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<筆頭著者プロフィール>

谷井久志：1992年大阪大学医学部卒業．大阪大学大学院医学研究科を経て，1997年より2年間，スウェーデン・カロリンスカ研究所へ留学．大阪大学医学部精神医学教室勤務を経て2004年1月より現職（三重大学大学院医学系研究科神経感覚医学講座精神病態学分野講師）．研究テーマは，パニック障害の臨床遺伝的研究，精神薬理学（悪性症候群），老年精神医学（アルツハイマー病，老年期うつ病）などである．臨床応用に繋がるような疾患研究を志している．

特集：第28回日本生物学的精神医学会シンポジウム（1）

1-7

シンポジウム：不安障害の生物学ー最前線

パニック障害の遺伝子探索

谷井久志[1)]，井上　顕[1)]，西村幸香[1)]，梶木直美[1)]
貝谷久宣[2)]，佐々木　司[3)]，岡崎祐士[1,4)]

Key words：panic disorder, cormordity, NIRS, catecol-O-methyl-transferase, monoamine oxidase A

1．はじめに

パニック障害は動悸，発汗，胸部不快感，めまい感など自律神経系の異常を中心とする複数の身体的な症状を伴いつつ予期しない不安発作が繰り返されることを特徴とする（American Psychiatric Association, 1994）。パニック障害はしばしば慢性の経過を辿り，寛解と増悪を繰り返し，恐怖症や大うつ病など他の精神疾患に合併することも多い（Weissman, et al. 1997）。パニック障害の苦悩は深刻であり，医療への希求は強く，疾患の解明への当事者からの期待と要望は極めて強い。パニック障害は自殺企図が高頻度，救急受診が高頻度などの特徴があり，不安障害全体の直接治療費総額は，統合失調症とほぼ同額とされ，さらに，不安障害による生産性の低下と損失は，直接医療費と同規模の額と推計されている。以上のような観点からパニック障害を解明し，より良い治療に結びつける試みについては，本疾患の患者の苦痛の軽減など医療的な必要性のみならず，医療経済学観点からも社会的な意義が認められるものである。

2．パニック障害における遺伝学的研究の意義について

パニック障害など精神疾患は多因子が複雑に関与する疾患という特徴がある。そのゲノム研究とは形質を説明するゲノム情報を探る研究であり，その研究においては，①合理的な必要性，②適切な形質の定義，③稠密なDNA多型・変異マーカーと，④十分なサンプル数が必要であり，⑤核内遺伝子配列情報が否定される場合にも対応できる方法論（epigeneticsなど）を持つことが重要である。また，パニック障害を研究対象とする合理的必然性ついては，①遺伝因の関与，②表現型が簡潔・明瞭で妥当性を持つこと，③当事者の熱烈な要望があり，協力を得られやすいこと，④医療経費が膨大（罹患率2％程度，救急受診多い）であること，⑤十分なサンプル数を用いた研究が少な

1）三重大学大学院医学系研究科・神経感覚医学講座・精神病態学分野〔〒514-8504　三重県津市江戸橋2-174〕Hisashi Tanii, Ken Inoue, Yukika Nishimura, Naomi Kajiki, Yuji Okazaki：Department of Psychiatry, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan
2）医療法人和楽会・パニック障害研究センター　Hisanobu Kaiya：Warakukai Incorporated Medical Institution, Research Center for Panic Disorder
3）東京大学保健センター　Tsukasa Sasaki：Department of Psychiatry, Health Service Center, University of Tokyo
4）東京都立松沢病院　Yuji Okazaki：Tokyo Metropolitan Matsuzawa Hospital
【谷井久志　E-mail：h-tanii@clin.medic.mie-u.ac.jp】

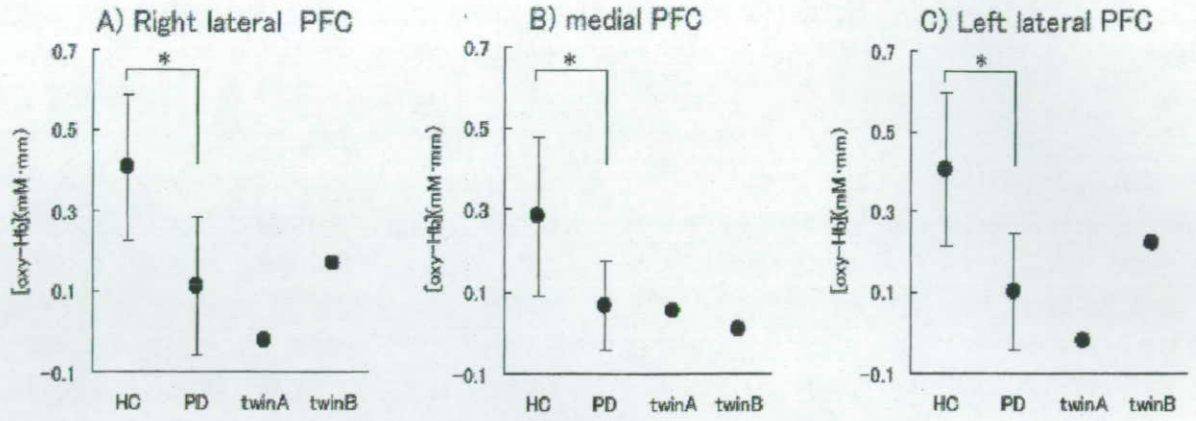

図1　一卵性双生児不一致例のNIRS（前頭葉機能）
Mean［oxy-Hb］changes during the word fluency task
Twin A：パニック障害　Twin B：健常者
＊：NC：normal control, PD：panic disorder　p<0.001 by t-test

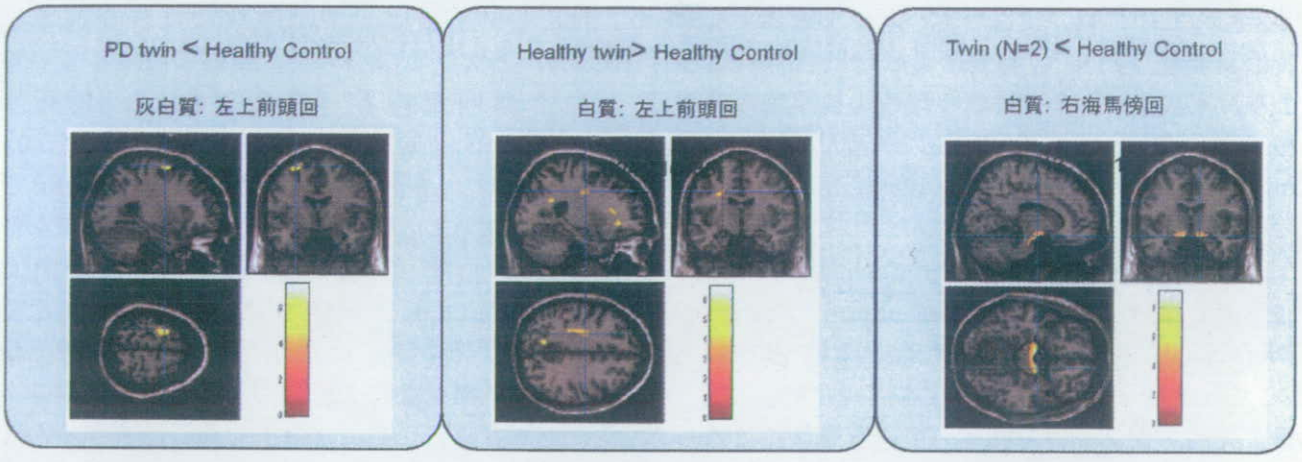

図2　一卵性双生児　パニック障害不一致例におけるVBM法を用いた脳形態画像解析

体であるが，その一方のみがパニック障害を発症している場合，すなわち一卵性双生児不一致例における両者の差異を検討することは疾患の原因を突き止める上で有力なアプローチの方法であるといえる。本研究では，パニック障害一卵性双生児不一致例（30歳男性ペア）についての光トポグラフィーやMRIを用いた検討を行った。両者において，各種心理検査結果については社会不安尺度であるSTAI-S以外には殆ど差を認めなかった。次いで，近赤外線スペクトロスコピー（NIRS）を用いた検討を，ETG-4000·日立メディコ社製の52ch NIRS装置を用いて行った。被験者への検査は語流暢課題を用いた。一卵性双生児不一致例について，課題開始30秒後のoxy-Hbの変化は**図1**に示した（Twin A: パニック障害，Twin B：健常者）。これらの結果からパニック障害における前頭葉機能（特に内側部）の低下が示唆された。次いで，MRIを施行し，一卵性双生児不一致例におけるVBM法を用いた脳形態画像解析を行った。ここで，パニック障害罹患者（A）では，健常男性群20名と比較して，左上前頭回灰白質の萎縮が認められたが，健常者（B）では，逆に左上前頭回白質において，健常男性群20名より大きかった。また双生児ペア（2名）と健常男性群20

いことや民族差の存在すること，⑥日本にパニック障害ゲノム研究に有利な条件があることがあげられる。

1．遺伝因子の関与について

以前より不安障害の家系研究において，家族集積性が認められることから遺伝的因子が関係していると推定されていた。パニック障害においても複数の家系研究がなされ，Crowe らはパニック障害患者の直系の子孫では第一度親族中の患者の割合は 17.3％であるのに比べ，正常対照群では約 1.8％と発病のリスクが高まる傾向を指摘している（Crowe et al., 1983）。その他，米国やベルギー，オーストラリアなどで行われた大規模調査において患者群の第 1 親等では対照群と比較して 8 倍の危険率になっている（Knowles, JA. & Weissman, MM, 1995）。Goldstein らによる発症年齢における研究では，20 歳以前の発症では家族性が強く 17 倍のリスクがあるのに対し，20 歳以後の発症では 6 倍のリスクであった（Goldstein et al., 1997）。親子間の発症年齢の差を見た研究でも，親の平均発症年齢が約 30.1 歳，子供では 20.8 歳であったが，評価のタイミングなどの要因がバイアスとなっているため表現促進効果（anticipation）については一概には評価できない状況である（Heiman et al., 1996）。双生児研究でも一卵性双生児の方が二卵性に比較して一致率が高くなっており（Torgersen, 1983），Kendler らの女児を用いた研究によると，一致率は一卵性 24％に対して二卵性は 11％であった（Kendler et al., 1992）。最近の双生児研究では全般性不安障害についての遺伝率は 32％に対して，パニック障害の遺伝率は約 48％と報告されている（Hettema et al., 2001）。以上をまとめると，パニック障害の遺伝的因子の関与についての疫学的な知見については第一度親族の罹患危険率が有意に高いこと，同胞相対危険が高いこと，双生児研究での一致率や遺伝率の高さがあげられる。

2．パニック障害の表現型について

パニック障害の表現系として呼吸器症状，自律神経症状，循環器症状があり，その表現型が簡潔・明瞭で妥当性があることを特徴としている。その主要症候は予期できないパニック発作，その反復・それに続く予期不安；高頻度の空間恐怖，抑うつの合併がある。また，パニック発作＝簡潔明瞭な症候をもち，短期間の脳機能異常を反映すると考えられ，その他として誘発物質の存在（CO_2，乳酸）や主要症候への SSRI などの治療薬が有効な反応を示すことなどがある。我々は 1000 例以上のパニック障害患者の初発時パニック発作の症状別頻度を検討した（梶木ら，2005）。その結果として，14 番目までの症状（30％以上の患者に存在）のうち，13 個は国際的精神疾患診断基準の症状と共通，一つ（口渇）は異なり（10 位）で，日本人に特有の民族差と考えられた。また性差（男性＜女性）と発症年齢（青年後期～ 30 代半ば好発）は国際的に共通と判断された。

3．日本特有の条件と研究計画について

今日までのパニック障害の連鎖研究における家系の総数は不十分であり，更に対象家系（患者数）を増やした形での研究が必要とされている。わが国にはパニック障害専門クリニックの存在（関東・東海）という特別な臨床フィールドの存在があり，既に 8,000 人以上が登録され，現在 4,000 人が受診中であり，世界最大と考えられている。これらを対象に既に 500 人以上のサンプリングが行われており，合計 1,000 人のサンプリングを計画している。双生児の発見も既に 5 組となっている。パニック障害は両親の協力も得られやすく，また罹患同胞対が統合失調症よりも発見しやすいという臨床的な印象もあり，短期間のサンプリングを可能にする要素に恵まれている。本研究においては TDT によるゲノムスキャンを先行させ，罹患同胞対による連鎖研究と染色体スクリーニングを併行する計画で進められており，パニック障害に関する疾患感受性遺伝子の解明を目標としている。

3．パニック障害の遺伝学的研究の紹介

1．一卵性双生児不一致例についての検討

一卵性双生児は，遺伝的には全く相等しい二個

名を比較した結果では，右海馬傍回を中心とした領域で萎縮が認められた。以上の結果はパニック障害患者における海馬傍回を含む辺縁系の変化については疾患感受性などの脆弱性を示し，パニック障害の発症に関与するのは前頭葉であるという可能性を示唆するものであった。

2．家族歴の有無による検討

100例以上のパニック障害患者に対する近赤外線スペクトロスコピーによる検討では，全体に賦活が小さく，また語流暢課題遂行中の脳血流量の増加が遅延傾向にあることが示され，前頭葉機能の低下が考えられた（Nishimura et al., 2006)。ここで，パニック障害・第一度親族にパニック障害が1人以上いる群（N=9）と近親者にパニック障害の家族歴および精神科受診や服薬のない群（N=83）との比較，すなわちパニック障害・精神疾患家族歴（PD）の有無による比較を行ったところ，家族歴のあるパニック障害の前頭葉の内側部において有意に［oxy-Hb］の賦活が小さいことが示され，何らかの遺伝的関与が考えられた。

3．Catecol-O-methyl-transferase（COMT）(22q11-13）多型および monoamine oxidase A (MAOA)（Xp11.23）の多型における検討

パニック障害におけるCOMT多型による分類：群別プロフィールについては有意な差は認められなかったが，COMT多型別にみた語流暢課題遂行中の前頭葉機能（NIRS）に変化が見られた。特にパニック障害との関与が示されているLL型で異なる賦活が示された。また，パニック障害（男性）においてMAOA多型別にみた語流暢課題遂行中の前頭葉機能（NIRS）で，一部のCH（部位）において，MAOA 3 repeatタイプ（N=7）の［oxy-Hb］の賦活が小さいことが示され，前頭葉機能において何らかの遺伝的因子の関与が示唆された。

ハミルトンらは連鎖研究においてCOMT遺伝子およびその近傍のSNPs（COMT Val158Met多型を含む）における連鎖を報告した（Hamilton et al., 2002)。Rotondoらは双極性障害とパニック障害の合併/非合併例において，catecol-O-methyl-transferase（COMT） Val158Met多型との関連についての検討を行い，パニック障害を合併しない双極性障害において最も強い相関を認めた（Rotondo et al., 2003)。我々が行ったパニック障害とCOMT L/L多型との関連について（Tawara et al., unpublished data)，Wooらの先行研究と同様の結果が得られ（Woo et al., 2002, Woo et al., 2004)，COMTLAlleleがパニック障害の疾患感受性と相関していることが追試された。また，パニック障害が女性に多いという疫学的な統計があり，パニック障害という表現型が男女で不均等に分布する理由を説明する上でCOMTは一つの有力な候補遺伝子である。事実，COMT欠失マウスの行動プロフィールにおいてオスはより「攻撃的」で，メスはより「不安が強い」傾向があるという性差が認められている。そして，ヒトにおいてはCOMT遺伝子型が男性では強迫性障害（OCD）と女性ではパニック障害に関連しているという傾向がある（Gogos et al., 1998；Karayiorgou et al.1999；Hamilton et al., 2002)。115人のパニック障害患者を用いたDomschkeらの研究において，COMT Val158Met多型が女性におけるパニック障害と関連があるとされた（Domschke et al., 2004)。性差がCOMT遺伝子型に関連する表現型で重要な役割を果たす可能性があるため，我々は性別を一致させた健常者コントロールを用いて，性が遺伝子効果に影響するかを検討した。しかし，性別と診断によって対象者を検討したところ，男女ともパニック障害ではL/L遺伝子型が増加する傾向があったが，有意差は認められなかった。女性においてパニック障害と関連する遺伝子部位を見出すことは今後の課題である。また，COMT遺伝子型の分布は民族の人口で異なる。COMTLの対立遺伝子座頻度は，Caucasiansでは約50％と，中国人では18％，日本人では29％である。L/L遺伝子型の頻度は，Caucasiansでは約25％，中国人で3％，日本人では6％であった（Eaton et al., 1994, Li et al., 1997, Ohara et al., 1998)。韓国では，COMTLの割合は23.0％，L/L遺伝子型は4.9％であった。この比率は他のアジアの国においてもほぼ同等である。本研究においてはL/L型は4.3％であった。

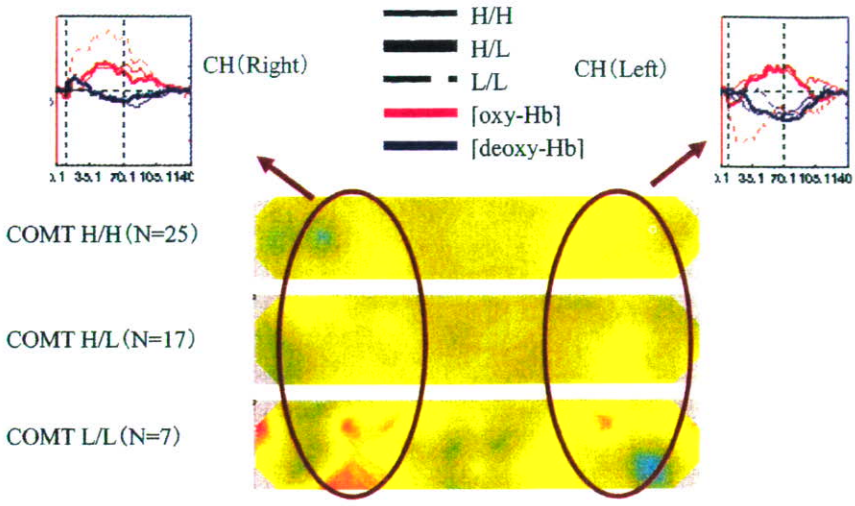

図3　COMT多型別にみた語流暢課題遂行中の前頭葉機能（NIRS）

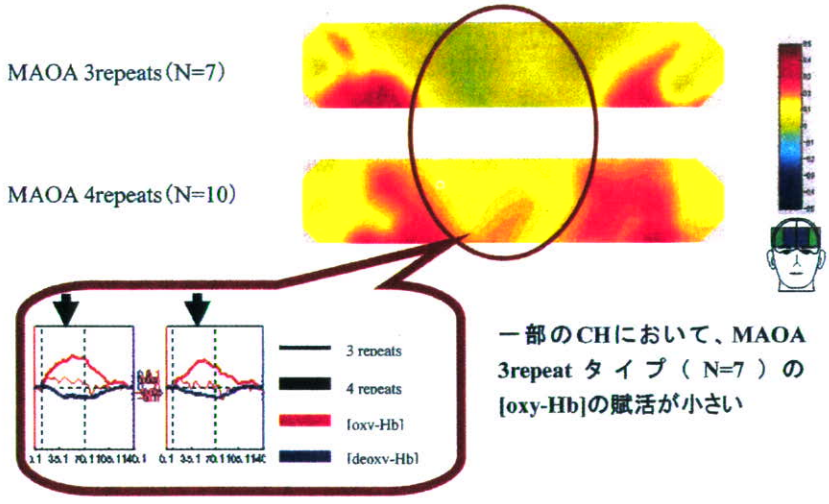

図4　MAO多型別にみた語流暢課題遂行中の前頭葉機能（NIRS）

アジア人においてCOMTL頻度は比較的低くなっており，パニック障害におけるCOMT遺伝子多型の果たす役割を示唆している。台湾，韓国，および日本のパニック障害の生涯有病率は各々，0.4％，1.7％，1－3％と低くなっている。一方，米国における生涯有病率は3.5％と高値を示す（Aoki et al., 1994, Weissman et al., 1997, Hwu et al., 1998, Kaiya et al., 2005）。したがって，アジア人における，COMTL遺伝子型の頻度が低くなっていることはパニック障害の有病率に民族差のあることを説明する可能性がある。

また，精神疾患とMAOAとの関連については，双極性障害における自殺（Ho et al., 2000）や反復性の大うつ病性障害（Schulze et al., 2000）において女性との関連が見出されている。パニック障害が女性に多いという疫学的な統計とMAOA遺伝子X染色体上にあることへの関連性が考えられるため，パニック障害とX染色体との関連については十分な検討がなされるべき課題であると思われる。この関連の報告としては，Deckertらがドイツ人（n=80）とイタリア人（n=129）のサンプルにおいてモノアミンオキシダーゼA遺伝子（Xp）のプロモーター領域の繰り返し配列の伸長した多型が主に女性のパニック障害患者で認められ多く

見られることを報告し（Deckert et al., 1999），伸長した場合にルシフェラーゼ活性を高めることから，モノアミンオキシダーゼAの活性の増加がパニック障害と関連していると考察している。

4．ま と め

以上の結果はパニック障害における前頭葉機能の低下が遺伝的因子によっても制御される可能性を示している。Gormann らによるパニック障害の神経解剖学的仮説では PET，SPECT 研究でパニック障害患者の前頭葉機能低下が示唆され，発作に対する“破滅的な”解釈や回避に関連すると考えられている（Gormann et al., 2000）。パニック障害は遺伝的負因とともに他の身体疾患との関連性や身体的な症状が特徴的であり，発現する症状による分類など新たなアプローチの方法も考慮しつつ研究が進められることが重要である。今後，このような遺伝的研究が更に発展することにより，パニック障害についての的確な診断が可能となることで，脆弱性因子の解明に基づく予防，そしてより個体の状況に合わせた効果的な治療などが期待される。

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ORIGINAL ARTICLES

Evidence for Acquired Pregenual Anterior Cingulate Gray Matter Loss from a Twin Study of Combat-Related Posttraumatic Stress Disorder

Kiyoto Kasai, Hidenori Yamasue, Mark W. Gilbertson, Martha E. Shenton, Scott L. Rauch, and Roger K. Pitman

Background: Controversy exists over the nature and origin of reduced regional brain volumes in posttraumatic stress disorder (PTSD). At issue is whether these reductions represent preexisting vulnerability factors for developing PTSD upon traumatic exposure or acquired PTSD signs due to the traumatic stress that caused the PTSD or the chronic stress of having the disorder (or both). We employed a case–control design in monozygotic twin pairs discordant for combat exposure to address the preexisting versus acquired origin of brain morphometric abnormalities in PTSD.

Methods: We used voxel-based morphometry to search for gray matter density reductions in magnetic resonance imaging (MRI) data obtained in a previous study of combat-exposed Vietnam veteran twins with (n = 18) versus without (n = 23) PTSD and their "high-risk" versus "low-risk" (respectively) identical combat-unexposed cotwins.

Results: Compared with the combat-exposed twins without PTSD, the combat-exposed twins with PTSD showed significant gray matter density reductions in four predicted brain regions: right hippocampus, pregenual anterior cingulate cortex (ACC), and left and right insulae. There was a significant PTSD Diagnosis × Combat Exposure interaction in pregenual ACC in which combat-exposed PTSD twins had lower gray matter density than their own combat-unexposed cotwins as well as than the combat-exposed twins without PTSD and their cotwins.

Conclusions: The results point to gray matter volume diminutions in limbic and paralimbic structures in PTSD. The pattern of results obtained for pregenual ACC suggests that gray matter reduction in this region represents an acquired sign of PTSD consistent with stress-induced loss.

Key Words: Anterior cingulate gyrus, brain, magnetic resonance imaging, monozygotic twins, posttraumatic stress disorder

Several structural magnetic resonance imaging (MRI) studies employing anatomic segmentation have found lower gray matter volumes in the hippocampus in posttraumatic stress disorder (PTSD) stemming from various traumatic events (1). One segmentation study found diminished gray matter volumes in pregenual anterior cingulate cortex (ACC) and subcallosal cortex but not dorsal ACC (2), whereas another did find dorsal ACC reduction (3). Decreased pregenual ACC activation in response to trauma-related stimuli is a prominent functional neuroimaging finding in PTSD (4–5).

The technique of voxel-based morphometry (VBM) allows an automated examination of structural brain differences using statistical parametric mapping (SPM) techniques. The validity of the VBM technique for assessing regional gray matter density compared with conventional region of interest measurements has been confirmed in several previous studies (6–9). Employing VBM, the first authors found reduced dorsal ACC gray matter density in victims of an urban terrorist attack with PTSD (10). Another recent study that employed VBM found gray matter density reduction in pregenual ACC but not dorsal ACC, although manual segmentation did not confirm volumetric reduction in the former structure (11). That study also found gray matter density reduction in left insula. Yet another recent PTSD VBM study found gray matter density reductions in hippocampus, pregenual ACC, and insula (12).

Controversy exists over the nature and origin of reduced regional brain volume in PTSD. Thus far the debate has focused on the hippocampus (13). At issue is whether reduced volume represents an acquired PTSD sign, for example, is due to the traumatic stress that caused the PTSD or the chronic stress of having PTSD (or both) or is a preexisting vulnerability factor for developing PTSD upon traumatic exposure. We have been employing a case–control design in monozygotic twin pairs discordant for combat exposure in Vietnam to address the preexisting versus acquired origin of biological abnormalities found in PTSD (14). In a structural MRI study that manually traced the outlines of the right and left hippocampus, we found that lower total hippocampal volume constituted a "familial" vulnerability factor for PTSD because it was found in both the combat-exposed twins with PTSD and their "high-risk" combat-unexposed cotwins whose hippocampal volumes were lower than those of the combat-exposed twins without PTSD and their "low-risk," combat-unexposed cotwins (15). (Note that the term "familial" includes both heredity and shared environment, i.e., environmental experiences that both members of a twin pair have had in common.)

In this study, we applied a VBM analysis to MRI data from the same twin sample to conduct a search throughout the entire brain for regional gray matter structural differences. On the basis of the published structural imaging studies just reviewed, we

From Department of Neuropsychiatry (KK, HY), Graduate School of Medicine, University of Tokyo, Tokyo, Japan; Research Service (MWG), VA Medical Center, Manchester, New Hampshire; Psychiatry Neuroimaging Laboratory (MES), Department of Psychiatry, and Surgical Planning Laboratory (MES), MRI Division, Department of Radiology, Brigham & Women's Hospital, Boston; McLean Hospital (SLR), Belmont, Department of Psychiatry (RKP), Massachusetts General Hospital, Boston; and Department of Psychiatry (MWG, MES, SLR, RKP), Harvard Medical School, Boston, Massachusetts.

Address reprint requests to Roger K. Pitman, M.D., at Massachusetts General Hospital, Room 2616, Building 149, 13th Street, Charlestown, MA 02129; E-mail: roger_pitman@hms.harvard.edu.

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predicted lower gray matter density in combat-exposed twins with PTSD compared with combat-exposed twins without PTSD in the following regions: hippocampus, dorsal ACC, pregenual ACC, subcallosal cortex, and insula. In an attempt to clarify the origin of any such differences, we used the data from the combat-unexposed cotwins. Gray matter diminution that confers familial vulnerability to PTSD would be expected in the high-risk, compared with the low-risk, combat-unexposed twins. In contrast, diminution that reflects acquired damage in PTSD would be expected to be manifest in a Diagnosis × Combat Exposure interaction in which the combat-exposed twins with PTSD had lower gray matter density than their own high-risk, combat-unexposed cotwins as well as the combat-exposed twins without PTSD and their low-risk cotwins.

Methods and Materials

Subjects

The strategy for subject ascertainment and recruitment has been presented elsewhere (16). The sample was described in detail in the report of our previous hippocampus manual tracing study (15). This study reanalyzed the same MRI scans from the same subjects. In the previous study, one combat-exposed twin with PTSD and his combat-unexposed cotwin were removed from the analysis because the former was an extreme, asymmetrical outlier for manually traced hippocampal volume. This subject and his cotwin were included in the current study. (Exclusion of this pair did not alter the conclusions.) The protocol was approved by the institutional review board of the Manchester, New Hampshire, VA Medical Center. All subjects had previously given written informed consent before participation after a complete description of the procedures.

MRI Data Preanalysis

The MRI acquisition techniques were described in the previous report (15). The methods used to analyze these data in the present study were similar to those reported elsewhere (10). Image analysis was performed using ANALYZE PC 3.0 (Mayo Foundation, Rochester, Minnesota) and SPM 99 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, United Kingdom) running in MATLAB 6.1 (Mathworks, Sherborn, Massachusetts). In ANALYZE, image data were resampled using an algorithm to make them isotropic, with the sides measuring .9375 mm, and then stored. Resampled images were first spatially normalized into the standard MNI152 template (17,18). Normalized images were then segmented into gray matter, white matter, cerebrospinal fluid, and skull and scalp compartments using an automated, operator-independent process (19). The segmentation step also incorporated an image density nonuniformity correction to address image density variations caused by various positions of cranial structures within the MRI head coil (20). The spatially normalized segments of the gray matter were smoothed with a 12-mm full-width at half-maximum isotropic Gaussian kernel to accommodate individual variability in sulcal and gyral anatomy. For medial temporal regions (e.g., hippocampus), a 4-mm smoothing kernel was used instead, as has been recommended (21). By smoothing the data, the partial volume effect was used to create a spectrum of gray matter densities. Gray matter density is equivalent to the weighted average of the gray matter voxels located in the volume defined by the smoothing kernel. Because previous studies have shown a fair correlation between regional gray matter density determined by VBM and structural volumes measured by conventional, manual tracing (7,9,22), the regional gray matter density can be considered to represent the local volume of gray matter.

Statistical Analyses

Demographic and psychometric data were analyzed by means of a mixed model that treated Diagnosis (in the combat-exposed twin) as a between-pairs fixed effect, Combat Exposure as a within-pairs fixed effect (repeated measure), and pairs as a random effect (16). This model analysis yields a t statistic. Gray matter density was estimated on a voxel-by-voxel basis using SPM 99 (23). Contrasts were made between the 18 combat-exposed twins with PTSD and the 23 combat-exposed twins without PTSD, and separately between their high- and low-risk cotwins, using independent t tests, adjusted for individual intracranial volume. Diagnosis × Exposure interactions were evaluated by means of a mixed, multigroup (Diagnosis), conditions (Combat Exposure), and covariates (intracranial volume) model in which one twin pair was treated as though one subject with two conditions. In this analysis, 82 covariates were entered corresponding to 82 images ([18 + 23] × 2). For each of the foregoing analyses, a statistical parametric map (SPM) of the t statistic (SPM{t}) was created, and the SPM{t} values were transformed to the normal distribution (SPM{z}). The statistical significance threshold was set at $p < .05$ corrected for multiple comparisons using the False Discovery Rate (FDR) (24).

The anatomic locations of peak coordinates were initially defined using the latest version of Talairach Daemon Client (25). These localizations were then confirmed by visually inspecting the coordinates overlaid on the mean structural image of the study sample. For peaks located within predicted brain regions, small volume correction was applied using the following a priori volume approximations from the literature: hippocampus 3.5 mL each side; insula 8 mL each side; dorsal ACC 10 mL bilaterally; pregenual ACC 5 mL bilaterally; and subcallosal cortex 5 mL bilaterally (total volume of predicted brain regions = 43 mL). For peaks located outside predicted regions, correction for whole brain was employed. Because the predictions were directional, namely, lower gray matter density in combat-exposed subjects and PTSD pairs, the tests were one-tailed, and only results in the predicted direction(s) are reported.

Results

Demographics and Psychometrics. Group mean age, Combat Severity Score (26), total Clinician-Administered PTSD Scale (CAPS) score (27), number of potentially traumatic lifetime noncombat events (16), total Michigan Alcoholism Screening Test (MAST) score (28), and Symptom Check List-90-Revised (SCL-90-R) Depression scale score (29), along with statistical analyses, are presented in Table 1. It may be seen that age was similar among subject groups. Combat-exposed twins with PTSD had more severe combat exposure than combat-exposed twins without PTSD. As expected by virtue of selection, the former had greater combat-related symptom severity on the CAPS. Twin pairs with PTSD (i.e., twin pairs in which the combat-exposed twin was diagnosed with current, combat-related PTSD) reported more potentially traumatic lifetime noncombat events than non-PTSD pairs (i.e., twin pairs in which the combat-exposed twin was diagnosed with neither current nor past combat-related PTSD). Combat-exposed twins also reported more potentially traumatic lifetime noncombat events than their combat-unexposed cotwins. Combat-exposed twins with PTSD had more severe alcoholism histories than the other three groups. Combat-exposed twins with PTSD also reported more depression than

Table 1. Group Means (SD) of Combat-Exposed Vietnam Veterans with and without Posttraumatic Stress Disorder (PTSD) and Their Combat-Unexposed, Identical Cotwins

	PTSD Pairs[a]		Non-PTSD Pairs[b]		Mixed Model						Independent *t* Tests			
					Diagnosis		Exposure		Interaction		Exposed		Unexposed	
	Exposed (n = 18)	Unexposed (High Risk) (n = 18)	Exposed (n = 23)	Unexposed (Low Risk) (n = 23)	t 39	p	t 40	p	t 39	p	t 39	p	t 39	p
Age (years)[c]	52.8 (3.4)	52.8 (3.4)	51.8 (2.3)	51.8 (2.3)	—	—	—	—	—	—	1.1	.27	1.1	.27
CAPS[d]	73.3 (16.9)	—	6.2 (7.3)	—	—	—	—	—	—	—	17.2	<.001	—	—
Combat Severity[e]	7.7 (2.1)	—	3.5 (2.6)	—	—	—	—	—	—	—	5.6	<.001	—	—
Traumatic Events[f]	8.1 (2.6)	5.2 (3.7)	5.1 (4.0)	4.2 (3.0)	2.6	.01	2.4	.02	1.3	.20	2.8	.009	1.0	.34
MAST[g]	19.1 (17.6)	6.4 (10.2)	2.4 (4.5)	2.5 (4.0)	4.5	<.001	2.8	.007	2.8	.007	4.4	<.001	1.7	.10
Depression[h]	2.5 (.9)	.3 (.5)	.6 (.7)	.4 (.5)	4.6	<.001	5.3	<.001	6.9	<.001	7.4	<.001	.7	.51

[a]As determined by the presence of current combat-related PTSD in the combat-exposed twin.
[b]As determined by the absence of current or past combat-related PTSD in the combat-exposed twin.
[c]As of October 1, 2000.
[d]Clinician-Administered PTSD Scale (range 0–136).
[e]18-item measure (range 0–18).
[f]Number of potentially traumatic lifetime noncombat events.
[g]Michigan Alcoholism Screening Test (range 0–25).
[h]Symptom Check List-90-Revised Depression Subscale (range 0–4).

the other three groups. The Pearson Correlation Coefficient between total CAPS score and SCL-90-R Depression was large (r = .86) in the PTSD pairs but negligible (r = −.06) in the non-PTSD pairs.

Gray Matter Density

Table 2 presents the results of the contrasts between combat-exposed twins with versus without PTSD. For the sake of a complete exposition of these data, all results statistically significant at the liberal threshold of uncorrected $p < .001$ with spatial extent of $k > 10$ voxels are shown. Of the seven peaks that met this threshold, four were located in predicted brain regions, namely, right hippocampus, pregenual ACC, right midinsula, and left anterior insula (shown in Figure 1). Each of these four peaks also met the statistical significance threshold of $p < .05$ with small volume correction for the a priori size of the structure (as shown in the second column of Table 3). No voxels in nonpredicted brain regions met the threshold of $p < .05$ corrected for whole brain in these, or any other, analyses.

At the right hippocampus, pregenual ACC, left anterior insula, and right midinsula loci shown in Table 2, within the 18 PTSD combat veterans, we examined the correlations between gray matter density and total CAPS score, as well as SCL-90-R Depression score. Because these analyses involved single voxels, the significance threshold was $p < .05$ uncorrected. None of these correlations were significant. We also performed the same correlations for CAPS A (reexperiencing), B (avoidance/numbing), and C (hyperarousal) symptom cluster subscores; for these analyses we applied a Bonferroni correction to the significance threshold, namely, $p < .017$ (.05/3). The only significant correlations were between symptom cluster B (reexperiencing) and gray matter density in pregenual ACC (r = −.57, p = .008), left anterior insula (r = −.53, p = .0130), and right midinsula (r = −.59, p = .006).

The contrasts between the high-risk, combat-unexposed cotwins of the combat-exposed twins with PTSD versus the low-risk, combat-unexposed cotwins of the combat-exposed twins without PTSD did not identify any voxels that met the statistical significance threshold of $p < .05$, even with small volume corrections. The only statistically significant Diagnosis × Exposure interaction was found in pregenual ACC ([8 50 12], z = 3.32, k = 16, p = .02 corrected for the a priori size of this structure). The location of this cluster is shown in Figure 2. Scatterplots of individual subjects' values at the [8 50 12] pregenual ACC locus are shown in Figure 3. There were no significant correlations between gray matter density in the 18 PTSD combat veterans minus gray matter density in their combat-unexposed cotwins, and total CAPS score, SCL-90-R Depression, or (with Bonferroni corrections) any of the CAPS symptom cluster subscores.

The mixed model and t test analyses that yielded the statistically significant results described earlier were repeated entering the following possibly confounding variables into the respective models as covariates: age, combat severity (in the exposed twin), number of potentially traumatic lifetime noncombat events, MAST score, and SCL-90-R Depression score. To control for a possibly confounding role of childhood physical or sexual abuse, the data were re-analyzed deleting pairs within which either member had such a history. All these results are shown in Table 3.

Table 2. Loci Showing Gray Matter Density Reductions in Combat-Exposed Twins with versus without Combat-Related Posttraumatic Stress Disorder That Were Significant at Uncorrected $p < .001$

z	p_{svc}	k	[x y z]	Brain Region
4.50	**.001**	**248**	**[44 −2 −14]**	**Right midinsula**
4.47		145	[−62 −56 −4]	Left middle temporal gyrus
4.39	**.001**	**27**	**[34 −28 −16]**	**Right hippocampus**
3.95	**.005**	**236**	**[−36 10 −4]**	**Left anterior insula**
3.71	**.004**	**185**	**[0 46 10]**	**Pregenual anterior cingulate cortex**
3.52		35	[−46 42 −14]	Left inferior frontal gyrus
3.47		22	[−64 −44 10]	Left superior temporal gyrus

k, cluster size; p_{svc}, significance level with small volume correction on the basis of the a priori size of the structure; [x y z] = Montréal Neurological Institute coordinates of peak voxel.
Predicted areas appear in boldface.

Discussion

Of the seven loci at which combat-exposed twins with PTSD had lower gray matter density than combat-exposed twins without PTSD at a liberal threshold of $p < .001$, four were located in predicted brain regions, namely, right hippocampus, pre-

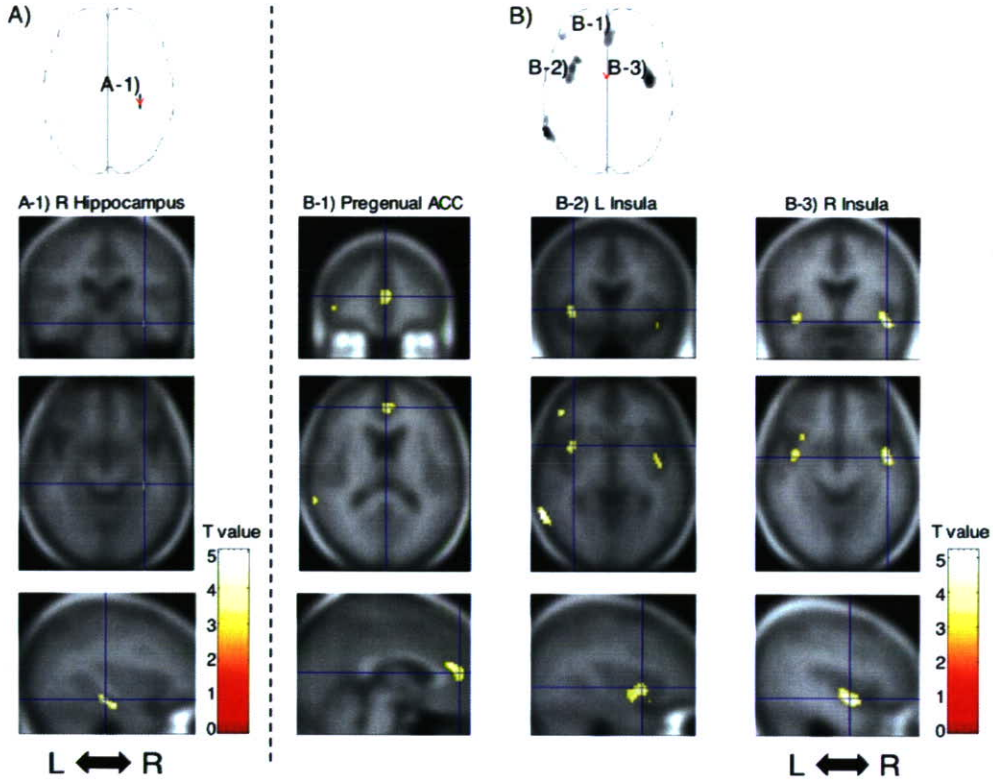

Figure 1. Brain regions showing diminution in gray matter density in combat-exposed twins with posttraumatic stress disorder (PTSD) versus those without PTSD. **(A)** Statistical parametric mapping (SPM) analysis with 4-mm Gaussian smoothing kernel revealed statistically significant reduced gray matter density shown in the axial projection. **(A-1)** Regional gray matter density reduction in the right hippocampus is rendered onto orthogonal slices of the averaged magnetic resonance image of the present study's subjects. **(B)** SPM analysis with 12-mm Gaussian smoothing kernel revealed statistically significant reduced gray matter density shown in the axial projections. Regional gray matter density reductions in the following areas are rendered: **(B-1)** pregenual anterior cingulate cortex; **(B-2)** left insula; **(B-3)** right insula. L, left hemisphere; R, right hemisphere; ACC, anterior cingulate cortex.

genual ACC, and left anterior and right midinsula, even though the predicted brain regions occupy less than 10% of total gray matter volume. This regional specificity supports the validity of our results and implicates limbic and paralimbic structures as the major sites of gray matter density reductions in combat-related PTSD. Gray matter reductions in pregenual ACC and both insulae significantly correlated only with the cluster B "reexperiencing" symptoms of PTSD.

The ACC, especially its pregenual (or "affective") division, and insula are components of the anterior "paralimbic belt," are strongly interconnected to each other and to the amygdala, and are highly involved in emotional aspects of brain function (30–32). Impaired pregenual ACC function is one of the most robust neuroimaging findings in PTSD (4–5). A neurocircuitry model of PTSD posits that the ventromedial prefrontal cortex, including pregenual ACC, inhibits the expression of classically conditioned fear responses by the amygdala (33). Thus impairment in this brain region might be expected to most affect the DSM-IV symptoms that are putatively most closely related to conditioned fear, namely, the cluster B symptoms (especially B.4 and B.5, that is, intense psychological distress (and/or) physiological reactivity on exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event). To the extent that diminished structure implies diminished function, reduced pregenual ACC gray matter density is consistent with this neurocircuitry model.

Table 3. Results Adjusted for Potentially Confounding Variables

Unadjusted	Age	Combat Severity[a]	Traumatic Events[b]	MAST Score[c]	Childhood Abuse[d]	SCL-90-R Depression[e]	
$z\ (p_{svc})$	$z\ (p_{svc})$	$z\ (p_{svc})$	$z\ (p_{svc})$	$z\ (p_{svc})$	$z\ (p_{svc})$	$z\ (p_{svc})$	Brain Region
Combat-Exposed Twins: PTSD vs. non-PTSD							
4.50 (.001)	4.43 (.001)	3.23 (.05)	3.98 (.005)	3.39 (.02)	3.19 (.04)	2.07 (.49)	Right midinsula [44 −2 −14]
4.39 (.001)	4.36 (.001)	3.05 (.11)	4.11 (.004)	4.63 (<.001)	3.56 (.04)	1.52 (.99)	Right hippocampus [34 −28 −16]
3.95 (.005)	3.98 (.005)	3.32 (.03)	3.06 (.07)	3.26 (.04)	3.63 (.01)	2.15 (.44)	Left anterior insula [−36 10 −4]
3.71 (.004)	3.59 (.009)	2.50 (.18)	2.79 (.08)	2.40 (.14)	2.92 (.03)	.93 (.99)	Pregenual anterior cingulate cortex [0 46 10]
Diagnosis × Combat Exposure Interaction							
3.32 (.02)	3.39 (.02)	3.32 (.02)[f]	2.76 (.10)	2.77 (.10)	3.42 (.01)	1.81 (.53)	Pregenual anterior cingulate cortex [8 50 12]

p_{svc}, significance level with small volume correction; PTSD, posttraumatic stress disorder.
[a]18-item measure (range 0–18).
[b]Number of potentially traumatic lifetime noncombat events.
[c]Michigan Alcoholism Screening Test (range 0–25).
[d]Deleting six PTSD pairs and five non-PTSD pairs within which either twin had a history of childhood abuse.
[e]Depression subscale of Symptom Check List 90—Revised.
[f]Covariate is value in combat-exposed twin.

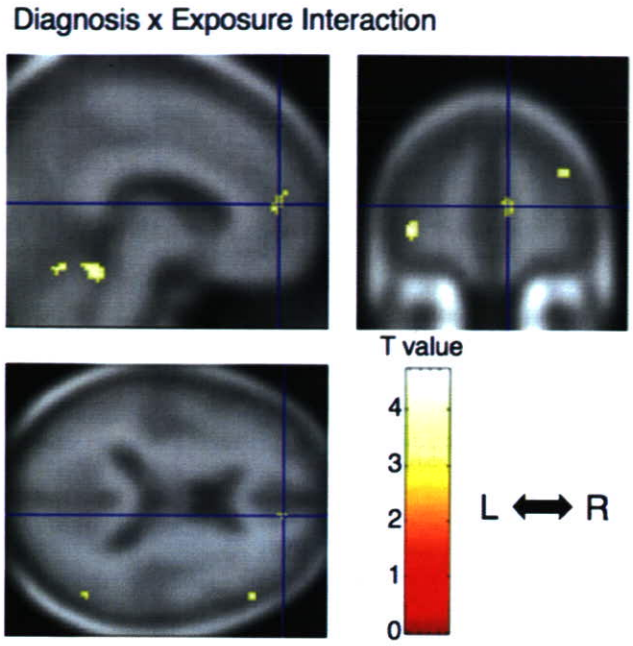

Figure 2. Brain region showing posttraumatic stress disorder Diagnosis × Combat Exposure interaction. Regional interaction for gray matter density in pregenual anterior cingulate cortex is rendered onto orthogonal slices of the averaged magnetic resonance image of the study subjects. Crosshairs indicate the peak coordinate of the interaction [8 50 12]. L, left hemisphere; R, right hemisphere.

Functional neuroimaging studies of the hippocampus in PTSD are less common, but they, too, support impairment in this brain region (34–36). Hippocampal impairment may contribute to PTSD by reducing the ability to construct declarative narratives that bind the affect associated with the traumatic event (37), by the ability to recognize safe contexts (33), or by other unknown mechanisms. The reduced gray matter density found in bilateral insulae is paradoxical in light of studies that have generally found hyperactivity in this brain region in PTSD (38) and other anxiety conditions (39). One model of anterior insula function posits that this structure detects the difference between an observed and expected body state and generates an interoceptive prediction signal that triggers anxiety (39). A structurally compromised insula may be less inhibited in generating such signals in PTSD, but this is in the realm of speculation.

The most interesting result from our study is the significant Diagnosis × Exposure interaction in the pregenual ACC, with combat-exposed PTSD twins having lower gray matter density than their own combat-unexposed cotwins and than the combat-exposed twins without PTSD and their cotwins, supporting the inference that pregenual ACC gray matter reduction is an acquired sign of PTSD. In animals, exposure to chronic stress has been shown to damage not only the hippocampus in rodents (40) and primates (41) but also the ACC in rodents (42,43) and primates (44). It has been hypothesized that such damage may provide a basis for structural changes observed in PTSD (42,45). A recent study of mentally healthy persons that used automated segmentation found that those who reported early life stressors had smaller ACCs than those who did not (46). However, causal inferences are difficult to draw from the cross-sectional study of nontwins.

When the Diagnosis × Exposure interaction at [8 50 12] was adjusted for MAST score, its statistical significance level was reduced to corrected $p = .10$, which falls short of statistical significance. We did not obtain data regarding recent alcohol consumption. This is a limitation considering that imaging findings related to alcohol may be more sensitive to recent as opposed to more remote intake. On the other hand, the likelihood that increased alcohol use by the PTSD veterans accounts for the gray matter density reduction in their ACCs is diminished by the consideration that if the PTSD subjects studied here had consumed enough alcohol to damage their brains, evidence for this should have been found in other brain regions known to be affected by alcohol, including superior, motor, and other areas of the frontal cortex and cerebellum (47,48), none of which (except for a small cluster in left inferior frontal cortex) showed volumetric reduction in the PTSD compared with the non-PTSD combat veterans at even the liberal threshold of uncorrected $p < .001$. Thus the specificity of volumetric diminution to our predicted brain regions argues against a global effect such as alcohol-induced brain damage. Finally, a recent manual tracing study found comparably (and significantly) reduced ACC volume in subgroups of PTSD veterans with and without a history of lifetime alcohol abuse or dependence, in comparison to non-PTSD veterans (49).

When the Diagnosis × Exposure interaction in pregenual ACC was adjusted for number of potentially traumatic lifetime noncombat events, its statistical significance level was also reduced to corrected $p = .10$. This means we cannot be fully confident that stressful events other than military combat do not account for the reduced ACC gray matter density in the PTSD veterans. However, even if such events did contribute, this would still not be inconsistent with stress-induced diminution of this structure. When the Diagnosis × Exposure interaction in pregenual ACC was adjusted for depression, it was no longer significant. This is not surprising given the high association between depression and PTSD in our sample, in which self-

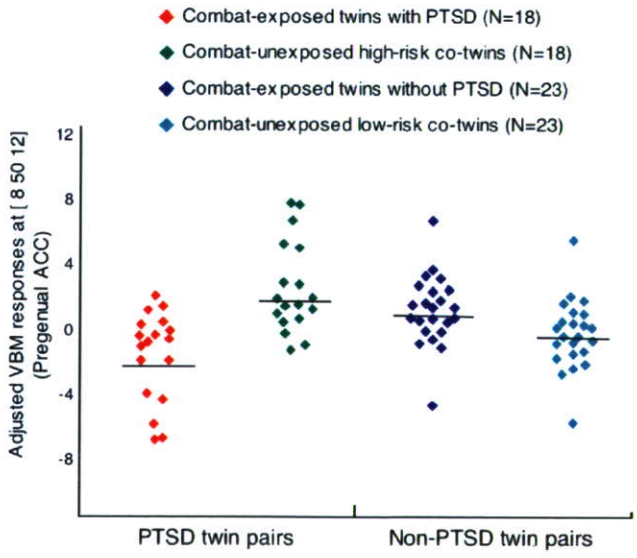

Figure 3. Scatterplots of individual subjects' adjusted voxel-based morphometry responses. Shown at the site of the posttraumatic stress disorder Diagnosis × Combat Exposure interaction in pregenual anterior cingulate cortex [8 50 12]. Means are represented by solid horizontal lines drawn on each group's distribution.

reported depression appears to have been acquired along with PTSD, making the two likely facets of the same posttraumatic psychopathology.

However, because the most salient, common difference in our study was the presence of combat-related PTSD in the former, and because, as noted earlier, the observed effects remained significant or nearly significant after considering the contributions of several important potentially confounding variables, it is reasonable to attribute this lower gray matter density to the presence of combat-related PTSD.

Combat-exposed twins with PTSD also had lower gray matter density than combat-exposed twins without PTSD in right hippocampus and left anterior and right midinsula, as well as at another site within pregenual ACC, replicating previous studies. These results could not be explained by group differences in age, combat severity, number of potentially traumatic lifetime noncombat events, alcoholism, or child abuse. Unfortunately, the analyses that included the data from the combat-unexposed cotwins were unable to shed light on the origin of these gray matter reductions in the combat-exposed twins with PTSD, because they failed to yield either a significant difference between high- and low-risk combat-unexposed cotwins (which would support a pretrauma vulnerability factor) or a significant Diagnosis × Exposure interaction (which would support an acquired abnormality). Finally, these results were unable to replicate previously reported segmentation and voxel-based morphometric findings of gray matter reduction in subcallosal cortex and dorsal ACC.

In the same twin sample studied here, we previously found manual tracing evidence that diminished hippocampal volume represents a pretrauma vulnerability factor for PTSD, rather than an acquired PTSD sign (15). In contrast, our VBM results suggest that diminished volume in pregenual ACC is acquired as a result of the combat exposure that led to PTSD, the PTSD itself, or both. We have no ready explanation as to why diminutions in these two structures should have different origins. As noted earlier, however, the origin of gray matter density reduction in the pregenual ACC site other than the one that showed the significant interaction could not be explicated by our data; it is possible that it represents a PTSD vulnerability factor. Additional techniques that may help to clarify this uncertainty in future studies include cortical parcellation (segmentation) and magnetic resonance spectroscopy.

It has been suggested that VBM may not detect very small, localized gray matter volume reductions because false-negative VBM findings may arise from the changes in the shape or displacement of structures in the course of spatial normalization (7). Additionally, VBM may be biased against finding group differences in areas that are spatially complex (50). Inversely, we cannot rule out the possibility that the abnormalities detected by VBM in our study reflected group differences in the shape of brain structures rather than their volume (11), although even shape differences may have functional consequences. The failure of VBM to find a significant hippocampal gray matter reduction in the high- versus low-risk, combat-unexposed cotwins contrasts with our positive result in the same sample using manual segmentation of hippocampus (15) suggests that the latter technique may be more sensitive to reduced volume in this structure than the voxel-based approach. Similarly, we are unable to rule out the possibility that subtle group differences in other brain regions in this study remained below the sensitivity of VBM or the detection power conferred by our sample.

KK and HY contributed equally to this work.

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Gender-Common and -Specific Neuroanatomical Basis of Human Anxiety-Related Personality Traits

Hidenori Yamasue[1], Osamu Abe[2], Motomu Suga[1], Haruyasu Yamada[2], Hideyuki Inoue[1], Mamoru Tochigi[1], Mark Rogers[1], Shigeki Aoki[2], Nobumasa Kato[1] and Kiyoto Kasai[1]

[1]Department of Neuropsychiatry and [2]Radiology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Exploration of the relationships between regional brain volume and anxiety-related personality traits is important for understanding preexisting vulnerability to depressive and anxiety disorders. However, previous studies on this topic have employed relatively limited sample sizes and/or image processing methodology, and they have not clarified possible gender differences. In the present study, 183 (male/female: 117/66) right-handed healthy individuals in the third and fourth decades of life underwent structural magnetic resonance imaging scans and Temperament and Character Inventory. Neuroanatomical correlates of individual differences in the score of harm avoidance (HA) were examined throughout the entire brain using voxel-based morphometry. We found that higher scores on HA were associated with smaller regional gray matter volume in the right hippocampus, which was common to both genders. In contrast, female-specific correlation was found between higher anxiety-related personality traits and smaller regional brain volume in the left anterior prefrontal cortex. The present findings suggest that smaller right hippocampal volume underlies the basis for higher anxiety-related traits common to both genders, whereas anterior prefrontal volume contributes only in females. The results may have implications for why susceptibility to stress-related disorders such as anxiety disorders and depression shows gender and/or individual differences.

Keywords: anxiety, gender, hippocampus, MRI, prefrontal cortex

Introduction

Anxiety is a universal mechanism for generating adaptive behavior as it guides appropriate responses to environmental cues such as danger and threat; however, in humans, an excessive amount of anxiety predisposes individuals toward psychiatric conditions such as phobia, depression, and post-traumatic stress disorder (PTSD) (reviewed in Gross and Hen 2004). Recent structural magnetic resonance imaging (MRI) studies have revealed significant neural correlates of these anxiety and depressive disorders in hippocampus, amygdala, and prefrontal cortex (reviewed in Pitman et al. 2001; Hasler et al. 2004). However, whether or not brain functional/structural deviations represent a predispositional vulnerability to develop the disorders remains unclear.

A previous volumetric MRI twin study reported smaller-than-normal hippocampal volume as a preexisting vulnerability factor for PTSD after exposure to psychological trauma rather than a shrinkage resulting from strong and chronic stress (Gilbertson et al. 2002). Based on this notable finding, healthy individuals who are vulnerable to develop anxiety disorders should show smaller-than-normal hippocampal volume. Evaluating healthy individuals may have advantage in avoiding a number of potential confounds that could affect regional brain volume in studies of patients with anxiety and depression, such as alcohol abuse (Agartz et al. 1999), psychiatric comorbidity (Schuff et al. 2001), chronic illness (Sheline et al. 1999), and chronic medication (Vermetten et al. 2003). However, neuroanatomical correlates of human anxiety-related personality traits remain unclear.

Recent functional neuroimaging studies have revealed that individual differences in anxiety-related traits are associated with differences in neural response to emotional activation in the amygdala and prefrontal regions among healthy individuals (Grachev and Apkarian 2000; Hariri et al. 2002, 2005; Etkin et al. 2004; Milad et al. 2005; Pezawas et al. 2005; Most et al. 2006). In addition, a recent animal study reported a negative correlation between hippocampal volume and trait anxiety in rats with normal anxiety-related behavior (Kalisch et al. 2006). In contrast, a limited number of previous studies have examined relationships between brain morphological variability, a highly heritable trait marker (e.g., Lyons et al. 2001; Thompson et al. 2001), and anxiety-related traits in healthy human adults (Knutson et al. 2001; Pujol et al. 2002; Omura et al. 2005; Wright et al. 2006). Previous studies reported that high anxiety-related traits correlated with small whole brain volume ($n = 86$ [male/female = 38/48]; Knutson et al. 2001) and large anterior cingulate surface area ($n = 100$ [50/50]; Pujol et al. 2002). More recent studies employed computational morphological analysis to identify regional correlates of anxiety-related traits throughout the entire brain, although the study sample sizes were relatively small ($n = 41$ [19/22] in Omura et al. 2005; $n = 28$ [11/17] in Wright et al. 2006). Because the previous human studies employed relatively limited image processing methodology and/or small sample size as overviewed above, the relationship between regional brain volume and anxiety-related traits has not yet been examined in the whole brain in a study employing a large sample size.

Among healthy individuals, one of the major factors contributing to individual difference in anxiety-related traits is gender difference. Previous studies have reported sex dimorphism in both anxiety-related traits (e.g., Cloninger et al. 1993; Farmer et al. 2003) and brain anatomy (e.g., Good et al. 2001; Luders et al. 2004). Furthermore, a few studies have suggested gender differences in neural correlates of emotional modulation (Canli et al. 2002). The above findings suggest it is necessary to consider gender effects in attempting to uncover the neuroanatomical underpinnings of human anxiety-related personality traits.

The use of self-report questionnaires such as Temperament and Character Inventory (TCI) has been well established as a means to assess individual differences in behavioral traits (Cloninger 1987; Cloninger et al. 1993). Cloninger and

colleagues describe 4 dimensions of temperament in the TCI including harm avoidance (HA), a frequently measured anxiety-related personality trait, as a marker of genetic and biological origins (Cloninger et al. 1993). In accordance with the theory, previous studies have reported high heritability of HA (e.g., Farmer et al. 2003) and significant early environmental and genetic backgrounds, an example of the latter being serotonin transporter promoter polymorphism (5-HTTLPR) (e.g., Lesch et al. 1996). However, no specific genetic variants contributing to the traits have been conclusively identified (reviewed in Sen et al. 2004). Furthermore, previous studies have reported that individuals with panic disorder, generalized anxiety disorder (Starcevic et al. 1996), PTSD (Richman and Frueh 1997), and depression, as well as those with genetic vulnerability to depression (Farmer et al. 2003), scored high in HA. Therefore, the HA of TCI is suitable as a probe to index neuroanatomical correlates of individual differences in anxiety-related personality traits.

The present study was thus designed to use computational voxel-by-voxel morphometric analysis to explore the relationship between individual differences in HA scores and regional gray matter volumes in the hippocampus, amygdala, and prefrontal cortex as well as throughout the whole gray matter in 183 healthy young adults. Furthermore, the gender difference in the correlation between anxiety-related traits and regional gray matter volume was also examined. The possible confounding effects of aging, handedness, and psychiatric illness were controlled in order to identify neuroanatomical correlates of personality traits as directly as possible.

Materials and Methods

Subjects and Clinical Evaluation

One hundred and eighty three right-handed Japanese subjects (117 males/66 females), mainly college students, hospital staff, and their acquaintances, participated in the present study. Because the present study was concerned with trait aspects of brain morphology and personality, the age of the subjects was restricted to the third and fourth decades of life to minimize the effects of aging and the menopause on brain morphology. The socioeconomic status (SES) and parental SES were assessed using the Hollingshead scale (Hollingshead 1965). Handedness was assessed based on the Edinburgh Inventory (Oldfield 1971). The participants were interviewed by a trained psychiatrist (H.Y. or M.S.) to be screened for the presence or absence of neuropsychiatric disorders through the structured clinical interview for DSM-IV axis I disorder, non-patient edition (First et al. 1997). The exclusion criteria were current or past DSM-IV Axis I or II psychiatric disorder including alcohol/substance-related disorders in themselves, neurological illness, and traumatic brain injury with any known cognitive consequences or loss of consciousness for more than 5 min. All participants had to have IQ greater than 75. These interviews were performed on the same day as MR scanning. The ethical committee of the University of Tokyo Hospital approved this study. After a complete explanation of the study to the subjects, written informed consent was obtained.

Personality Assessment

A valid Japanese translation (Kijima et al. 2000) of TCI (Cloninger 1987; Cloninger et al. 1993) was used for measuring the personality trait of each subject. Each subject completed a 240-item TCI questionnaire within 3 months before or after MR scan. In this study, we focused on the HA subscale of the TCI.

MRI Acquisition

The method of 1.5-mm-slice high-spatial resolution MRI acquisition was the same as that of our previous study (Yamasue et al. 2003). Briefly, the MRI data were obtained using a 1.5-T scanner (General Electric Signa Horizon Lx version 8.2, GE Medical Systems, Milwaukee, WI). Three-dimensional Fourier transform spoiled gradient recalled acquisition with steady state was used because it affords excellent contrast between the gray matter and white matter in the evaluation of brain structures. The repetition time was 35 ms, the echo time 7 ms with one repetition, the nutation angle 30 degrees, the field of view 24 cm, and the matrix 256 × 256 (192) × 124. A trained neuroradiologist (H.Y. or O.A.) evaluated the MRI scans and found no gross abnormalities in any of the subjects.

Image Processing for Voxel-Based Morphometry

Image processing for voxel-based morphometry (VBM) (Ashburner and Friston 2000; Good et al. 2001), a fully automatic technique for computational analysis of differences in local brain tissue volume throughout the entire brain, was conducted using SPM 2 (Institute of Neurology, London, UK). This method involves the following steps: 1) spatial normalization of all images to a standardized anatomical space by removing differences in overall size, position, and global shape; 2) extraction of gray and white matter from the normalized images; and 3) analysis of differences in local gray and white matter volume across the whole brain (Ashburner and Friston 2000). Spatial normalization to the standard anatomical space was performed in a 2-stage process. In the first step, each image was registered to the International Consortium for Brain Mapping template (Montreal Neurological Institute, Montreal, Canada), which approximates Talairach space. This step applied a 12 parameter affine transformation to correct for image size and position. Regional volumes were preserved while corrections for global differences in whole brain volume were made. The normalized images of all participants were averaged and smoothed with a Gaussian kernel of 8 mm full-width at half-maximum (FWHM) and then used as a new template with reduced scanner- and population-specific bias. In the second normalization step, we locally deformed each image of our entire group to the new study-specific template using a nonlinear spatial transformation. This accounts for the remaining shape differences between the images and the template and improves the overlap of corresponding anatomical structures. Finally, using a modified mixture model cluster analysis, normalized images were corrected for nonuniformities in signal intensity and partitioned using study-specific customized prior probability map into gray and white matter, cerebrospinal fluid, and background. To remove unconnected nonbrain voxels (e.g., rims between brain surface and meninges), a series of morphological erosions and dilations to the segmented images were applied (Good et al. 2001). In an intensity modulation step, voxel values of the segmented images were multiplied by the measure of warped and unwarped structures derived from the nonlinear step of the spatial normalization (Jacobian determinant). This step converts relative regional gray matter density to absolute gray matter density expressed as the amount of gray matter per unit volume of brain tissue prior to spatial normalization. The resulting modulated gray matter images were smoothed with a Gaussian kernel of 12 mm FWHM.

Statistical Analysis of VBM

Statistical analyses were performed using an analysis of covariance model (Friston et al. 1990). To account for global anatomical variations, the intracranial volume calculated from VBM procedure was treated as a confounding covariate. To detect the neuroanatomical correlates of individual differences in HA, statistical analysis treated intracranial volume as confounding covariate and the score of HA in TCI as the covariate of interest. To test hypotheses with respect to regionally specific association with HA, the estimates were compared using 2 linear contrasts. The resulting set of voxel values for each contrast constituted a statistical parametric map of the t-statistic (SPM[t]). The SPM(t)s were displayed at an uncorrected threshold of $P < 0.001$ for graphical reporting. We only discuss results in the text and in tables that survive a correction at 0.05 for the search volumes. The statistics in the tables are transformed to a Z-score to make them more intuitive. The significance of each region was corrected for multiple comparisons using false discovery rate (FDR) because previous literature suggests that multiple hypothesis testing (Bonferroni type) family-wise error (FWE) correction tends to wipe out both false and true positives when

applied to the entire data in neuroimaging (Genovese et al. 2002). The innovation of FDR is that they control the expected proportion of the rejected hypotheses that are falsely rejected. Thus, the statistical significance level was set at FDR-corrected $P < 0.05$. Whereas significant effects were explored throughout the entire gray matter regions, small volume correction was employed in predicted regions based on previous literature: hippocampus (Gilbertson et al. 2002), amygdala (Hariri et al. 2002; Pezawas et al. 2005), and prefrontal cortex (Grachev and Apkarian 2000; Canli et al. 2002; Yamasue et al. 2003; Milad et al. 2005). In contrast to the whole gray matter exploration, FWE-corrected P was conservatively employed to detect findings within the searched volumes (SVs) (hippocampus: 3.5 ml; amygdala: 2 ml; Prefrontal cortex: 60 ml, bilaterally).

Furthermore, the gender difference in the correlation between HA and regional gray matter volume was tested using the condition by covariates interaction analysis. This interaction analysis treated gender as a condition, the score of HA as the covariate of interest, and intracranial volume as confounding covariate. The threshold for statistical significance was the same as that in the correlational analysis between the score of HA and regional gray matter volume. Once a significant interaction was found, post hoc correlational analysis between the score of HA and regional gray matter volume was then conducted in each gender separately.

Results

Although the group mean score of HA was higher in the female group than in the male group, a Mann-Whitney test revealed that the group difference did not reach statistical significance ($P = 0.58$). Whereas the score of HA showed no significant correlations with age, self-SES, parental-SES, and handedness ($0.032 <$ Spearman's rho < -0.124, $0.095 < P < 0.668$), Mann-Whiteny test showed significant gender differences in age ($P = 0.01$) and self-SES ($P = 0.002$). (Table 1) To control the gender differences in age and self-SES, the VBM interaction analysis between gender and HA employing these variables as confounding as covariates was added.

The VBM revealed that the score of HA showed a significant negative correlation with regional gray matter volume in the right hippocampus (peak coordinate = [34, -36, -6], $z = 3.57$, FWE-corrected $P = 0.005$ with 3.5 ml SV, cluster size = 1096 mm^3) (Fig. 1, Table 2). The regional gray matter volume in the other brain regions showed no significant correlation with the score of HA in the male and female combined group.

Table 1
Subject characteristics

Variable	Male (n = 117)		Female (n = 66)		Mann-Whitney	
	Mean	SD	Mean	SD	Z value	P
Demographic variables						
Age (range)	29.2 (21-40)	4.1	27.8 (22-40)	4.2	−2.60	0.009
Handedness (range)[a]	95.7 (25-100)	10.9	96.2 (50-100)	9.8	−1.34	0.18
SES[b]	1.44	0.5	1.77	0.7	−3.07	0.002
Parental SES[b]	2.09	0.6	2.14	0.6	−0.47	0.64
TCI						
HA	16.4	7.2	16.7	6.6	−0.56	0.58
Novelty seeking	22.3	5.9	22.3	5.3	−0.17	0.87
Reward dependence	15.1	3.4	16.9	3.2	−3.25	0.001
Persistence	4.7	1.8	4.6	1.6	0.00	1
Self direcctedness	29.6	6.5	31.0	6.4	−1.19	0.24
Cooperativeness	28.9	5.2	30.4	5.3	−1.87	0.061
Self transcendence	9.0	4.9	11.4	6.2	−2.40	0.017

Note: SD, standard deviation.
[a]Determined using Edinburgh Inventory (Oldfield 1971): Scores greater than 0 indicate right handedness. A score of 100 indicates strong right handedness.
[b]Assessed using the Hollingshead scale (Hollingshead 1965). Higher scores indicate lower educational and/or occupational status.

A significant gender difference in the correlation with HA was found in regional gray matter volume of the left anterior prefrontal cortex ([-20, 56, -2], $z = 4.11$, FWE-corrected $P = 0.008$ with 60 ml SV, cluster size = 1016 mm^3). Consequently, post hoc correlational analyses showed a significant negative correlation between the score of HA and the regional gray matter volume in left anterior prefrontal cortex only in the female ([-18, 56, 2], $z = 3.58$, FWE-corrected $P = 0.046$ with 60 ml SV, cluster size = 632 mm^3) but not in the male subjects ([-18, 56, 2], $z = 1.83$, FWE-corrected $P = 0.82$ with 60 ml SV) (Fig. 2, Table 2). The interaction remained significant after the effect of aging, and self-SES was eliminated. The regional gray matter volume in the other brain regions, including right hippocampus ([34, -36, -6], $z = 0.88$, FWE-corrected $P = 0.55$ with 3.5 ml SV), shows no significant gender difference in correlation between HA and regional brain volume.

Neither significant correlation nor significant gender difference in the correlation was observed between HA and regional gray matter volume in amygdala of 12-mm FWMH smoothed images, although a trend level negative correlation was found in the left amygdala of 4-mm FWHM smoothed images of female subjects ([-16, -12, -16], $z = 2.73$, FWE-corrected $P = 0.08$ with SV 2 ml).

To compare the current results with previous findings, the correlation with voxel density was additionally examined. Then, HA showed no significant correlation or gender difference in the correlation with the regional white or gray matter density, which should mainly reflect probability of tissue existence rather than regional brain volume.

Discussion

The present study demonstrated evidence that smaller right hippocampus is a gender common neuroanatomical correlate of higher anxiety-related traits in a relatively large sample of young healthy individuals. Of note, a personality trait, a behavioral index thought to have multiple, complex determinants, showed a statistically significant association with a localized brain region, the right hippocampus, which was common to both genders. In contrast, the current analysis also revealed that regional brain volume in the left anterior prefrontal cortex showed a negative correlation with HA that was present only in the female group.

The negative association between right hippocampal volume and anxiety-related traits revealed by the current study is in line with previous reports of smaller hippocampal volume in patients with PTSD (reviewed in Pitman et al. 2001) and depression (reviewed in Hasler et al. 2004). In particular, patients with long-lasting PTSD symptoms consistently demonstrated smaller-than-normal hippocampus volume, although several previous studies examining acute and shortly recovered patients with PTSD reported no significant volume decrease in patients with PTSD compared with healthy individuals (Bonne et al. 2001; Yamasue et al. 2003). In addition, a previous study reported that high HA predicts increased PTSD symptom severity (Richman and Frueh 1997). The current study reveals that small right hippocampal volume predicts high HA in healthy young individuals and further supports the suggestion by a twin study that smaller-than-normal right hippocampus is a preexisting vulnerable factor to develop long lasting and severe PTSD after exposure to psychological trauma (Gilbertson et al. 2002).

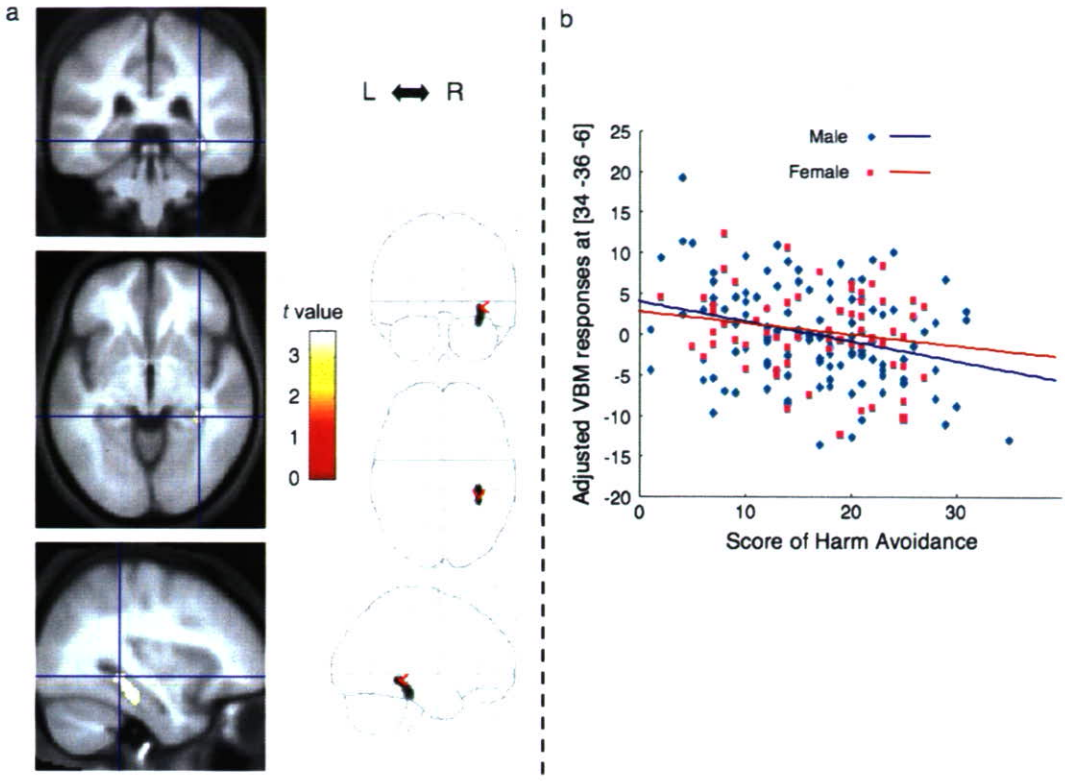

Figure 1. Gender-common negative correlation between HA and regional gray matter volume in the right hippocampus. (*a*) Gray matter regions showing significant correlations with the individual variability of HA were rendered in the Montreal Neurological Institute space. (Right) Statistical parametric map in the 3 orthogonal projections shows voxels where negative correlations with HA emerged. (Left) The gray matter voxels showing negative correlations with the individual variability of HA were rendered onto the averaged images of the whole sample ($N = 183$) (voxel threshold: uncorrected $P < 0.001$). L, left; R, right. (*b*) Scatter plots depicting correlations between regional gray matter volume at the peak voxel [34, −36, −6] and individual variability in HA in females ($N = 66$) and males ($N = 117$).

Table 2
Neuroanatomical correlates of HA

Anatomical location	Peak coordinate			*Z* score	Correlation coefficient	Corrected *P*	Cluster size (mm^3) (voxel threshold: uncorrected $P < 0.001$)
	x	*y*	*z*				
Negative correlation ($n = 183$) (Fig. 1)							
Right hippocampus	34	−36	−6	3.57	−0.26	0.005*	1096
Interaction with gender on the correlation between HA and regional brain volume** ($n = 183$)							
Left anterior prefrontal (Fig. 2)[a]	−20	56	−2	4.11	—	0.008**	1016
Post hoc analyses: Negative correlation in female ($n = 66$)							
Left anterior prefrontal (Fig. 2)[b]	−18	56	2	3.58	−0.43	0.046**	632

[a]In contrast to the anterior prefrontal, the interaction with gender on the correlation between HA and right hippocampal volume did not reach statistically significant level ([34, −36, −6], $z = 0.88$, corrected $P = 0.55$ with 3.5 ml SV).

[b]In contrast to the correlation in female, the correlation between HA and left anterior prefrontal volume did not reach statistically significant level in male ($n = 117$, [−18, 56, 2], $z = 1.83$, corrected $P = 0.82$ with 60 ml SV).

*FWE-corrected *P* with 3.5 ml SV.

**FWE-corrected *P* with 60 ml SV.

The current study is also consistent with previously reported associations between anxiety-related traits and hippocampal function and chemical condition in healthy human subjects (Gallinat et al. 2005) and experimental animals (Kalisch et al. 2006). Gallinat et al. (2005) reported a significant correlation between lower *N*-acetylaspartate, a putative neural integrity marker, and higher trait anxiety, using MR spectroscopy and state and trait anxiety inventory, in 38 healthy subjects. Kalisch et al. (2006) recently reported a negative correlation between hippocampal volume and trait anxiety in normal anxiety-related behavior rats, although they found a positive correlation in extreme anxiety-related behavior rats. Animal studies (e.g., Nakao et al. 2004) and recent functional MRI studies (e.g., Dolcos et al. 2004; Strange and Dolan 2004) further reported a modulating role for hippocampus in processing of emotional memory interacting with amygdala. The current human in vivo finding is consistent with these suggestions. The present study identified possible involvement of the hippocampus in trait anxiety even at the brain structural level, a static trait marker, in healthy young human individuals.

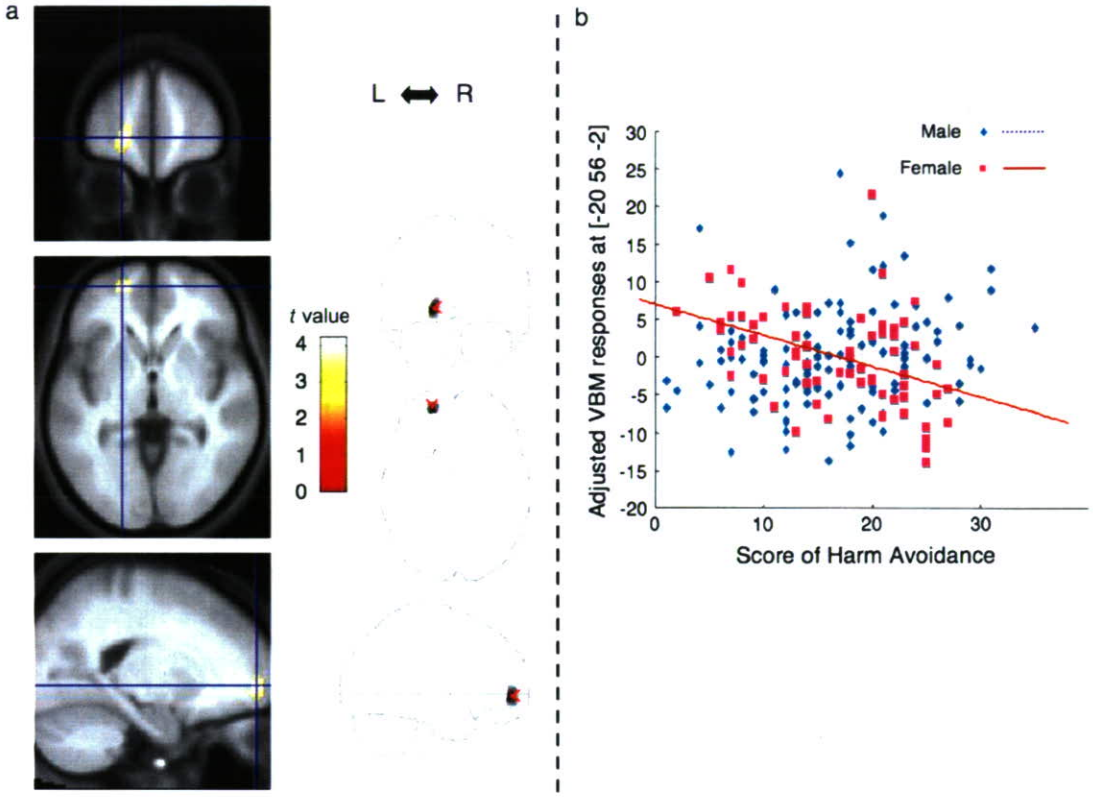

Figure 2. Female-specific negative correlation between HA and regional gray matter volume in the left anterior prefrontal cortex. (*a*) The gray matter regions where interaction between gender and HA was found are rendered in the Montreal Neurological Institute space. (Right) Statistical parametric map in the 3 orthogonal projections shows voxels where interaction between gender and HA emerged. (Left) The gray matter voxels showing interactions between gender and the individual variability of HA were rendered onto the averaged images of the whole sample ($N = 183$) (voxel threshold: uncorrected $P < 0.001$). (*b*) Scatter plots depicting correlations between regional gray matter volume at the peak voxel [−20, 56, −2] and individual variability in HA in females ($N = 66$) and males ($N = 117$).

Moreover, the present study revealed a significant female-specific association between HA and regional volume in the left anterior prefrontal cortex. Previous postmortem and brain activation studies have reported a similar location to that in the current study, anterior prefrontal as well as frontopolar cortex, as a neural substrate of emotional modulation, anxiety, and depression. A previous postmortem brain study reported a significant decrease in growth-associated protein levels and related mRNA expression in anterior prefrontal cortex of suicide brains compared with controls (Hrdina et al. 1998). In addition, Merali et al. (2004) reported that corticotropin-releasing hormone (CRH) levels were elevated in frontopolar and dorsomedial prefrontal cortex of suicide victims relative to the comparison group. Conversely, using quantitative polymerase chain reaction analyses, it was observed that mRNA for CRH1 receptors was reduced in frontopolar cortex of suicide brains. The same research group (Merali et al. 2006) further reported that immunoreactivity levels of CRH among brains of suicides were elevated in several brain regions including frontopolar cortex. Using single-photon emission-computed tomography, Segawa et al. (2006) reported that an improvement of depressive symptom severity due to electroconvulsive therapy showed a correlation with the change in regional cerebral blood flow of left frontopolar cortex. Papousek and Schulter (2002) reported that a frontopolar activation revealed by electroencephalography is related to emotional modulation. Urry et al. (2006) also revealed an activation of anterior prefrontal cortex (Brodmann's area 10) using functional MRI, which was associated with regulation of negative affect. Of note, Wright et al. (2006) recently found an inverse correlation between neuroticism scores and cortical thickness of the anterior portion of the left orbitofrontal cortex in a location close to that of the current finding. Moreover, related to sex dimorphism, a recent lesion study reported a gender difference in the role of the anterior portion of ventromedial prefrontal cortex in emotional and social dysfunction (Tranel et al. 2005). In addition, it was further reported that both ongoing self evaluation of emotional experience and subsequent memory performance for the highly emotionally arousing pictures showed correlations with activations in more extensive brain regions including anterior cingulate cortex of women than in those of men (Canli et al. 2002). They suggested that greater overlap in brain regions sensitive to current emotion and contributing to subsequent memory may be a neural mechanism for emotions to enhance memory more powerfully in women than in men. The present study supports this notion and further extends these gender differences in emotional processing at the brain structural level. Individual differences in emotional modulation in females are more likely to reflect individual differences at the level of brain structure. The present findings at least partially explain individual and gender differences in the susceptibility to develop anxiety and depressive disorders. Female individuals with smaller anterior prefrontal cortex as well as higher anxiety-related