patients were diagnosed according to the criteria in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) (American Psychiatric Association 1994). These included both outpatients and inpatients of the Division of Neuropsychiatry of the Yamaguchi University Hospital. The extent of depressive state was assessed by a 21-item Hamilton Depression Rating Scale (HDRS). Subjects were regarded as under a current depressive state when they showed a score of more than 20 on HDRS and met the DSM-IV criteria for major depressive episode. Subjects were regarded to be in remission when they showed a score of less than 6 on HDRS and did not show any symptoms of the major depressive episode in the DSM-IV criteria for more than 2 months. A group of individuals with mood disorder in a current depressive state was assessed every 2 months for 6 months to investigate gene expression alteration during recovery from depressive states. Antidepressant medications for these subjects are summarized in Table 1. Individuals were excluded from the present study when they showed abnormal physical examinations or abnormal results for routine medical laboratory tests such as a complete blood count and renal, liver, and thyroid function. Female subjects who were pregnant or took oral contraceptives were also excluded. First-degree relatives who had no significant current or past medical or neurological illness, significant alcohol or drug abuse, and past or current Axis I psychiatric illness were enrolled (Table 2). All normal control subjects were screened to exclude significant current or past medical or neurological illness, significant alcohol or drug abuse, and past or current Axis I psychiatric illness. This protocol was approved by the Institutional Review Board of Yamaguchi University Hospital. Informed written consent was obtained for all subjects.

Blood Sample Preparation

Blood was obtained by venipuncture between 10:00 A.M. and 11:00 A.M. and processed to determine plasma cortisol concentration and total RNA purification.

RNA Isolation and Complementary DNA Synthesis

Total RNA was prepared from blood samples using QIAamp RNA Blood Mini kit (Qiagen, Chatsworth, California). The total RNA yield was determined by OD260. One microgram of total RNA was used for complementary DNA (cDNA) synthesis by random hexamer and Omniscript reverse transcriptase (Qiagen). The cDNA was stored at −80°C until use.

Real-Time Quantitative Polymerase Chain Reaction

Real-time quantitative polymerase chain reaction (PCR) was performed on cDNA with LightCycler (Roche Molecular Biochemicals, Germany) using the QuantiTect SYBR Green PCR kit (Qiagen) according to the manufacturer's manual. Polymerase chain reaction conditions were 15 minutes at 95°C, 35 to 45 cycles of 15 seconds at 95°C, 20 seconds at 55°C, and 10 seconds at 72°C. Cycle number was optimized for each primer set corresponding to GRα, GRβ, and gylceraldehyde-3-phosphate dehydrogenase (GAPDH). Used primer sets were 5-gaactggcagcggttttatc-3 and 5-tctcggggaattcaatactca-3 for GRα, 5-ccattgtcaagagggaagga-3 and 5-tgtgtgagatgtgctttctgg-3 for GRβ, and 5-cagcctcaagatcatcagca-3 and 5-tgtggtcatgagtccttcca-3 for GAPDH. Amplification of the single PCR product was confirmed by monitoring the dissociation curve. Amplification curves were visually inspected to set a suitable baseline range and threshold level. To generate standard curves, different concentrations of cDNA made from total RNA isolated from human lymphoma cell line Jurkat cells were used in each PCR reaction. The number of cycles required to reach the threshold fluorescence level was scored and used for generating standard curves and interpolating mRNA concentration levels. The relative quantification method was employed for quantification of target molecules according to the manufacturer's protocol, in which the ratio between the amount of target molecule and a reference molecule within the same sample was calculated. At a minimum, all measurements were performed in duplicate. The GAPDH mRNA level was used

Table 2. Demographic and Clinical Characteristics of Subjects

		Patients					
		MDD		BPD		Relatives	
	Control Subjects $n = 31$	Depressed $n = 18$	Remission $n = 38$	Depressed $n = 13$	Remission $n = 35$	MDD $n = 17$	BPD $n = 15$
Mean Age (years)	49.9 ± 1.7	52.9 ± 3.9	58.3 ± 2.1	55.5 ± 3.7	52.9 ± 2.4	48.8 ± 3.6	44.7 ± 4.8
Gender (Female/Male)	15/16	9/9	14/24	11/2	29/6	13/4	10/5
HDRS		26.9 ± 2.0	3.7 ± .32	24.6 ± 1.1	3.2 ± .33		
Serum Cortisol (μg/dL)	8.8 ± .79	10.9 ± 3.5	10.2 ± .76	9.4 ± 2.1	11.5 ± .97		

Includes subjects with bipolar disorder, major depressive disorder, first-degree relatives of BPD and MDD, and normal control subjects. MDD, major depressive disorder; BPD, bipolar disorder; HDRS, Hamilton Depression Rating Scale.

for normalization. Expression value was normalized by dividing the mean of the value of control subjects.

Plasma Cortisol Determination

Plasma cortisol concentration was measured with radioimmunoassay by the laboratory of SRL Corporation (Tokyo, Japan).

DEX/CRH Test

A subgroup of subjects in a current depressive state underwent the DEX/CRH test as previously reported with minor modifications (Heuser et al 1996). Mood disorder patients were pretreated with an oral dose of 1 mg of dexamethasone (DEX) (Dexamethasone, Asahikasei Pharmaceutical Corporation, Tokyo, Japan) at 11:00 P.M. The next day, intravenous cannulation was carried out at 12:30 P.M. and 100 μg of human CRH (hCRH, Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 1:00 P.M., immediately after the first blood collection. Blood specimens were drawn through the intravenous catheter 15 minutes, 30 minutes, 60 minutes, and 120 minutes later. Blood samples were immediately centrifuged and stored at −20°C. Plasma levels of cortisol and ACTH were measured with radioimmunoassay (SRL Corporation). We defined nonsuppressors as those individuals whose post-DEX plasma cortisol levels were more than 5 μg/dL.

Data Analysis

Data are presented as means ± standard error of mean (SEM) unless otherwise specified. Distributions for each variable were examined for normality using Shapiro and Wilk's test. When homogeneity of variances and a normal distribution of data were detectable, one-way analysis of variance (ANOVA) and post hoc test (Tukey test) were used for statistical analysis. When significant deviations from normality were found ($p < .05$), nonparametric statistics were applied. When deviation from normality and lack of homogeneity of variances occurred, Kruskal-Wallis one-way analysis of variance was used for statistical analysis and then, if significant, the Steel-Dwass test was used for group comparisons. The Spearman rank correlation was calculated to assess the correlation between data. Two group comparisons, such as the effect of antidepressant usage or gender on GR mRNA expression, were performed using the Student t test. For categorical variables, the chi-square test was used. The analysis of covariance (ANCOVA) using age as a covariate was performed to assess GR mRNA expression levels between suppressors and nonsuppressors of the DEX-CRH test. For all statistical analysis, $p < .05$ was considered significant.

Results

The mean ages were not significantly different between major depressive disorder patients, bipolar disorder patients, and normal control subjects ($F = 1.95$, $df = 4,131$, $p = .106$) (Table 2). Regarding the gender distribution, bipolar disorder patients showed a larger ratio of female to male ($X^2 = 12.7$, $df = 4$, $p = .013$). Levels of plasma cortisol did not differ between major depressive disorder patients, bipolar disorder patients, and normal control subjects ($F = .92$, $df = 4,58$, $p = .456$). Real-time PCR revealed that the expression level of GRα mRNA was decreased in major depressive disorder patients in a current depressive state and in remission, compared with normal control subjects ($F = 8.13$, $df = 4,131$, $p < .0001$, post hoc $p = .028$, and $p = .011$, respectively) (Figure 1). The levels of GRα mRNA expression of major depressive disorder patients showed no significant difference between the depressive state and remission ($p = .382$).

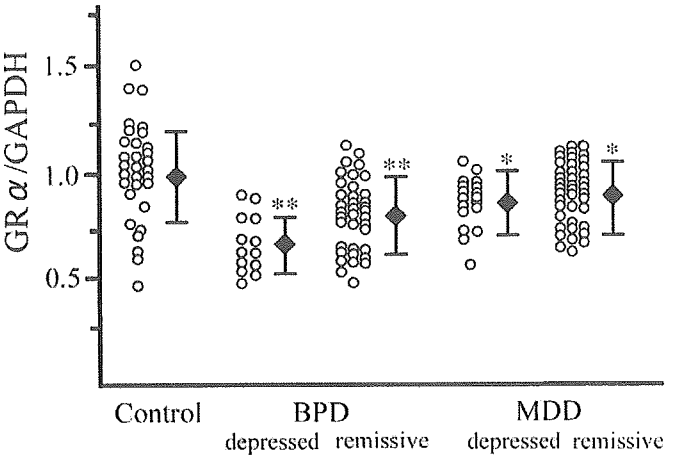

Figure 1. Quantitative RT-PCR revealed that reduced GRα mRNA expression was shown in major depressive disorder patients in a current depressive state ($n = 18$), major depressive disorder patients in remission ($n = 38$), in bipolar disorder patients in a current depressive state ($n = 13$), and in bipolar patients in remission ($n = 35$) compared with normal control subjects ($n = 31$). Values are mean ± standard error. $^{*}p < .05$, $^{**}p < .01$. RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger RNA; MDD, major depressive disorder; BPD, bipolar disorder.

Reduced GRα mRNA expression was also recognized in bipolar disorder patients in a current depressive state as well as in remission (post hoc $p < .0001$ and $p = .0005$, respectively) (Figure 1). The GRα mRNA expression levels of bipolar disorder patients showed no significant difference between depressive state and remission ($p = .238$). There was no significant correlation between GRα mRNA level and plasma cortisol concentration of normal control subjects, major depressive disorder patients, and bipolar disorder patients ($r = -.010$, $p = .967$; $r = .037$, $p = .508$; $r = .269$, $p = .238$, respectively). Gender difference did not produce significant effects on GRα mRNA expression of normal control subjects ($t = -.641$, $df = 29$, $p = .526$), major depressive disorder patients ($t = -.266$, $df = 54$, $p = .792$), and bipolar disorder patients ($t = -.291$, $df = 46$, $p = .772$). No significant correlation between age and GRα mRNA level was shown in normal control subjects ($r = .144$, $p = .439$), major depressive disorder patients in a current depressive state ($r = -.108$, $p = .671$), major depressive disorder patients in remission ($r = -.111$, $p = .508$), bipolar disorder patients in a current depressive state ($r = -.278$, $p = .357$), and bipolar disorder patients in remission ($r = -.004$, $p = .839$).

In contrast to GRα, there was no significant difference of GRβ mRNA expression between mood disorder patients in a current depressive state and normal control subjects ($F = .27$, $df = 2,59$, $p = .762$) (Figure 2). Also, no significant difference in GRβ mRNA expression was found between the first-degree relatives of mood disorder patients and normal control subjects ($F = 1.71$, $df = 2,58$, $p = .190$) (data not shown). A strong negative correlation was found between the expression levels of GRα mRNA and of GRβ mRNA of control individuals ($r = -.463$, $p = .009$) (Figure 3). However, there were no significant correlations between the expression levels of GRα mRNA and of GRβ mRNA of both major depressive disorder patients in a current depressive state ($r = .169$, $p = .502$) and bipolar disorder patients in a current depressive state ($r = .349$, $p = .243$) (Figure 3). There was no significant correlation between GRβ mRNA level and plasma cortisol concentration of normal control subjects, major depressive disorder patients, and bipolar disorder patients ($r = .242$,

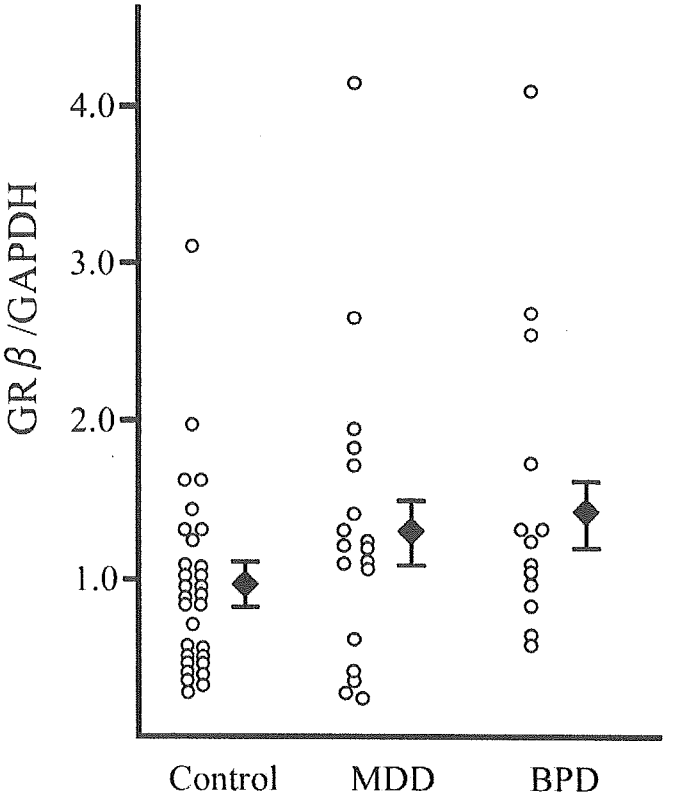

Figure 2. Quantitative RT-PCR revealed that GRβ mRNA levels of subjects with major depressive disorder (n = 18) and bipolar disorder (n = 13) did not show significant differences from that of normal control subjects (n = 31). Values are mean ± standard error. RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger RNA; MDD, major depressive disorder; BPD, bipolar disorder.

p = .304; r = −.632, p = .253; r = .400, p = .600, respectively). Gender difference did not produce a significant effect on GRβ mRNA expression of normal control subjects (t = .968, df = 29, p = .341), major depressive disorder patients in a current depressive state (t = .265, df = 16, p = .794), and bipolar disorder patients in a current depressive state (t = −.794, df = 11, p = .444). No significant correlation between age and GRβ mRNA level was shown in normal control subjects (r = −.136, p = .466), major depressive disorder patients in a current depressive state (r = −.157, p = .533), and bipolar disorder patients in a current depressive state (r = −.154, p = .614).

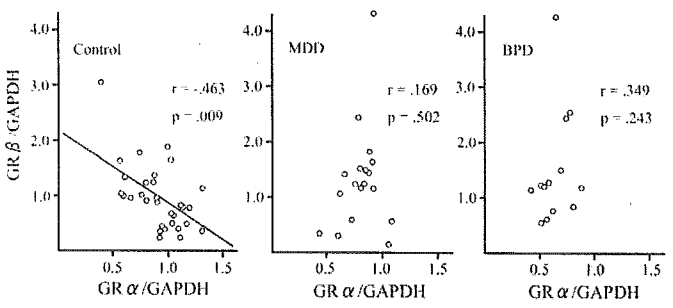

Figure 3. Significant correlation between GRα mRNA levels and GRβ mRNA levels was recognized in normal control subjects (n = 31) but not in major depressive disorder patients (n = 18) or bipolar disorder patients in a current depressive state (n = 13). mRNA, messenger RNA; MDD, major depressive disorder; BPD, bipolar disorder.

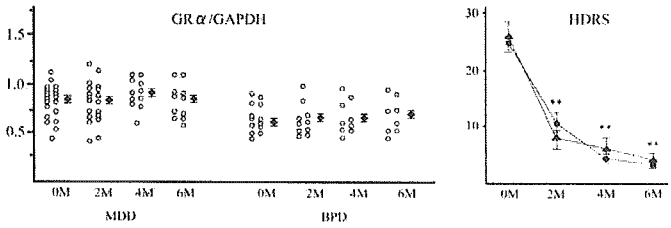

Figure 4. During recovery from a depressive state, stable reduction of GRα mRNA expression was shown at 0 (n = 20), 2 (n = 19), 4 (n = 11), and 6 months (n = 11) in subjects with major depressive disorder and at 0 (n = 13), 2 (n = 10), 4 (n = 9), and 6 months (n = 8) in subjects with bipolar disorder. HDRS values of major depressive patients (circle) and of bipolar disorder patients (triangle) were significantly reduced at 2, 4, and 6 months compared with 0 months. Values are mean ± standard error. ** p <0.01. mRNA, messenger RNA; MDD, major depressive disorder; BPD, bipolar disorder; HDRS, Hamilton Depression Rating Scale.

To investigate whether GRα mRNA levels vary during recovery from depressive state, individuals with major depressive disorder (n = 20) and individuals with bipolar disorder (n = 13) in a current depressive state were assessed for GRα mRNA expression every 2 months for 6 months. No significant difference of GRα mRNA level was detected between 0 (n = 20), 2 (n = 19), 4 (n = 11), and 6 (n = 11) months in major depressive disorder patients (F = 1.38, df = 3,57, p = .258). No significant difference of GRα mRNA level was detected between 0 (n = 13), 2 (n = 10), 4 (n = 9), and 6 (n = 8) months in bipolar disorder patients (F = .32, df = 3,36, p = .810). Hamilton Depression Rating Scale values were significantly reduced at 2 months in major depressive disorder patients and bipolar disorder patients (F = 47.2, df = 3,57, p < .0001, post hoc p < .0001 and F = 24.5, df = 3,36, p < .0001, post hoc p < .0001, respectively) (Figure 4).

Next, to examine whether reduced GRα expression is present in trait-dependent change of mood disorder, GRα mRNA expressions were determined in first-degree relatives of major depressive disorder patients (n = 17) and bipolar disorder patients (n = 15). Reduced GRα mRNA expression was recognized in the first-degree relatives of bipolar disorder patients but not in the first-degree relatives of major depressive disorder patients (F = 3.30, df = 2,60, p = .043, post hoc p = .040, and p = .968, respectively) (Figure 5). There were no significant differences in GRα mRNA expression regarding gender of first-degree relatives of major depressive disorder patients (t = .157, df = 3, p = .205) and those of bipolar disorder patients (t = 1.05, df = 13, p = .312). No significant correlation between age and GRα mRNA level was shown in first-degree relatives of major depressive disorder patients (r = −.283, p = .270) and those of bipolar disorder patients (r = .285, p = .303).

To examine whether the reduction of GRα mRNA expression in mood disorders would have an influence on the HPA axis activity, GRα and GRβ mRNA levels of mood disorder patients in a current depressive state were compared between suppressors (n = 13, 10 major depressive disorder patients and 3 bipolar disorder patients) and nonsuppressors (n = 16, 8 major depressive disorder patients and 8 bipolar disorder patients) of the DEX/CRH test. The mean age of nonsuppressors was significantly higher than suppressors (59 ± 3.3 vs. 44 ± 5.5; t = 2.47, df = 29, p = .020) with similar gender distribution (X^2 = 3.84, df = 1, p = .11), which is consistent with a previous report (Heuser et al 1994). Thus, we applied analysis of covariance using age as a covariate, finding no significant difference in GRα and GRβ mRNA expression level between suppressors and nonsuppres-

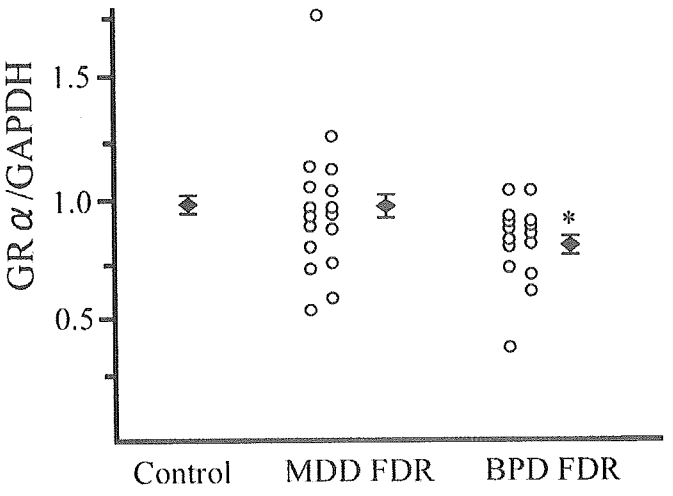

Figure 5. Quantitative RT-PCR revealed that GRα mRNA expression was reduced in first-degree relatives of bipolar disorder patients (n = 15) but not in first-degree relatives of major depressive disorder patients (n = 17) compared with normal control subjects (n = 31, data shown in Figure 1). Values are mean ± standard error. * $p < .05$. RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger RNA; MDD FDR, first-degree relatives of major depressive disorder; BPD FDR, first-degree relatives of bipolar disorder patients.

sors (F = 1.04, df = 1,26, p = .319; F = 1.97, df =1,26, p = .179, respectively) (Figure 6).

Finally, to examine the effect of antidepressants on GRα mRNA expression, bipolar disorder patients in remission were divided into two groups regarding antidepressant medication and assessed for GRα mRNA expression. No significant difference in GRα mRNA was shown between bipolar disorder patients in remission (n = 18) medicated with both antidepressants and mood stabilizers and those (n = 18) medicated with mood stabilizers only (t = 1.30, df = 1,34, p = .20). Also, seven drug-free patients (bipolar disorder n = 1; major depressive disorder n = 6) showed significant GRα mRNA reduction compared with normal control subjects (t = 2.14, df = 1,35, p = .038).

Discussion

In this study, we demonstrated that GRα mRNA level reduction occurs in peripheral blood cells of individuals with major depressive disorder and bipolar disorder, in both a depressive state as well as in remission. Reduced GRα mRNA expression was also shown in first-degree relatives of bipolar disorder patients but not in those of major depressive disorder patients. Based on these and previous findings that GRα mRNA reduction occurred in the cerebral cortex, hippocampus, and amygdala of mood disorder brains (Webster et al 2002; Knable et al 2004; Perlman et al 2004), we propose that the GRα mRNA level in mood disorder patients is decreased in multiple systems. Glucocorticoid receptor plays multiple roles in the brain, such as modulation of neural activity and regulation of the HPA axis, as well as in the immunological system (Riccardi et al 2002). The GRα mRNA reduction in the peripheral blood cells of mood disorder patients may be associated with the immunological alteration of mood disorder patients such as increased plasma concentration of interleukin-1 (IL-1) and interleukin-6 (IL-6) (Maes et al 1995; Owen et al 2000; Anisman and Merali 2003), although there was controversy about immunological alteration of mood disorder patients.

The present findings of GRα mRNA reduction in mood disorder patients in remission and continuous GRα mRNA reduction during recovery from a depressive state show that altered GRα mRNA levels are not restricted to depressive states but continue after recovery. Furthermore, first-degree relatives of bipolar disorder patients also showed reduced GRα mRNA levels. These results suggest that GRα mRNA reduction is not a state-dependent finding but a trait-dependent finding of mood disorder, especially in bipolar disorder. Together with previous findings that remissive bipolar disorder patients and first-degree relatives of bipolar disorder patients showed disturbed GR function assessed by cortisol concentration after the DEX/CRH test (Holsboer et al 1995; Watson et al 2004), first-degree relatives of bipolar disorder patients as well as remissive bipolar disorder patients may have GR dysregulation.

Although GRα mRNA level could be influenced by plasma cortisol, plasma cortisol concentrations at 10:00 A.M. were not different between individuals with mood disorders and normal control subjects, which is consistent with previous reports (Young et al 1994, 2001). Both GRα mRNA and GRβ mRNA levels did not correlate with plasma cortisol concentrations of normal control subjects as well as mood disorder patients, suggesting that GRα mRNA and GRβ mRNA levels were not simply determined by plasma cortisol concentrations. One cannot deny the possibility that altered baseline plasma cortisol concentrations of mood disorder patients, which was not determined in the present study, may reduce GRα mRNA levels. However, there have been inconsistent reports that evening baseline plasma concentrations of mood disorder patients either increased (Young et al 1994, 2001) or decreased (Vythilingam et al 2004). Furthermore, increased glucocorticoid concentrations do not result in the down-regulation of GRα, since a normal number of glucocorticoid-binding sites were shown in recovered depressive patients with sustained high plasma cortisol concentrations (Hunter et al 1988) and Cushing's disease patients (Invitti et al 1999; Huizenga et al 2000). Thus, our finding of GRα mRNA reduction in mood disorder patients may not be a direct consequence of elevated cortisol concentrations.

Although most individuals in our study with mood disorders were on antidepressant medication, which can influence GRα mRNA expression, drug-free patients also showed GRα mRNA reduction. Furthermore, GRα mRNA reduction of bipolar disor-

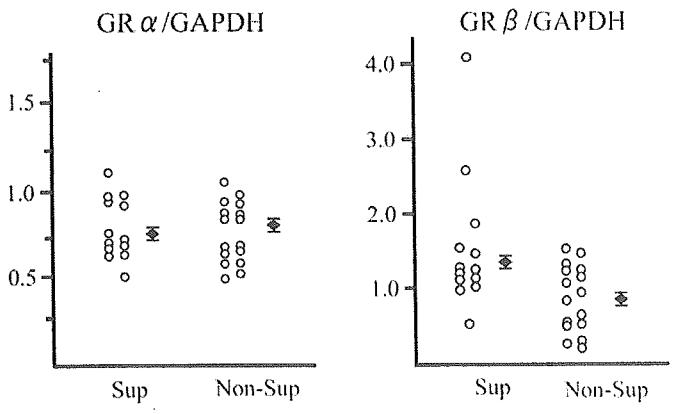

Figure 6. No significant difference in GRα mRNA or GRβ mRNA expression of mood disorder patients was shown between suppressors (n = 13) and nonsuppressors (n = 16) of the DEX/CRH test. mRNA, messenger RNA; DEX/CRH, dexamethasone/corticotropin-releasing hormone; Sup, suppressors; Non-Sup, nonsuppressors.

der patients in remission occurred regardless of antidepressant medication. These results are consistent with the report of the lack of statistical difference between GRα mRNA levels in the brain of subjects on antidepressant medication and subjects free of antidepressant medication (Webster et al 2002). In vivo and in vitro studies on the effect of antidepressants on GRα expression showed an increase or unaltered expression of GRα (Seckl and Fink 1992; Barden et al 1995; Pariante et al 1997; Okugawa et al 1999; Vedder et al 1999; Pariante and Miller 2001), although there was a report that some antidepressants reduced GRα mRNA in blood cells (Heiske et al 2003). Thus, antidepressant medication alone might not be a major determinant of GRα mRNA reduction in the peripheral blood cells. This is supported by the present result that first-degree relatives of bipolar disorder patients with no exposure to antidepressant medication also showed a reduction in GRα mRNA.

The present result showed no significant difference in GRα and GRβ mRNA expression levels between suppressors and nonsuppressors of the DEX/CRH test in mood disorder patients. Nonsuppression of the DEX/CRH test is thought to be indicative of a blunted response to increased glucocorticoid via GRα-mediated transcription (Barden et al 1995; Holsboer 2000; Pariante and Miller 2001). The finding of unaltered GRβ expression between suppressors and nonsuppressors is not consistent with the proposed function for GRβ as an antagonist for GRα-mediated transcription. There was a report that GRβ does not act as an antagonist for GRα-mediated transcription (Lange et al 1999). Thus, the biological function of GRβ remains controversial (Carlstedt-Duke 1999; Vottero and Chrousos 1999). Both GRβ mRNA and GRβ protein have been repeatedly reported to be extremely low in lymphocytes, as well as in the brain, compared with GRα (DeRijk et al 2003; Pedersen and Vedeckis 2003). Together with the present results of unaltered GRβ expression in mood disorder patients, GRβ may not play an important role in the pathogenesis of mood disorders. However, it is possible that GRβ may exert some effects on the dysregulation of the HPA axis in mood disorder patients.

It is not surprising there was no detectable difference in GRα mRNA regardless of the results of the DEX/CRH test, which is indicative of GRα-mediated transcription, because GRα-mediated transcription is modified by many factors including GR phosphorylation, nuclear localization, and interaction with other molecules such as AP1, NFκ-B, and GRβ (Oakley et al 1999; Hayashi et al 2004). Moreover, aberrant HPA axis function of mood disorder is thought to be related to many neurotransmitters and hormones such as arginine vasopressin (AVP) (Holsboer and Barden 1996; Holsboer 2001), gamma-aminobutyric acid (GABA), and glutamate (Herman et al 2004). Furthermore, a recent report has shown discrepancies between the GR mRNA level, GR protein level, and GR function in peripheral blood mononuclear cells (Torrego et al 2004). Future studies should examine whether GRα mRNA reduction is associated with reduced GRα protein expression and disturbed GRα function.

Another finding of our study is the inverse correlation between the amount of GRα and GRβ mRNA in normal control subjects but not in mood disorder patients. The GRβ mRNA is produced from pre-mRNA common to GRα mRNA by alternative splicing associated with SRp30c (Xu et al 2003). Although regulatory mechanisms of alternative splicing remains to be clarified, the present result suggests that GRα may suppress GRβ production by regulating the alternative splicing in normal control subjects. If this hypothesis is valid, the lack of a significant correlation between GRα and GRβ recognized in mood disorder patients suggests aberrant alternative splicing associated with GRα-mediated transcription in mood disorder patients. It is also possible that an alternative splicing mechanism itself is disturbed in mood disorder patients (Lee and Irizarry 2003).

Although the pathological significance of reduced GRα mRNA expression in mood disorder patients is a problem, heterozygous mice of the GR-deficient mouse showed normal behavior at baseline with enhanced helplessness and despair in response to stress and nonsuppression to the DEX/CRH test (Ridder et al 2005). Thus, reduced expression of GRα mRNA could be one factor that leads individuals to be susceptible to stress and mood disorders.

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American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* Washington, DC: American Psychiatric Press.

Anisman H, Merali Z (2003): Cytokines, stress and depressive illness: Brain-immune interactions. *Ann Med* 35:2–11.

Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB (1999): The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol* 160:1–12.

Bamberger CM, Bamberger AM, de Castro M, Chrousos GP (1995): Glucocorticoid receptor β, a potential endogenous inhibitor of glucocorticoid action in humans. *J Clin Invest* 95:2435–2441.

Barden N, Reul JM, Holsboer F (1995): Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system? *Trends Neurosci* 18:6–11.

Calfa G, Kademian S, Ceschin D, Vega G, Rabinovich GA, Volosin M (2003): Characterization and functional significance of glucocorticoid receptors in patients with major depression: Modulation by antidepressant treatment. *Psychoneuroendocrinology* 28:687–701.

Carlstedt-Duke J (1999): Glucocorticoid Receptor β: View II. *Trends Endocrinol Metab* 10:339–342.

De Kloet ER (2003): Hormones, brain and stress. *Endocr Regul* 37:51–68.

DeRijk RH, Schaaf M, Stam FJ, de Jong IEM, Swaab DF, Ravid R, et al (2003): Very low levels of the glucocorticoid receptor β isoform in the human hippocampus as shown by Taqman RT-PCR and immunocytochemistry. *Brain Res Mol Brain Res* 116:17–26.

Gormley GJ, Lowy MT, Reder AT, Hospelhorn VD, Antel JP, Meltzer HY (1985): Glucocorticoid receptors in depression: Relationship to the dexamethasone suppression test. *Am J Psychiatry* 142:1278–1284.

Hayashi R, Wada H, Ito K, Adcock IM (2004): Effects of glucocorticoids on gene transcription. *Eur J Pharmacol* 500:51–62.

Heiske A, Jesberg J, Krieg JC, Vedder H (2003): Differential effects of antidepressants on glucocorticoid receptors in human primary blood cells and human monocytic U-937 cells. *Neuropsychopharmacology* 28:807–817.

Herman JP, Mueller NK, Figueiredo H (2004): Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. *Ann N Y Acad Sci* 1018:35–45.

Heuser IJ, Gotthardt U, Schweiger U, Schmider J, Lammers CH, Dettling M, et al (1994): Age-associated changes of pituitary-adrenocortical hormone regulation in humans: Importance of gender. *Neurobiol Aging* 15:227–231.

Heuser IJ, Schweiger U, Gotthardt U, Schmider J, Lammers CH, Dettling M, et al (1996): Pituitary-adrenal-system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and normal comparison subjects. *Am J Psychiatry* 153:93–99.

Holsboer F (2000): The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23:477–501.

Holsboer F (2001): Stress, hypercortisolism and corticosteroid receptors in depression: Implications for therapy. *J Affect Disord* 62:77–91.

Holsboer F, Barden N (1996): Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr Rev* 17:187–205.

Holsboer F, Lauer CJ, Schreiber W, Krieg JC (1995): Altered hypothalamic-pituitary-adrenocortical regulation in healthy subjects at high familial risk for affective disorder. *Neuroendocrinology* 62:340–347.

Huizenga NA, De Herder WW, Koper JW, de Lange P, v D Lely AJ, Brinkmann AO, et al (2000): Decreased ligand affinity rather than glucocorticoid receptor down-regulation in patients with endogenous Cushing's syndrome. *Eur J Endocrinol* 142:472–476.

Hunter R, Dick H, Christie JE, Goodwin GM, Fink G (1988): Lymphocyte glucocorticoid receptor binding in depression: Normal values following recovery. *J Affect Disord* 14:155–159.

Invitti C, Redaelli G, Baldi G, Cavagnini F (1999): Glucocorticoid receptors in anorexia nervosa and Cushing's disease. *Biol Psychiatry* 45:1467–1471.

Karst H, Karten YJ, Reichardt HM, de Kloet ER, Schutz G, Joels M (2000): Corticosteroid actions in hippocampus require DNA binding of glucocorticoid receptor homodimers. *Nat Neurosci* 3:977–978.

Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF (2004): Molecular abnormalities of the hippocampus in severe psychiatric illness: Postmortem findings from the Stanley Neuropathology Consortium. *Mol Psychiatry* 9:609–620.

Lange P, Koper JW, Brinkmann AO, Jong FH, Lamberts SW (1999): Natural variants of the β isoform of the human glucocorticoid receptor do not alter sensitivity to glucocorticoids. *Mol Cell Endocrinol* 153:163–168.

Lee CJ, Irizarry K (2003): Alternative splicing in the nervous system: An emerging source of diversity and regulation. *Biol Psychiatry* 54:771–776.

Leung DY, Hamid Q, Vottero A, Szefler SJ, Surs W, Minshall E, et al (1997): Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor β. *J Exp Med* 186:1567–1574.

Lopez JF, Chalmers DT, Little KI, Watson SJ (1998): Regulation of serotonin 1A, glucocorticoid and mineralcorticoid receptor in rat and human hippocampus: Implications for the neurobiology of depression. *Biol Psychiatry* 43:547–573.

Lupien SJ, Fiocco A, Wan N, Maheu F, Lord C, Schramek T, et al (2005): Stress hormones and human memory function across the lifespan. *Psychoneuroendocrinology* 30:225–242.

Maes M, Meltzer HY, Bosmans E, Bergmans R, Vandoolaeghe E, Ranjan R, et al (1995): Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. *J Affect Disord* 34:301–309.

McEwen BS (2000): The neurobiology of stress: From serendipity to clinical relevance. *Brain Res* 886:172–189.

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002): Neurobiology of depression. *Neuron* 34:13–25.

Oakley RH, Jewell CM, Yudt MR, Bofetiado DM, Cidlowski JA (1999): The dominant negative activity of the human glucocorticoid receptor β isoform. *Specificity and mechanisms of action.* J Biol Chem 274:27857–27866.

Okugawa G, Omori K, Suzukawa J, Fujiseki Y, Kinoshita T, Inagaki C (1999): Long-term treatment with antidepressants increases glucocorticoid receptor binding and gene expression in cultured rat hippocampal neurones. *J Neuroendocrinol* 11:887–895.

Owen BM, Eccleston D, Ferrier IN, Young AH (2000): Raised levels of plasma interleukin-1β in major and post viral depression. *Acta Psychiatr Scand* 103:226–228.

Pariante CM, Miller AH (2001): Glucocorticoid receptors in major depression: Relevance to pathophysiology and treatment. *Biol Psychiatry* 49:391–404.

Pariante CM, Pearce BD, Pisell TL, Owens MJ, Miller AH (1997): Steroid-independent translocation of the glucocorticoid receptor by the antidepressant desipramine. *Mol Pharmacol* 52:571–581.

Pedersen KB, Vedeckis WV (2003): Quantification and glucocorticoid regulation of glucocorticoid receptor transcripts in two human leukemic cell lines. *Biochemistry* 42:10978–10990.

Perlman WR, Webster MJ, Kleinman JE, Weickert CS (2004): Reduced glucocorticoid and estrogen receptor alpha messenger ribonucleic acid levels in the amygdala of patients with major mental illness. *Biol Psychiatry* 56:844–852.

Riccardi C, Bruscoli S, Migliorati G (2002): Molecular mechanisms of immunomodulatory activity of glucocorticoids. *Pharmacol Res* 45:361–368.

Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, et al (2005): Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. *J Neurosci* 25:6243–6250.

Rupprecht R, Kornhuber J, Wodarz N, Lugauer J, Gobel C, Riederer P (1991): Lymphocyte glucocorticoid receptor binding during depression and after clinical recovery. *J Affect Disord* 22:31–35.

Sapolsky RM, Romero LM, Munck AU (2000): How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* 21:55–89.

Seckl JR, Fink G (1992): Antidepressants increase glucocorticoid and mineralcorticoid receptor mRNA expression in rat hippocampus in vivo. *Neuroendocrinology* 55:621–626.

Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH (2000): Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor β isoform. *J Allergy Clin Immunol* 105:943–950.

Strickland I, Kisich K, Hauk PJ, Vottero A, Chrousos GP, Klemm DJ, et al (2001): High constitutive glucocorticoid receptor β in human neutrophils enables them to reduce their spontaneous rate of cell death in response to corticosteroids. *J Exp Med* 193:585–593.

Torrego A, Pujols L, Roca-Ferrer J, Mullol J, Xaubet A, Picado C (2004): Glucocorticoid receptor isoforms α and β in in vitro cytokine-induced glucocorticoid insensitivity. *Am J Respir Crit Care Med* 170:420–425.

Vedder H, Bening-Abu-Shach U, Lanquillon S, Krieg JC (1999): Regulation of glucocorticoid receptor-mRNA in human blood cells by amitriptyline and dexamethasone. *J Psychiatr Res* 33:303–308.

Vottero A, Chrousos GP (1999): Glucocorticoid receptor β: View I. *Trends Endocrinol Metab* 10:333–338.

Vythilingam M, Vermetten E, Anderson GM, Luckenbaugh D, Anderson ER, Snow J, et al (2004): Hippocampal volume, memory, and cortisol status in major depressive disorder: Effects of treatment. *Biol Psychiatry* 56:101–112.

Wassef A, Smith EM, Rose RM, Gardner R, Nguyen H, Meyer WJ (1990): Mononuclear leukocyte glucocorticoid receptor binding characteristics and down-regulation in major depression. *Psychoneuroendocrinology* 15:59–68.

Watson S, Gallagher P, Ritchie JC, Ferrier N, Young AH (2004): Hypothalamic-pituitary-adrenal axis function in patients with bipolar disorder. *Br J Psychiatry* 184:496–502.

Webster JC, Oakley RH, Jewell CM, Cidlowski JA (2001): Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative β isoform: A mechanism for the generation of glucocorticoid resistance. *Proc Natl Acad Sci U S A* 98:6865–6870.

Webster MJ, Knable MB, O'Grady J, Orthmann J, Weickert CS (2002): Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. *Mol Psychiatry* 7:985–994.

Whalley LJ, Borthwick N, Copolov D, Dick H, Christie JE, Fink G (1986): Glucocorticoid receptors and depression. *Br Med J* 292:859–861.

Wodarz N, Rupprecht R, Kornhuber J, Schmitz B, Wild K, Braner HU (1991): Normal lymphocyte responsiveness to lectins but impaired sensitivity to in vitro glucocorticoids in major depression. *J Affect Disord* 22:241–248.

Wodarz N, Rupprecht R, Kornhuber J, Schmitz B, Wild K, Riederer P (1992): Cell-mediated immunity and its glucocorticoid-sensitivity after clinical recovery from severe major depressive disorder. *J Affect Disord* 25:31–38.

Xu Q, Leung DYM, Kisich KO (2003): Serine-arginine-rich protein p30 directs alternative splicing of glucocorticoid receptor pre-mRNA to glucocorticoid receptor β in neutrophils. *J Biol Chem* 278:27112–27118.

Yehuda R, Boisoneau D, Mason JW, Giller EL (1993): Glucocorticoid receptor number and cortisol excretion in mood, anxiety and psychotic disorders. *Biol Psychiatry* 34:18–25.

Young EA, Carlson NE, Brown MB (2001): Twenty-four-hour ACTH and cortisol pulsatility in depressed women. *Neuropsychopharmacology* 25:267–276.

Young EA, Haskett RF, Grunhaus L, Pande A, Weinberg VM, Watson SJ (1994): Increased evening activation of the hypothalamic-pituitary-adrenal axis in depressed patients. *Arch Gen Psychiatry* 51:701–707.

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Frontal lobe function in bipolar disorder: A multichannel near-infrared spectroscopy study

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Frontal lobe dysfunction has been implicated as one of the pathophysiological bases of bipolar disorder. Detailed time courses of brain activation in the bipolar disorder group were investigated using multichannel near-infrared spectroscopy (NIRS), a recently developed functional neuroimaging technology with a high time resolution, and were compared with those in the major depression and healthy control groups. Seventeen patients with bipolar disorder, 11 equally depressed patients with major depression, and 17 healthy controls participated in the study. Changes in oxy hemoglobin concentration ([oxy-Hb]) during cognitive and motor tasks were monitored using frontal and temporal probes of two sets of 24-channel NIRS machines. [oxy-Hb] increases in the bipolar disorder group were smaller than those in the healthy control group during the early period of a verbal fluency task, larger than those in the major depression and healthy control groups during the late period of this task, and were smaller than those in the major depression group during a finger-tapping task. Depressive symptoms and antidepressant dosages did not correlate with [oxy-Hb] changes in the two patient groups. Bipolar disorder and major depression were characterized by preserved but delayed and reduced frontal lobe activations, respectively, in the present high-time-resolution study by multichannel NIRS.

Keywords: Near-infrared spectroscopy; Cerebral blood volume; Bipolar disorder; Major depression; Verbal fluency task; Diagnosis

Introduction

Bipolar disorder and major depressive disorder (major depression) are two of the principal disorders among mood disorders. Although their etiology and pathophysiology have not yet been completely elucidated, a number of structural and functional neuroimaging studies suggest the importance of the frontal lobe. For example, a reduction in the volume of cerebral regions (Beyer and Krishnan, 2002; Fossati et al., 2004; Sheline, 2003; Strakowski et al., 2002), particularly the gray matter and glial cell density (Davidson et al., 2002) in the frontal lobe, has been reported in structural neuroimaging studies. In functional neuroimaging studies using positron emission tomography (PET), single-photon emission computed tomography (SPECT), or functional magnetic resonance imaging (fMRI), abnormal changes in cerebral glucose metabolism and cerebral blood flow have been demonstrated, particularly in the prefrontal cortex (Drevets, 2000; Stoll et al., 2000; Videbech, 2000), and they were often reported to be associated with cognitive dysfunctions in some studies (Sweeney et al., 2000; Veiel, 1997).

In many of the functional neuroimaging studies demonstrating abnormal prefrontal functions, mixed patients with bipolar disorder and major depression were examined; that is, different diagnostic groups (e.g., bipolar disorder and major depression) with various mood states (e.g., depressed and manic) were often classified into one patient group (Strakowski et al., 2000). Differences in abnormalities in frontal lobe functions between patients with bipolar disorder and those with major depression have been suggested in recent studies, in which depressed patients with bipolar disorder and those with major depression were examined separately. Decreased prefrontal activity (hypofrontality) both at rest and during an activation task has been consistently demonstrated in depressed patients with major depression in a number of PET, SPECT, and fMRI studies (Brody et al., 2001; Drevets, 2000; Liotti and Mayberg, 2001; Malhi et al., 2004b; Rogers et al., 2004).

On the other hand, in depressed patients with bipolar disorder, the reported changes in the frontal lobe function during an activation task are so far inconsistent (Strakowski et al., 2000, 2004) although changes at rest are consistent in showing decreased activity (Blumberg et al., 2002): increased activity (visuospatial working memory task, Chang et al., 2004), decreased activity

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(Stroop task, Blumberg et al., 2003; auditory discrimination continuous performance task, Ketter et al., 2001; positive affect induction, Malhi et al., 2004a; emotional recognition, Yurgelun-Todd et al., 2000), and unchanged frontal lobe functions (semantic decision task, Curtis et al., 2001) compared to healthy controls have been reported, even within the same task, that is the verbal fluency task (increased activity, Curtis et al., 2001; decreased activity, Matsuo et al., 2002, 2004; unchanged function, Dye et al., 1999).

As far as the authors surveyed, only two research groups have directly contrasted the frontal lobe functions between depressed patients with bipolar disorder and those with major depression, but these studies showed inconsistent results. In near-infrared spectroscopy (NIRS) studies, Matsuo et al. found reduced [oxy-Hb] increases in the prefrontal region during a verbal fluency task in both the bipolar disorder and major depression groups compared with the healthy control groups, and found no significant differences between the bipolar disorder and major depression groups (Matsuo et al., 2000, 2002, 2004, 2005). However, in fMRI study, Lawrence et al. (2004) found larger prefrontal activations in response to emotional stimuli in the bipolar disorder group than in the major depression group. The reasons for the differences in the results of these research groups have not been clarified.

NIRS is a recently developed noninvasive functional neuroimaging technique. NIRS can detect regional cerebral blood volume (rCBV) changes in terms of changes in oxy hemoglobin concentration ([oxy-Hb]) and deoxy hemoglobin concentration ([deoxy-Hb]). The principle of NIRS is based on the modified Lambert–Beer law, and NIRS monitors the absorption of near-infrared light by oxy and deoxy hemoglobin using two different wavelengths. Both the [oxy-Hb] increase and [deoxy-Hb] decrease detected by NIRS have been shown to reflect cortical activation by simultaneous measurements using other methodologies (Hock et al., 1997; Kleinschmidt et al., 1996; Mehagnoul-Schipper et al., 2002; Toronov et al., 2001). The correlations with cerebral blood flow have been shown to be stronger for [oxy-Hb] than for [deoxy-Hb] (Malonek et al., 1997; Strangman et al., 2002b). In an animal study using a perfused brain rat model, [oxy-Hb] was also demonstrated to be the most sensitive marker of CBF changes among [oxy-Hb], [deoxy-Hb], and [total-Hb] (Hoshi et al., 2001).

NIRS has some advantages and disadvantage over other functional neuroimaging methodologies such as PET, SPECT, and fMRI. The three advantages of NIRS are (1) the complete noninvasiveness of the measurement enabling repeated measurements, (2) the high time resolution of 0.1 s enabling a detailed clarification of temporal changes in rCBV, and (3) the portability and compactness of its apparatus enabling measurements under natural conditions with subjects sitting on a comfortable chair. The disadvantages of NIRS are that it measures hemoglobin concentrations (1) only as relative changes, not as absolute values, (2) only in the cortex immediately beneath the probes but not in deeper brain structures, (3) with a high time resolution but with a low spatial resolution, and (4) not only in the brain but also in other surface structures, such as the skin and skull. Considering the advantages and disadvantages described above, NIRS is assumed to be particularly useful in assessing the dynamic aspects of cortical activation in rather broad areas.

NIRS has been demonstrated to enable the detection of brain activations during cognitive tasks in healthy controls (reviewed by Hoshi, 2003; Obrig and Villringer, 2003; Strangman et al., 2002a). For mood disorders, several NIRS studies have been conducted. Okada et al. (1996) reported no dominant hemispheric changes in [total-Hb] in the prefrontal area of patients with major depression during a mirror drawing task. In addition to Matsuo et al. (2000, 2002, 2005) as described above, both Suto et al. (2004) and Herrmann et al. (2004) reported reduced frontal activation during a verbal fluency task in patients with major depression. Eschweiler et al. (2000) found that reduced [oxy-Hb] increases predict a good therapeutic efficacy of repetitive transcranial magnetic stimulation in patients with major depression.

In the present study, we evaluated the spatial and temporal characteristics of rCBV changes during cognitive activation in patients with bipolar disorder by multichannel NIRS, and compared them with those in patients with major depression. The verbal fluency task was employed as cognitive activation and the finger-tapping task as cognitively undemanding control activation. The inconsistency in frontal lobe activation in bipolar disorder has not been clarified in any functional neuroimaging methodologies as described above, and there have been no studies that assessed the temporal characteristics of cerebral activation in mood disorders except a NIRS study in our laboratory (Suto et al., 2004). The objectives of the present study are (1) to clarify the characteristics of brain activations in patients with bipolar disorder along the task time course with the aid of the high time resolution of NIRS and (2) to compare them with those in healthy controls as well as patients with major depression of similar psychopathology. We hypothesized that (1) the characteristics of the frontal lobe function are expressed more clearly in cognitive activation than in control motor activation, (2) cognitive activations in bipolar disorder are consistent in some time segments and inconsistent in other time segments with those in major depression, and (3) such differences in activations along the time course can explain, at least in part, the inconsistent results in cognitive activation regarding bipolar disorder.

Materials and methods

Subjects

Seventeen patients with bipolar disorder, 11 patients with major depression, and 17 healthy controls participated in the present study (Table 1). The patients with bipolar disorder and those with major depression were recruited among the outpatients and inpatients at Gunma University Hospital, and were diagnosed according to the criteria in the *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* (American Psychiatric Association 1994).

The patients with bipolar disorder included 11 males and 6 females (age: mean, 40.9 years; SD, 13.3; range, 20–62), and 4 patients with bipolar I disorder and 13 patients with bipolar II disorder. At the time of the study, all the subjects were euthymic to subdepressive as indicated by their scores in the 24-item Hamilton Rating Scale for Depression (HRSD, Hamilton, 1960; mean, 9.4; SD, 6.5; range, 1–22), and were on medication with mood stabilizers and/or antidepressants.

The patients with major depression, including 9 males and 2 females (age: mean, 44.8 years; SD, 13.1; range, 24–59), were euthymic to subdepressive at the time of the study (HRSD score: mean, 10.4; SD, 9.5; range, 0–26) with the same severity as the patients with bipolar disorder ($t = -0.32$, $P = 0.76$), and were on

Table 1
Characteristics of subjects

Case	Age	Sex	Subtype	HRSD	Performance	Total imipramine equivalent dose mg/day	Medication (imipramine equivalent dose) mg/day
Bipolar disorder (n = 17)							
1	39	M	II	12	8	243.8	Clomipramine 75 (93.8), paroxetine 40 (150), lithium 800, levomepromazine 15
2	27	F	I	10	11	0	Lithium 600, risperidone 2
3	33	M	II	11	11	125	Maprotiline 50 (50), milnacipran 75 (75), lithium 400, bromocriptine 5
4	43	F	II	11	17	325	Amitriptiline 150 (150), amoxapine 100 (100), maprotiline 75 (75), lithium 600, bromocriptine20
5	57	F	II	10	21	60	Milnacipran 60 (60), lithium 600
6	38	M	II	3	18	240	Imipramine 60 (60), maprotiline 150 (150), milnacipran 30 (30), sodium valproate 400
7	49	M	II	19	21	0	Chlorpromazine 50, lithium 400, sodium valproate 800
8	33	M	II	1	19	0	Lithium 600
9	48	M	II	10	17	200	Imipramine 125 (125), paroxetine 20 (75), lithium 1000
10	28	M	II	17	10	287.5	Clomipramine 200 (250), trazodone 75 (37.5), lithium 600, risperidone 1
11	62	M	II	17	20	125	Paroxetine 30 (112.5), trazodone 25 (12.5)
12	57	M	I	3	14	162.5	Milnacipran 150 (150), trazodone 25 (12.5), lithium 800
13	20	F	II	5	13	12.5	Sulpiride 25 (12.5), lithium 200, sodium valproate 400
14	50	M	I	4	14	0	Lithium 1200
15	28	M	II	22	17	100	Dosulepin 50 (50), mianserin 10 (25), sulpiride 50 (25), levomepromazine 10
16	25	F	I	3	8	0	Lithium 400, sodium valproate 400
17	59	F	II	2	11	0	Carbamazepine 400, lithium 600
Mean	40.9	M11/F6	I4/II13	9.4	14.7	110.7	
SD	13.3			6.5	4.4	114.2	
Major depression (n = 11)							
1	54	M		12	9	175	Paroxetine 40 (150), trazodone 50 (25)
2	51	M		2	16	25	Amitriptyline 25 (25), lithium 800, bromocriptine 7.5
3	52	M		0	23	25	Clomipramine 10 (12.5), trazodone 25 (12.5)
4	24	M		6	14	200	Mianserin 20 (50), paroxetine 40 (150), carbamazepine 400
5	57	M		1	5	50	Mianserin 20 (50)
6	30	M		2	15	100	Amitriptyline 100 (100)
7	59	M		17	17	45	Milnacipran 45 (45)
8	37	F		8	9	156.3	Clomipramine 125 (156.3), levomepromazine 10
9	55	M		26	14	50	Dosulepin 50 (50)
10	26	F		14	11	125	Milnacipran 100 (100), sulpiride 50 (25)
11	48	M		26	23	75	Clomipramine 50 (62.5), trazodone 25 (12.5)
Mean	44.8	M9/F2		10.4	14.2	93.3	
SD	13.1			9.5	5.6	62.3	
Healthy controls (n = 17)							
Mean	42.8	M13/F4			16.5		
SD	4.5				3.6		

M, male; F, female; I, bipolar I disorder; II, bipolar II disorder; HRSD, 24-item Hamilton Rating Scale for Depression.

medication with antidepressants. Eight of the 11 patients were also included in our previous study (Suto et al., 2004): two patients in the study were excluded from the present one because more strict criteria for artifact rejection of body movements in NIRS measurements were employed in the present study, and three new patients were added.

The healthy controls included 13 males and 4 females (age: mean, 42.8 years; SD, 4.5; range, 36–52). They had no history of any major psychiatric disorders, neurological disorders, substance abuses, head injuries, or major physical illnesses, and were not on any psychotropic medications at the time of the study. Sixteen of the 17 healthy controls were also included in our previous study (Suto et al., 2004).

The mean ages and sex ratios were not significantly different among the three groups ($F = 0.44$, $P = 0.65$; chi square = 1.14, $P = 0.57$). All the subjects were right-handed as indicated by their Edinburgh scores (Oldfield, 1970; mean, 95.1; SD, 12.0; range, 33.3–100). The present study was approved by the Institutional Review Board of Gunma University Graduate School of Medicine, and written informed consent was obtained from all the subjects prior to the study.

Activation tasks

Hemoglobin concentration changes were measured during cognitive and motor activations. The subjects sat on a comfortable

chair in a daylight room with their eyes open throughout the measurements. The cognitive activation consisted of a 30-s pretask baseline, a 60-s verbal fluency task, and a 60-s post-task baseline. During the verbal fluency task, the subjects were instructed to generate as many words whose initial syllable was either /a/, /ka/, or /sa/ as they could. The three initial syllables were employed in this order and changed every 20 s during the 60-s task to reduce the time during which the subjects remained silent. The number of words generated during the verbal fluency task was determined as a measure of task performance. The subjects were instructed to repeat the syllables /a/, /i/, /u/, /e/, and /o/ during the pretask and post-task baseline periods as a Japanese phrase for 'A, B, C' in English.

The motor activation consisted of a 30-s pretask rest, a 40-s right-finger-tapping task, and a 30- s post-task rest. The subjects were instructed to tap their four fingers with their thumb in turn as quickly and accurately as they could. They practiced the right-finger-tapping after receiving the instructions on the task, and it was confirmed that they could perform the task correctly.

NIRS measurements

NIRS machine

In this study, changes in [oxy-Hb], [deoxy-Hb], and [total-Hb] were measured using two 24-channel NIRS machines (Hitachi ETG-100) at two wavelenghts of near-infrared light (780 and 830 nm) whose absorption was measured, and [oxy-Hb] and [deoxy-Hb] were calculated. [total-Hb] was calculated as the sum of [oxy-Hb] and [deoxy-Hb]. The distance between the pair of emission and detector probes was 3.0 cm, and it was considered that the machines measure points at 2–3 cm depth from the scalp, that is, the surface of cerebral cortices (Hock et al., 1997; Toronov et al., 2001).

Probe positions and measurement points

The probes of the NIRS machines were placed on the subject's frontal and bilateral temporal regions. The frontal probes measured the hemoglobin concentration changes at 24 measurement points in a 9×9 cm^2 area, with the lowest probes positioned along the Fp_1–Fp_2 line according to the international 10/20 system used in electroencephalography. Each set of bilateral temporal probes measured the hemoglobin concentration changes at 12 measurement points in a 6×6 cm^2 area, with the central probe positioned at the midpoint between the vertex and the external ear hole. These measurement points were labeled F1–F24, L1–L12, and R1–R12 for the frontal, left temporal, and right temporal channels, respectively, from top to bottom.

The correspondence of the probe positions and the measurement points on the cerebral cortex was confirmed by superimposing the probe positions on a magnetic resonance image of a three-dimensionally reconstructed cerebral cortex of a representative subject in the healthy control group (Figs. 1–3), and the correspondence was also supported by a multisubject study of anatomical cranio-cerebral correlation (Okamoto et al., 2004).

Measurement parameters

The absorption of near-infrared light was measured with a time resolution of 0.1 s. The obtained data were analyzed using the "integral mode": the pretask baseline was determined as the mean

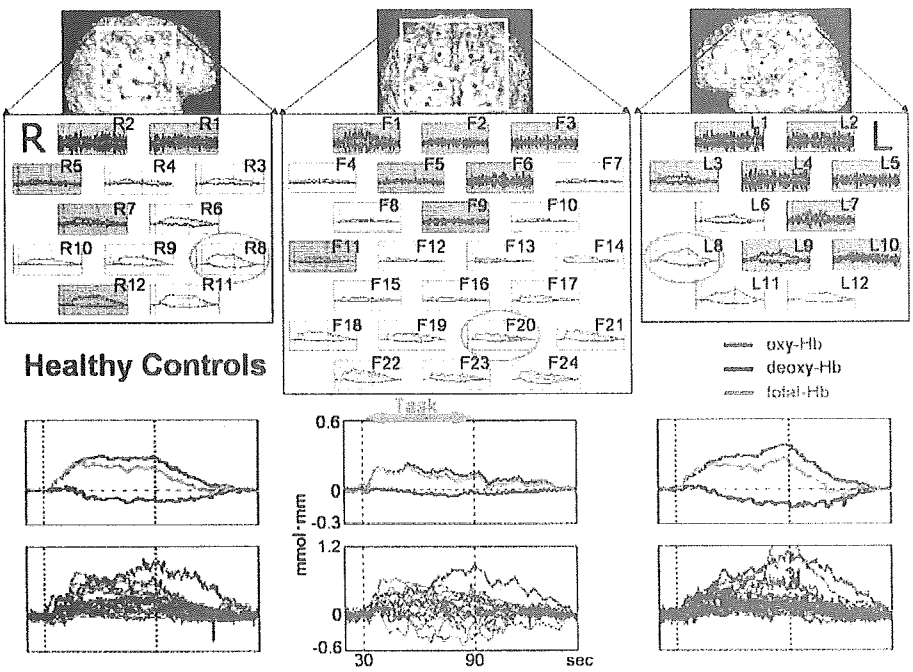

Fig. 1. Grand averaged waveforms of hemoglobin concentration changes during cognitive activation in the healthy control group. Grand averaged waveforms of [oxy-Hb] (red line), [deoxy-Hb] (blue line), and [total-Hb] (green line) changes during cognitive activation (between two vertical dotted lines) measured by the frontal (center) probe and the left (right) and right temporal (left) probes in the healthy control group. The channels with low signal-to-noise ratios were presented with gray meshing. Three sets of the grand averaged waveforms and superimposed individual waveforms of [oxy-Hb] changes in representative channels (circled in orange) are enlarged below. The upper figures show the measurement positions of the NIRS machines, which were superimposed on a magnetic resonance image of a reconstructed cerebral cortex of a representative subject.

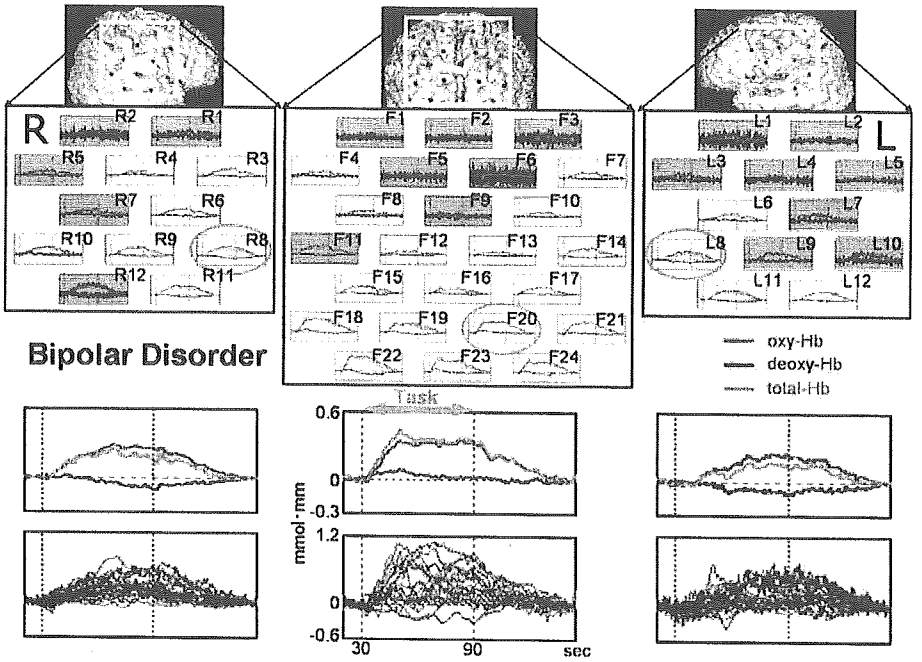

Fig. 2. Grand averaged waveforms of hemoglobin concentration changes during cognitive activation in the bipolar disorder group. Grand averaged waveforms of [oxy-Hb] (red line), [deoxy-Hb] (blue line), and [total-Hb] (green line) changes during cognitive activation (between two vertical dotted lines) measured by the frontal (center) probe and the left (right) and right temporal (left) probes in the bipolar disorder group. The channels with low signal-to-noise ratios were presented with gray meshing. Three sets of the grand averaged waveforms and superimposed individual waveforms of [oxy-Hb] changes in representative channels (circled in orange) are enlarged below. The upper figures show the measurement positions of the NIRS machines, which were superimposed on a magnetic resonance image of a reconstructed cerebral cortex of a representative subject.

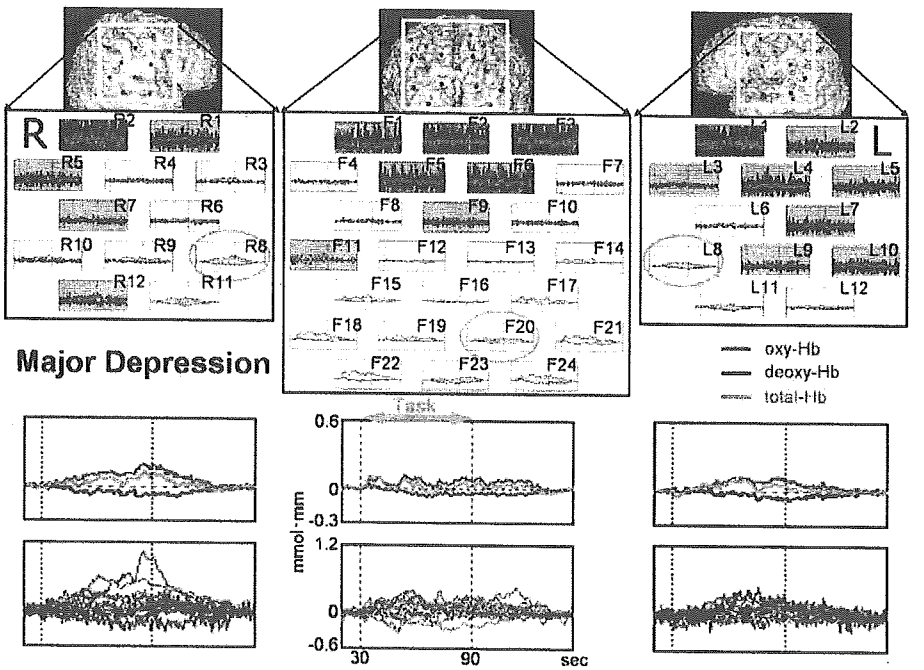

Fig. 3. Grand averaged waveforms of hemoglobin concentration changes during cognitive activation in the major depression group. Grand averaged waveforms of [oxy-Hb] (red line), [deoxy-Hb] (blue line), and [total-Hb] (green line) changes during cognitive activation (between two vertical dotted lines) measured by the frontal (center) probe and the left (right) and right temporal (left) probes in the major depression group. The channels with low signal-to-noise ratios were presented with gray meshing. Three sets of the grand averaged waveforms and superimposed individual waveforms of [oxy-Hb] changes in representative channels (circled in orange) are enlarged below. The upper figures show the measurement positions of the NIRS machines, which were superimposed on a magnetic resonance image of a reconstructed cerebral cortex of a representative subject.