Brain Derived Neurotrophic Factor Val66Met Polymorphism, the Five Factor Model of Personality and Hippocampal Volume: Implications for Depressive Illness

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Abstract: Altered hippocampal volume, the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism, and neuroticism have each been implicated in the etiology of psychiatric disorders, especially depression. However, the relationship between these variables is not well understood. Here, we determined the effects of the BDNF Val66met polymorphism on the five-factor personality dimensions (assessed using the NEO-FFI), trait depression (assessed with the DASS-21) in a cross-sectional cohort of 467 healthy volunteers. A large matched subset of this cohort was also assessed for grey matter volume of the hippocampus and contiguous temporal cortical regions using magnetic resonance imaging. In Met carriers, elevations in neuroticism and trait depression and stress were associated with lower *mean* hippocampal volume, but there were no such associations in Val homozygotes. Trait depression, in particular, was found to moderate the effects of BDNF genotypes on hippocampal volume. Met carriers with high trait depression showed a reduction in grey matter volume of the *mean* hippocampus compared with Val homozygotes. These findings suggest that even in otherwise healthy subjects, trait depression may contribute to the susceptibility of Met carriers to hippocampal grey matter loss. *Hum Brain Mapp* 30:1246–1256, 2009. ©2008 Wiley-Liss, Inc.

Key words: brain derived neurotrophic factor Val66Met polymorphism; BDNF; hippocampus; neuroticism; five factor model; depression

Contract grant sponsor: ARC-linkage; Contract grant number: LP0455104; Contract grant sponsor: European Molecular Biology Organization; Contract grant number: ALTF 166-2004; Contract grant sponsor: Pfizer foundation; Contract grant sponsor: NHMRC; Contract grant numbers: No. 157209, No. 358770.

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Received for publication 28 October 2007; Revised 26 February 2008; Accepted 20 March 2008

DOI: 10.1002/hbm.20592

Published online 11 June 2008 in Wiley InterScience (www.interscience.wiley.com).

INTRODUCTION

Current theories on the etiology of major depression include a focus on stress-related reductions in hippocampal volume which contribute to the alterations in emotional and cognitive processes that characterize its symptoms [McEwen, 2005; Williams, 2006]. These theories provide a framework for examining trait risk factors for depression in otherwise healthy subjects. Neuroticism is a personality trait which reflects stress sensitivity and emotional instability, and shares up to 50% of the genetic risk for major depression [Kendler et al., 1993]. While the evidence remains insufficient, the Brain Derived Neurotrophic Factor (BDNF) Val66Met polymorphism has been implicated in higher trait depression and neuroticism [Gatt et al., 2007; Sen et al., 2003], as well as reductions in hippocampal volume [Bueller et al., 2006; Nemoto et al., 2006; Pezawas et al., 2004; Schofield et al., 2008; Szeszko et al., 2005]. While these relationships suggest a role for BDNF Val66Met in the etiology of depression, associations between this polymorphism, trait depression, neuroticism, and hippocampal grey matter have not yet been examined in the same subjects.

Evidence for the importance of stress-related mechanisms in the development of depression comes from both behavioral measures of personality, and from neuroimaging and molecular measures of hippocampal structure and function. In terms of personality, neuroticism is a highly heritable (40–50%) and stable trait which contributes substantially to genetic risk for depression. Indeed, high neuroticism scores are one of the most powerful predictors of future onset of depression [Jang et al., 1996; Kendler et al., 1994; Viken et al., 1994]. Neuroticism is the most extensively studied of the core dimensions in the five factor model of personality, which also comprises replicated dimensions of extraversion, openness to experience, agreeableness, and conscientiousness [Costa and McCrae, 1992].

In terms of biological measures, a number of studies have reported a reduction in hippocampal volume in patients with major depression, although there are also some null findings [Videbech and Ravnkilde, 2004 for review]. These reductions are consistent with theories that propose that the development of depression is at least in part due to stress-induced reductions in hippocampal neurogenesis [McEwen, 2005].

Brain derived neurotrophic factor (BDNF) has been implicated in the process of hippocampal neurogenesis and in neuroticism, and thus may moderate the stress-related mechanisms of depression. BDNF is a neurotrophin expressed widely in the mature brain, particularly in the hippocampus and prefrontal cortex [Lewin and Barde, 1996]. It plays an important role in neuronal function [Baquet et al., 2004; Gorski et al., 2003; Huang et al., 1999]. BDNF Val66Met is a single nucleotide polymorphism produced by an amino acid substitution of valine for methionine at codon 66 in the BDNF gene. It has been found to alter hippocampal function and memory processes [Bueller

et al., 2006; Egan et al., 2003; Hariri et al., 2003; Nemoto et al., 2006; Pezawas et al., 2004; Szeszko et al., 2005]. Thus, while there remains insufficient data for a direct link between BDNF variation and depression, indirect effects may occur via hippocampal mechanisms [Nakata et al., 2003; Neves-Pereira et al., 2002; Sklar et al., 2002].

The BDNF Met allele has been found to make a small but significant contribution to neuroticism in healthy individuals in one study to date [Sen et al., 2003]. While others have revealed null results [Lang et al., 2005; Willis-Owen et al., 2005] and contrary findings for the interaction of the BDNF Met allele and a dopamine transporter variant [Hunneke et al., 2007], BDNF effects on neuroticism may occur via effects on hippocampal systems.

A reduction in hippocampal grey matter has been associated with both the Met allele of the BDNF Val66Met polymorphism and with depression. Using magnetic resonance imaging (MRI), BDNF Met carriers have shown reductions in volume of the hippocampus [Bueller et al., 2006; Nemoto et al., 2006; Pezawas et al., 2004; Schofield et al., 2008; Szeszko et al., 2005] and the interconnected dorsolateral prefrontal cortex [Szeszko et al., 2005]. The BDNF Met allele has also been linked to higher trait depression via its effects on neural activity [Gatt et al., 2007]. These human neuroimaging findings are consistent with the role of this allele in hippocampal neurogenesis, although we note that this implication can currently only be tested directly in animal models. With this caveat in mind, associations between the BDNF Met allele and hippocampal shrinkage are consistent with theories of depression in which a mutual cycle of excessive stress (cytokine production and overactivity of the hypothalamic-pituitary-adrenal, HPA axis) may reduce both hippocampal neurogenesis and BDNF production. In turn, BDNF reduction may contribute to further hippocampal shrinkage and HPA overactivity [Duman, 2004; Duman et al., 2000]. Notably, BDNF levels may be normalized with antidepressants [Gonul et al., 2003; Karege et al., 2002]. Antidepressants also increase neurogenesis, which may be their common mechanism of action in treating depression [Duman, 2004].

While the above findings point to a role for neuroticism and hippocampal gray matter in susceptibility for depression, the role of the BDNF Met allele in these relationships remains unresolved. Here, we determined whether the BDNF Val66Met genotypes show distinctive associations between hippocampal grey matter and trait neuroticism and depression in healthy volunteers. Examining degrees of depression within a nonclinical population is consistent with the dimensional nature of depression, and enables observation of the associations between factors involved in the underlying predisposition for depression without confounds of clinical state (e.g., medication and chronicity) [Hankin et al., 2005; Slade and Andrews, 2005].

It was predicted that the BDNF Met allele would be associated with higher neuroticism and depression, and reduced hippocampal grey matter. In turn, reductions in

grey matter were expected to be most apparent for BDNF Met individuals with higher neuroticism and depression. The BDNF Met allele has also been associated with decreased grey matter in temporal cortical regions contiguous with the hippocampus in other conditions such as schizophrenia [Ho et al., 2006]. Thus, to provide a broader context for interpretation, we also examine contributions from temporal cortical regions contiguous to the hippocampus, and the other five factor personality dimensions, in relation to BDNF genotypes.

METHODS

Participants

The sample comprised 467 non-clinical caucasian subjects of European ethnicity (243 males, 224 females, mean age 36.8 ± 13.1 years) who participated in the Brain Resource International Database (BRID) (www.brainresource.com) [Gordon et al., 2005; Gordon, 2003]. Exclusion criteria were determined using the BRID personal history and screening assessments, which include the SPHERES [Hickie et al., 1998] and Patient Health Questionnaire (PHQ9) [Kroenke and Spitzer, 2002] to screen for symptoms of Axis-I disorder, the AUDIT (Alcohol Use Disorders Identification Test of the WHO) and Fagerstrom Tobacco Dependency Questionnaire, and items to screen for family history of psychiatric disorder (defined in terms of severity requiring medication and/or hospitalization), physical brain injury (causing loss of consciousness for 10 min or more), neurological disorder or other serious medical or genetic condition. All subjects were right-handed.

Written informed consent was obtained from all participants, according to human research ethical requirements.

Behavioral Measures

Participants completed the NEO-Five Factor Personality Inventory (NEO-FFI) [Costa and McCrae, 1992] which provides measures of the dimensions of neuroticism, extraversion, openness, agreeableness, and conscientiousness.

Trait depression and associated traits of anxiety and stress were assessed using the DASS-21, a shortened version of the Depression Anxiety Stress Scale (DASS-42) [Lovibond and Lovibond, 1995]. DASS Depression items assess symptoms typically associated with dysphoric mood, the Anxiety items capture specific symptoms of anxiety (such as physical arousal, panic attacks and fear), and the Stress items assess symptoms of generalized arousal (such as tension, irritability, and tendency to over-react to stressful situations). The DASS measure was developed to assess trait affect in both non-clinical and clinical populations, and has been validated against other commonly used measures such as the Beck Depression Inventory [Beck et al., 1961] and Beck Anxiety Inventory [Beck et al., 1988]. Established norms include Australian, US, and UK populations [Antony et al., 1998; Crawford and

Henry, 2003; Henry and Crawford, 2005; Lovibond and Lovibond, 1995]. Total scores for each DASS-21 subscale were doubled for comparison with DASS-42 norms, and raw scores were used for all analyses. Scores are categorized as follows: *Depression*, Normal 0–9, Mild 10–12, Moderate 14–20, Severe 21–27, and Extremely Severe 29+; *Anxiety*, Normal 0–7, Mild 8–9, Moderate 10–14, Severe 15–19, Extremely severe 20+; *Stress*, Normal 0–14, Mild 15–18, Moderate 19–25, Severe 26–33, Extremely severe 34+.

BDNF Genotypes

The BDNF Val66Met genotypes were determined by PCR amplification techniques followed by restriction digestion as previously described for the larger BRID cohort [Schofield et al., 2008]. DNA was extracted from cheek swab samples by a standard proteinase digestion and chloroform extraction procedure. PCR amplification of participant DNA was undertaken using primers 5' GTAT TCCTCCAGCAGAAAGAGAA 3' and 5' AAAGAAGCAA ACATCCGAGGAC 3' using standard conditions. The amplified fragment was digested with the restriction enzyme *Afl*III, which cleaves the Val allele and includes a positive digestion control in the PCR amplicon. PCR products were separated on 4% agarose gels. We have confirmed that the genotype frequencies are in Hardy-Weinberg equilibrium for this cohort ($\chi^2 = 2.59$, $P = 0.108$). There were 269 Val/Val, 179 Val/Met, and 19 Met/Met subjects in the sample. The Met/Met subjects were included with the Val/Met group (i.e., "Met carriers") consistent with previous studies [Gatt et al., 2007; Hariri et al., 2003], to maximize the equivalence of genotype group sizes for statistical analyses. Met carrier and Val/Val genotype groups did not differ in age ($t = 0.17$, $P = 0.87$), sex distribution ($\chi^2 = 0.31$, $P = 0.58$), or years of education ($t = -0.89$, $P = 0.38$).

Structural MRI

A subset of 113 subjects also underwent a structural MRI performed on a 1.5T Siemens Vision Plus scanner. This subset comprised 68 Val/Val and 45 Met carriers (43 Val/Met and 2 Met/Met genotypes), together which did not differ from each other in terms of global grey matter volume ($t = -1.39$, $P = 0.17$). A high resolution structural image was taken for each subject using a single T1 weighted volumetric MPRAGE sequence. Images were obtained in the sagittal plane, with scan parameters: TR = 9.7 ms, TE = 4 ms, TI = 200 ms, flip angle = 12° . A total of 180 contiguous 1 mm slices were acquired with a 256×256 matrix with an in plane resolution of $1 \text{ mm} \times 1 \text{ mm}$, resulting in isotropic voxels. The cross-site reliability of MR images acquired using this sequence has been established [Grieve et al., 2005].

Segmentation and spatial normalization of MRI data was performed using voxel based morphometry (VBM) in SPM2 (<http://www.fil.ion.ucl.ac.uk/spm/>) [Ashburner and

Friston, 2000]. SPM2 was used due to the availability of the toolbox for Automated Anatomical Labelling (AAL) [Tzourio-Mazoyer et al., 2002]. Images were smoothed using a FWHM 12 mm kernel, before volume calculation. Spatial normalization was undertaken by transforming each brain to a standardized stereotactic space based on the ICBM 152 template (Montreal Neurological Institute). Normalized images were resliced with $1.5 \times 1.5 \times 1.5$ mm voxels, then segmented into grey, white, CSF and non-brain portions based on a cluster analysis method to separate pixels based on intensity differences, together with *a priori* knowledge of spatial tissue distribution patterns in normal subjects. Customized templates created from the BRID subjects were used for normalization and segmentation processes [Grieve et al., 2005]. A correction was made to preserve quantitative tissue volumes following normalization procedure [Ashburner and Friston, 2000]. Grey matter volume was then generated for *a priori* regions of interest (ROIs), defined by standardized masks based on the neuroanatomical divisions of the Automated Anatomical Labelling (AAL) protocol [Tzourio-Mazoyer et al., 2002]. This automated masking procedure has previously been validated using manual tracing regions [Whitford et al., 2005]. Based on previous voxel-based findings for a reduction in both left and right hippocampus in BDNF Met carriers, extending across anterior and posterior regions [Schofield et al., 2008], the focal ROI was the hippocampus. To determine the specificity of findings to the hippocampus, we also included the parahippocampal gyrus, and contiguous temporal regions: superior, middle, and inferior temporal gyri.

Statistical Analyses

ANOVA models were first used to examine the effects of BDNF genotype on the dependent measures. Univariate ANOVA was used to examine BDNF genotype (Val/Val vs. Met carriers) differences in NEO-FFI personality dimensions and DASS-21 trait depression, anxiety and stress, with age and sex as covariates. Mixed design, repeated measures ANOVAs were then used to test the between-subjects effect of BDNF genotype on grey matter volume, with repeated measures factors of hippocampus (left, right), parahippocampus (left, right), superior temporal gyrus (left, right), superior temporal gyrus and superior temporal pole, middle temporal gyrus (left, right), middle temporal gyrus and middle temporal pole, and inferior temporal gyrus (left, right). Age and sex were included as covariates.

Correlation analyses were then used to determine the associations between NEO-FFI personality dimensions and DASS-21 depression, anxiety and stress, and grey matter volume, within the BDNF Val/Val and Met carrier groups. These correlations were undertaken for the mean grey matter volume for each of the five ROIs which represented within-subjects factors in the above ANOVAs (hippocampus, parahippocampus, and superior, middle and inferior temporal gyri).

Third, hierarchical multiple regression analyses were used to examine whether the effect of BDNF genotype on grey matter for ROIs depends on the level of NEO-FFI personality and DASS-21 measures. These regression analyses focused on NEO-FFI and DASS-21 measures which contributed to significant associations with grey matter in correlation analyses, and that were specific to one or the other genotype. Two regression models were tested for each ROI: the first examining the main effects of BDNF genotype (Val/Val and Met carriers) and NEO-FFI or DASS-21 measures, and the second examining the interaction of BDNF genotype and these measures. Age and sex were again covariates in these analyses.

Given the *a priori* predictions for the study significant effects for neuroticism, depression, and hippocampal grey matter measures were determined at an alpha level of 0.05. To provide a broader context for interpretation at a comparative threshold, an alpha level of 0.05 was also used to examine the contributions from the other measures of personality and grey matter volume in contiguous ROIs. Nonetheless, these additional results were interpreted with caution given their relatively exploratory nature.

RESULTS

Between-Group Effects of BDNF Genotype

No significant main effects of BDNF genotype group were evident for the NEO-FFI personality dimensions, or DASS-21 depression, anxiety, and stress measures (Table I).

Similarly, BDNF Met carrier versus Val/Val genotype groups did not differ significantly on *mean total* grey matter volume for hippocampal and related temporal cortical ROIs (Table I)¹, nor were there any BDNF genotype by region interactions.

Within-Group Correlations for Each BDNF Genotype

For BDNF Met carriers, higher NEO-FFI neuroticism was associated with higher DASS-21 Depression, Anxiety, and Stress (Table II), consistent with the view that neuroticism shares genetic risk for depressive symptoms. In this group, lower extraversion (or higher introversion) was also associated with higher scores on each DASS-21 measure (Table II), consistent with the social withdrawal features of depression.

In the BDNF Val/Val group, higher neuroticism showed a similar pattern of associations with higher Depression, Anxiety and Stress (Table II). In addition, lower agreeableness was correlated with higher Depression and Anxiety, and higher openness with higher stress scores (Table II). Thus, negative affect may be associated with a distinctive

¹BDNF genotype groups also did not differ on total cortical grey matter volume ($t = -1.39$, $P = 0.17$).

TABLE I. Means ($\pm$ standard error, SE) and summary of ANOVA results for BDNF genotype group differences on NEO-FFI personality dimensions, DASS-21 depression, anxiety and stress measures, and total grey matter volume for hippocampal and contiguous temporal cortical regions

	BDNF Val/Val	BDNF Met carriers	F	P
NEO-FFI				
Neuroticism	19.09 $\pm$ 0.47	17.91 $\pm$ 0.56	2.60	0.11
Extraversion	29.49 $\pm$ 0.39	30.00 $\pm$ 0.46	0.71	0.40
Openness	31.67 $\pm$ 0.40	30.84 $\pm$ 0.48	1.75	0.19
Agreeableness	31.52 $\pm$ 0.33	31.83 $\pm$ 0.40	0.35	0.56
Conscientiousness	31.25 $\pm$ 0.43	32.23 $\pm$ 0.51	2.19	0.14
DASS-21				
Depression	4.51 $\pm$ 0.34	3.81 $\pm$ 0.40	1.82	0.18
Anxiety	2.16 $\pm$ 0.21	1.95 $\pm$ 0.24	0.44	0.51
Stress	6.10 $\pm$ 0.38	5.22 $\pm$ 0.44	2.33	0.13
Grey matter volume (ml)				
Hippocampus	3133.25 $\pm$ 36.55	3184.73 $\pm$ 45.04	0.78	0.38
Parahippocampal gyrus	3742.46 $\pm$ 39.09	3795.98 $\pm$ 48.18	0.74	0.39
Superior temporal gyrus	6540.14 $\pm$ 81.69	6598.00 $\pm$ 100.68	0.20	0.66
Middle temporal gyrus	9266.57 $\pm$ 103.26	9355.44 $\pm$ 127.26	0.29	0.59
Inferior temporal gyrus	11280.69 $\pm$ 117.13	11484.86 $\pm$ 144.35	1.19	0.28

Note. NEO-FFI (df = 1,397), DASS-21 (df = 1,462), brain volume (df = 1,109). Differences on the NEO-FFI and DASS-21 were examined using univariate ANOVA, and repeated-measures was used for differences in terms of brain region, with age and gender included as covariates.

profile of personality traits in the Val/Val compared with Met carrier group.

In BDNF Met carriers, higher NEO-FFI neuroticism and DASS-21 depression and stress scores were also significantly associated with a reduction in *mean total* hippocampal grey matter *volume* (Table III). By contrast, no such associations were present in the Val/Val group (Table III). Figure 1 depicts the distinctive associations between

hippocampal volume and neuroticism, depression and stress in Met carriers versus the Val/Val group.

In addition, genotype groups showed a distinctive pattern of associations between other NEO-FFI personality dimensions and grey matter of temporal cortical regions. In Met carriers, higher DASS-21 stress was also associated with a reduction in *total volume* of the middle temporal gyrus, while higher NEO-FFI agreeableness was correlated with a reduction in *total volume* of the parahippocampal and superior temporal gyri (Table III). By contrast, in the Val/Val group, lower NEO-FFI openness was associated with reduced *mean total volume* of the parahippocampal, superior and middle temporal gyri, and higher agreeableness with reduced *mean total volume* of superior, middle, and inferior temporal gyri (Table III).

TABLE II. Bivariate correlation coefficients for associations between the NEO-FFI personality dimensions and DASS-21 depression, anxiety and stress measures within BDNF Val/Val and Met carrier groups

NEO-FFI	DASS-21		
	Depression	Anxiety	Stress
BDNF Val/Val			
Neuroticism	0.44*	0.31**	0.40***
Extraversion	-0.06	-0.01	0.10
Openness	0.20	0.18	0.27**
Agreeableness	-0.32**	-0.29**	-0.23
Conscientiousness	-0.07	-0.16	-0.12
BDNF Met carriers			
Neuroticism	0.44***	0.50***	0.42***
Extraversion	-0.40***	-0.47***	-0.33**
Openness	0.09	0.11	0.06
Agreeableness	-0.16	-0.07	-0.19
Conscientiousness	-0.09	-0.10	0.01

Note. N = 105 (Val/Val: n = 63; Met carriers: n = 42). Bolding indicates significant effects; *P < 0.001, **P < 0.05, ***P < 0.01.

Regression Analyses: Role of Personality and Depression in BDNF Genotype Effects on Grey Matter

Regression analyses focused on the NEO-FFI personality dimensions of neuroticism and openness, and DASS-21 measures of depression and stress which showed differential associations with grey matter for the BDNF genotypes (Tables II, III).

Personality Dimensions

BDNF genotype, examined as a main effect, did not predict grey matter volume for the ROIs when the contribution of Neuroticism was taken into account, consistent with the null effects evident in the repeated-measures

TABLE III. Bivariate correlation coefficients for associations between the NEO-FFI personality dimensions, DASS-21 depression, anxiety and stress measures, and mean grey matter for hippocampal and contiguous temporal cortical regions within BDNF Val/Val and Met carrier groups

NEO-FFI & DASS-21	Brain region (total grey matter volume)				
	Hippocampus	PHG	Superior temporal	Middle temporal	Inferior temporal
BDNF Val/Val					
NEO-FFI					
Neuroticism	-0.17	-0.18	-0.10	-0.08	-0.12
Extraversion	0.07	0.06	0.08	0.06	0.01
Openness	0.19	0.25*	0.27*	0.28*	0.23
Agreeableness	-0.24	-0.20	-0.29*	-0.29*	-0.30*
Conscientiousness	-0.11	-0.21	-0.13	-0.18	-0.20
DASS-21					
Depression	0.12	0.08	0.15	0.10	0.05
Anxiety	0.11	0.08	0.16	0.16	0.18
Stress	-0.12	-0.13	-0.04	-0.05	-0.08
BDNF Met carriers					
NEO-FFI					
Neuroticism	-0.31*	-0.27	-0.08	-0.04	-0.02
Extraversion	0.09	0.07	-0.02	-0.06	-0.09
Openness	0.00	0.06	0.18	0.17	0.24
Agreeableness	-0.28	-0.31*	-0.31*	-0.30	-0.42
Conscientiousness	0.04	0.07	-0.12	-0.16	-0.14
DASS-21					
Depression	-0.33*	-0.29	-0.17	-0.17	-0.18
Anxiety	-0.13	-0.04	-0.11	-0.12	-0.13
Stress	-0.33*	-0.28	-0.29	-0.32*	-0.26

Note. $N = 105$ (Val/Val: $n = 63$; Met carriers: $n = 42$). Bolding indicates significant effects: $*P < 0.05$. PHG = Parahippocampal gyrus.

ANOVAs. There was also no significant effect on ROI grey matter for the interaction of BDNF genotype and neuroticism.

Correspondingly, there were no significant effects for the contribution of openness as a main effect or interaction with BDNF genotype on ROI grey matter volume.

Depression and Stress

Similarly, BDNF genotype examined as a main effect, did not predict ROI grey matter when the contributions of depression and stress were taken into account, again consistent with repeated measures ANOVAs.

While there was also no significant effect on ROI grey matter for the interaction of BDNF genotype and stress, significant effects were revealed for the interaction of BDNF genotype and depression.

The interaction of BDNF genotype and level of trait depression was found to significantly predict reductions in grey matter volume for the left ($B = -21.81$, $P = 0.042$, $R^2 = 34.5\%$, $\Delta R^2 = 2.6\%$) and right ($B = -20.43$, $P = 0.044$, $R^2 = 34.9\%$, $\Delta R^2 = 2.5\%$) hippocampus. In each case, the reduction in hippocampal grey matter volume was predicted by BDNF Met carriers coupled with higher Depression. Figure 2 depicts this increasing reduction in hippocampal grey matter across DASS-21 categories of increasingly higher depression (see Fig. 2).

DISCUSSION

This study examined relationships between the BDNF Val66Met polymorphism, five factor personality dimensions, in particular neuroticism, symptoms of depression and grey matter of the hippocampus and contiguous temporal cortical regions, in a cohort of healthy volunteers. In BDNF Met carriers, lower *total* hippocampal grey matter volume was associated with higher neuroticism and depression, as well as related stress symptoms. These specific relationships were not present in BDNF Val/Val homozygotes. While there were no differences in hippocampal grey matter between BDNF Met carriers and Val/Val homozygotes considered as a total group, BDNF Met status predicted a reduction in *total* hippocampal volume for those Met carriers who also had a higher level of depression, rated as moderate to extremely severe. These findings suggest that otherwise healthy Met carriers who also have elevated depression may be vulnerable to hippocampal grey matter loss, while Val/Val homozygotes may be resistant to such loss even in the presence of higher depression. They have implications for elucidating risk factors involved in the etiology of major depressive disorder which implicates reductions in hippocampal neurogenesis.

In this study, Met carriers were not found to have an elevation in neuroticism or depressive symptoms in direct comparison with the Val/Val genotype group. A null effect for neuroticism has been observed in previous stud-

Figure 1.

Scatter-plots of associations between *total* hippocampal volume (mL) and behavioral measures, NEO-FFI neuroticism and DASS-21 depression and stress, within BDNF Met carriers and Val/Val homozygotes.

ies of the BDNF polymorphism and neuroticism in healthy volunteers [Lang et al., 2005; Willis-Owen et al., 2005], although a small but significant elevation of neuroticism in Met carriers has also been reported [Sen et al., 2003]. Within both BDNF Met carrier and Val/Val groups, higher neuroticism was also associated with higher trait depression and related symptoms of anxiety and stress, consistent with the shared genetic risk between these dimensions [Kendler et al., 1993, 1994]. We found a particularly strong correlation between depression scores and neuroticism with 7 of the twelve correlations between the DASS and NEO at the $P < 0.01$ level of significance (see Table II). We acknowledge that we have examined a trait measure of stress related temperament (neuroticism) plus a symptom based assessment of stress-related depression classified

according to a tripartite model of anhedonia (depression), generalized distress (stress) and specific psychosomatic symptoms of anxiety (anxiety). Future research should include more specific measures of experienced stress and its relationship to the symptom and trait measures as well as the biological measures examined in our study.

The distinctive loss in total hippocampal volume in BDNF Met carriers who also had elevated levels of trait depression (albeit not meeting diagnostic criteria) suggests that these individuals may be susceptible to the biological processes underlying major depressive disorder in a way that Val/Val homozygotes are not. Consistent with this proposal, we also observed specific associations between lower mean hippocampal grey matter and higher trait depression and stress, as well as neuroticism, in Met

Figure 2.

Line graphs depicting estimated marginal means from the multiple regression analysis, in which the interaction of BDNF Val66Met genotype status and DASS-21 level of depression predicted grey matter volume for the left and right hippocampus. BDNF Met carriers (grey line) with higher depression scores showed reduced hippocampal volume compared with Val/Val genotypes (black line) with similarly high depression scores.

carriers but not in the Val/Val group. These findings accord with evidence of grey matter volume reductions in the hippocampus in major depressive disorder [Videbech and Ravnkilde, 2004 for review], evidence implicating the Met allele in this disorder [Hwang et al., 2006; Jiang et al., 2005], and reports of an association between BDNF Met status and hippocampal grey matter reductions [Bueller et al., 2006; Nemoto et al., 2006; Pezawas et al., 2004; Schofield et al., 2008; Szeszko et al., 2005].

BDNF genotype groups also showed distinctive patterns of association between depressive traits and other dimensions of personality. For instance, in Met carriers, higher trait depression was also related to lower extraversion (or higher introversion), which may reflect associated traits of social withdrawal that go hand in hand with depressive symptoms. In addition, higher stress symptoms in Met carriers were related to a reduction in *total* grey matter of the middle temporal gyrus, which may point to further grey matter loss in regions contiguous with the hippocampus with the comorbid stress that accompanies depression. By contrast, in Val/Val homozygotes, lower levels of the NEO-FFI dimension of openness were associated with less *total* grey matter in parahippocampal, superior and middle temporal gyri, and higher agreeableness with lower volume of superior, middle, and inferior temporal gyri, suggesting that this genotype may contribute to grey matter variation in relation to different aspects of personality than those impacted by the Met allele.

The current findings are consistent with research that implicates hippocampal neurotoxicity and stress in the predisposition to depression [Belmaker and Agam, 2008; Lange and Irle, 2004]. It is possible that the BDNF Met allele predicts particularly severe forms of depression such as melancholic depression [Van Praag, 1987], consistent with the higher heritability of severe or recurrent sub-

types [Kendler et al., 1999], and the reduced hippocampal volumes found in more chronic cases [MacQueen et al., 2003; Sheline et al., 1999]. Melancholic depression is characterized by anhedonia, lack of mood reactivity to pleasurable stimuli, and somatic/psychomotor disturbances (e.g., weight loss, early morning awakening and psychomotor retardation); whereas the less severe nonmelancholic or atypical depression subtype is characterized by mood reactivity and positivity, increased appetite and excessive sleep, interpersonal rejection sensitivity, and often lifetime panic disorder or social phobia [Parker et al., 2002]. There is potential for these depression subgroups to be further characterized by additional gene by gene, or gene by environment interactions. For instance, BDNF and HTT are genes known to interact at multiple cellular levels [Malberg et al., 2000] and have been shown to interact together with early life stress to predict elevated depression symptoms in maltreated children [Kaufman et al., 2006]. Nonetheless, how these genetic and environmental factors interact to predict specific depression subtypes in adults remains to be determined. Further research is needed to validate specific subtypes of depression, which would facilitate prediction of individual treatment response.

Taken together, the present findings provide support for the view that the BDNF Val66Met polymorphism may be a factor in the reduced hippocampal neurogenesis associated with risk for depression [Jacobson and Sapolsky, 1991; Sapolsky, 2000a,b]. The deleterious impact of the BDNF Met allele on alterations in hippocampal volume may be apparent in otherwise healthy individuals who manifest a higher than normal level of trait depression. Nonetheless, it is important to exercise caution in extrapolating the findings to the aetiological processes of major depressive illness. This study was carried out in healthy volunteers

with no personal or family history of psychiatric illness, and BDNF genotype groups did not differ directly on measures of trait depression and neuroticism. It would therefore be important to demonstrate the relationships between BDNF Met status and grey matter loss in patients with diagnosed depressive illness who have extreme levels of depressive symptomatology. Moreover, while it is arguable that the results may have been biased by population stratification, there is growing recognition that this problem is generally quite minor or irrelevant for most association studies [Cardon and Palmer, 2003]. Further research into relationships between BDNF Val66Met genotypes, hippocampal and temporal cortical grey matter, and depression and associated risk factors such as neuroticism in both normative and patient samples has the potential to elucidate the gene-behavior mechanisms of major depression. Given that BDNF and hippocampal neurogenesis are also involved in antidepressant action [Duman et al., 2000; Gonul et al., 2003; Karege et al., 2002] this research may also help establish an evidence base for evaluating treatment effects and the factors which determine individual treatment response.

ACKNOWLEDGMENTS

We acknowledge BRC's in-kind support in providing access to the Brain Resource International Database (BRID). Access to the BRID is administered via a scientific network independent of BRC's operations, known as BRAINnet (<http://www.brainnet.net>). *Financial disclosure:* The Brain Resource Company (BRC) was the industry partner on the ARC-linkage grant which funded this study, but had no further role in design or implementation of the project. Partnership involved a cash contribution for a research assistant position and in-kind support from the Brain Resource International Database. EG is the CEO and Chairman of the BRC, and holds significant equity and stock options in the company. Professor Schofield holds stock options in the BRC. L.M.W. is a small equity holder in BRC. J.M.G. and S.K. are employed as postdoctoral researchers on the ARC-linkage grant which funded this study. A.H.K., R.H.P., R.J., and C.D.S. have no conflicts of interest for this study. L.M.W., R.H.P., and A.H.K. have received fees from BRC for research development and coordination unrelated to the study.

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