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No effect of 5HTTLPR or BDNF Val66Met polymorphism on hippocampal morphology in major depression

J. Cole^{*,†}, D. R. Weinberger[‡], V. S. Mattay[‡], X. Cheng[‡], A. W. Toga[§], P. M. Thompson[§], G. Powell-Smith[†], S. Cohen-Woods[†], A. Simmons^{¶,**,††}, P. McGuffin^{†,¶}, and C. H. Y. Fu^{†,¶}

[†]MRC Social, Genetic & Developmental Psychiatry Research Centre, Institute of Psychiatry, King's College London, London, UK

[‡]Clinical Brain Disorders Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA

[§]Laboratory of Neuro Imaging, Department of Neurology, University of California, Los Angeles, CA, USA

[¶]NIHR Biomedical Research Centre for Mental Health at South London and Maudsley NHS Foundation Trust, King's College London, London, UK

^{**}MRC Centre for Neurodegeneration Research, King's College London, London, UK

^{††}Department of Neuroimaging, Institute of Psychiatry, King's College London, London, UK

Abstract

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*Corresponding author: J. Cole, MRC Social Genetic & Developmental Research Centre, Institute of Psychiatry, King's College London, PO Box 80, London SE5 8AF, UK. james.h.cole@kcl.ac.uk.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1: Screenshot from MULTITRACER image analysis software. Software using for manual tracing was MULTITRACER (Woods, 2003). Magnetic resonance imaging data is displayed with the tracing orientation (coronal) in the main window. Lines in yellow and red represent the manual segmentation of the right and left hippocampus, respectively, in the given plane. Reference images in sagittal and coronal orientation are also shown, with yellow points representing the location where coronal traces intersect the other planes.

Figure S2: Hippocampal shape maps of 5HTTLPR and BDNF Val66Met genotypic comparisons in healthy individuals. (A) Superior view and (B) inferior view of 5HTTLPR comparison maps, regions in red–yellow (positive *P*-values) indicate points where L/L genotype showed increased surface distance measures and areas in pink–white (negative *P*-values) indicate reductions in L/L subjects compared with short allele carriers. (C) Superior and (D) inferior views of BDNF Val66Met maps, red–yellow points: Val/Val genotype showed increased surface distance measures. Pink–white: reductions in Val/Val subjects compared with Met allele carriers. (E) Superior view of reference maps labelled with hippocampal surface subfields. (F) Corresponding inferior view of reference maps. Superior and inferior view maps are presented with the right hippocampus on the left-hand side and vice versa.

Figure S3: Hippocampal shape maps of 5HTTLPR and IQ-matched BDNF Val66Met genotypic comparisons in recurrent MDD patients. (A) Superior view and (B) inferior view of 5HTTLPR comparison maps, regions in red–yellow (positive *P*-values) indicate points where L_A/L_A genotype showed increased surface distance measures and areas in pink–white (negative *P*-values) indicate reductions in L_A/L_A subjects compared with S allele carriers. (C) Superior view and (D) inferior view of IQ-matched BDNF Val66Met comparison maps, red–yellow points: Val/Val genotype showed increased surface distance measures. Pink–white: reductions in Val/Val subjects compared with Met allele carriers. Superior and inferior view maps are presented with the right hippocampus on the left-hand side and vice versa.

Table S1: Relationships between demographics and brain measures – healthy sample.

Table S2: Relationships between demographics and brain measures – MDD sample.

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Neuroimaging research implicates the hippocampus in the aetiology of major depressive disorder (MDD). Imaging genetics studies have investigated the influence of the serotonin transporter-linked polymorphic region (5HTTLPR) and brain-derived neurotrophic factor (BDNF) Val66Met polymorphism on the hippocampus in healthy individuals and patients with depression (MDD). However, conflicting results have led to inconclusive evidence about the effect of 5HTTLPR or BDNF on hippocampal volume (HCV). We hypothesized that analysis methods based on three-dimensional (3D) hippocampal shape mapping could offer improved sensitivity to clarify these effects. Magnetic resonance imaging data were collected in parallel samples of 111 healthy individuals and 84 MDD patients. Manual hippocampal segmentation was conducted and the resulting data used to investigate the influence of 5HTTLPR and BDNF Val66Met genotypes on HCV and 3D shape within each sample. Hippocampal volume normalized by intracranial volume (ICV) showed no significant difference between 5HTTLPR S allele carriers and L/L homozygotes or between BDNF Met allele carriers and Val/Val homozygotes in the group of healthy individuals. Moreover, there was no significant difference in normalized HCV between 5HTTLPR diallelic and triallelic classifications or between the BDNF Val66Met genotypes in MDD patients, although there was a relationship between BDNF Val66Met and ICV. Shape analysis detected dispersed between-group differences, but these effects did not survive multiple testing correction. In this study, there was no evidence of a genetic effect for 5HTTLPR or BDNF Val66Met on hippocampal morphology in either healthy individuals or MDD patients despite the relatively large sample sizes and sensitive methodology.

Keywords

BDNF; 5HTTLPR; hippocampus; major depressive disorder; MRI; shape mapping

Reduced hippocampal volume (HCV) is well documented in major depressive disorder (MDD) (McKinnon *et al.* 2009) and may be a factor in the pathogenesis of the disorder. Genes are aetiologically important in MDD (Levinson 2006) and for hippocampal structure (Peper *et al.* 2007); the two most examined being the serotonin transporter-linked polymorphic region (5HTTLPR) and brain-derived neurotrophic factor (BDNF) Val66Met single nucleotide polymorphism (SNP).

Serotonin has long been linked to MDD (Krishnan & Nestler 2008) and is believed to influence neurodevelopmental processes such as neurite outgrowth and synaptogenesis (Gaspar *et al.* 2003). Serotonin transporter-linked polymorphic region genotype may influence the hippocampus, the primary site of adult neurogenesis, by altering the levels of synaptic serotonin. However, research into 5HTTLPR variants and HCV has generated mixed results. Reduced volume has been associated with L or L_A homozygosity in MDD (Frodl *et al.* 2004, 2008a,b), but so has the S or L_G allele in both MDD (Eker *et al.* 2011) and healthy controls (Frodl *et al.* 2008a). Illness onset age may be important; an association between S/S genotype and reduced HCV has been reported in early onset patients, with the opposite pattern in late-onset patients (Taylor *et al.* 2005). Environmental influences, such as childhood emotional neglect, have also been shown to interact with 5HTTLPR genotype to impact HCV (Frodl *et al.* 2010). However, other reports have failed to find any genotypic effects in healthy individuals (Pezawas *et al.* 2005) or geriatric MDD and controls (Hickie *et al.* 2007).

Brain-derived neurotrophic factor is known to moderate neurogenesis and processes such as synaptic plasticity and dendritic morphology (Aan het Rot *et al.* 2009). Peripheral BDNF levels are decreased in MDD patients (Sen *et al.* 2008) and BDNF increase may be crucial for antidepressant efficacy (Santarelli *et al.* 2003). The Val66Met polymorphism influences activity-dependent secretion of BDNF, whereby Met allele carriers show reductions

compared with Val/Val homozygotes (Egan *et al.* 2003). The polymorphism has been associated with MDD (Hwang *et al.* 2006), although this has not been consistently replicated (Verhagen *et al.* 2010). In healthy individuals, the Met allele has been associated with HCV reductions using voxel-based morphometry (VBM) (Pezawas *et al.* 2004) and manual volumetrics (Bueller *et al.* 2006). In depression, Met allele carriers have shown bilateral volume reductions (Frodl *et al.* 2007), while Val/Val patients have displayed left hippocampal reductions (Gonul *et al.* 2010) and right hippocampal increases (Kanellopoulos *et al.* 2011). However, other studies observed no significant effects of BDNF genotype on HCV in MDD patients or healthy controls (Benjamin *et al.* 2010; Jessen *et al.* 2009).

Inconsistencies dominate the literature regarding the roles of 5HTTLPR and BDNF Val66Met in influencing magnetic resonance imaging (MRI)-derived HCV, possibly reflecting differing sample characteristics, reliance on gross volumetric measures or a failure to account for epistasis (Pezawas *et al.* 2008). In this study, we investigated the independent and interactive relationships between 5HTTLPR and BDNF Val66Met and the hippocampus in MDD using volumetrics and a more sensitive methodology which examines the three-dimensional (3D) morphology of the hippocampi (Cole *et al.* 2010).

Materials and methods

Participants

Two independent cohorts were recruited (Table 1): 111 right-handed healthy individuals [mean age 33.00 (SD 9.23) years] from the National Institute of Mental Health (NIMH), USA (Pezawas *et al.* 2008), and 84 MDD patients with recurrent unipolar depression [mean age 48.82 (SD 8.93) years] from the Institute of Psychiatry, King's College London, UK (Cohen-Woods *et al.* 2009; Uher *et al.* 2008). Ethical approval was granted by NIMH Institutional Review Board and the Outer South London Research Ethics Committee, respectively and written informed consent was provided by all participants prior to taking part in the study. All patients met DSM-IV criteria for recurrent MDD (Schedules for Clinical Assessment in Neuropsychiatry – Wing *et al.* 1990), with a mean Beck Depression Inventory (BDI) (Beck *et al.* 1961) score of 15.25 (SD 11.34). Fifty-nine of the MDD patients were taking antidepressant medications and 25 MDD patients had been medication-free for at least 4 weeks at the time of scanning. Exclusion criteria were history of mania, hypomania, schizophrenia or mood incongruent psychosis in the participant or a first-degree relative; a lifetime diagnosis of alcohol or substance abuse; depression only secondary to medical illness or medication; severe head trauma or neurological condition and any contraindications to magnetic resonance scanning. Healthy individuals were screened to ensure that they had no lifetime history of any psychiatric illness.

Genotyping

Serotonin transporter-linked polymorphic region and BDNF Val66Met genotyping were performed using either polymerase chain reaction or Taqman 5' exonuclease assay. Full details have been published previously for the healthy individuals (Pezawas *et al.* 2008) and MDD patients (Cohen 2008). None of the genotype frequencies for either sample violated Hardy–Weinberg equilibrium ($P > 0.05$) (Table 1). Major depressive disorder patients were further genotyped for the rs25331 SNP, an A to G substitution within the 5HTTLPR, which is believed to reduce transcriptional efficiency of the L allele (Hu *et al.* 2005; Nakamura *et al.* 2000). This enabled analysis of both the conventional diallelic classification and the triallelic classification in the MDD sample, whereby the L_G allele is classed as equivalent to the S allele.

A labelling error in the healthy sample for the BDNF Val66Met analysis prevented identification of two participants (one Val/Met and one Val/Val), thus this analysis was performed in 109 participants. In MDD patients, 5HTTLPR genotyping failed in three participants, leaving 81 patients for the diallelic analysis. Two further samples failed for rs25531, thus the triallelic analysis was restricted to 79 patients.

MRI acquisition and image pre-processing

In the healthy group, 3D spoiled gradient recalled (SPGR) T1-weighted scans were acquired at 1.5T (General Electric, Milwaukee, WI, USA). Acquisition parameters were TE = 5 ms, TR = 24 ms, flip angle = 45°, field of view = 24 × 24 cm, slice thickness = 1.5 mm, number of slices = 124 and image matrix = 256 × 256. In the MDD patient sample, using a custom-written pulse sequence, Magnetisation-Prepared Rapid Gradient Echo (MP-RAGE) T1-weighted scans were acquired at 1.5T (General Electric, WI, USA), with the parameters: TE = 3.8 ms, TR = 8.59 ms, flip angle = 8°, field of view = 24 × 24 cm, slice thickness = 1.2 mm, number of slices = 180 and image matrix = 256 × 256. Data were then reviewed to ensure good image quality (Simmons *et al.* 2009, 2011). All SPGR and MP-RAGE images were pre-processed prior to structural analysis using the Laboratory of Neuro Imaging (LONI) Pipeline (Rex *et al.* 2003). This involved an initial inhomogeneity correction (i.e. bias field correction, Shattuck & Leahy 2002), automated extraction of brain images using Brain Extraction Tool (BET – Smith 2002) and linear alignment to a standard template (ICBM452 brain – LONI, UCLA) using a six parameter rigid-body transformation to AC–PC orientation.

Volumetric analysis

Each hippocampus was traced manually using *MULTITRACER* (Woods 2003) with a well-established protocol (Narr *et al.* 2004) by a trained rater blinded to diagnostic group (J. C.). Reliability with the protocol was adequate [inter-rater; intraclass correlation coefficient (ICC) = 0.85] as was internal consistency (intra-rater; ICC, left = 0.93; right = 0.94), when compared with other similar research (Bueller *et al.* 2006; Taylor *et al.* 2005). Boundaries of the hippocampus were delineated in the coronal plane, with reference to sagittal and axial views, moving from anterior to posterior along contiguous image slices inclusive of all hippocampal grey matter including the dentate gyrus and subiculum. Best efforts were made to exclude hippocampal white matter regions such as the alveus and fimbria (Figure S1, Supporting information). Intracranial volume (ICV) measures were generated using the automated segmentation procedures in the *FREE SURFER* image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>). In brief, the procedure includes removal of non-brain tissue, automated atlas transformation, intensity normalization and the segmentation of grey matter and white matter, following intensity gradients to optimally define tissue borders (Dale *et al.* 1999; Fischl *et al.* 2002).

Statistical analysis

Analyses were conducted within each sample as the healthy and MDD groups differed in age, location and MRI acquisition parameters, so they could not be pooled together. *SPSS* v. 19.0 (SPSS Inc, Chicago, IL, USA) was used for all analyses, except for power analysis which utilized *G*POWER* v3.1 (Faul *et al.* 2007). Normality of the data was tested using the Shapiro–Wilk test and relationships between demographic measures and HCV were tested using Pearson's correlations and χ^2 tests. For each genotype, a multivariate analysis of variance (*ANOVA*) was conducted, with HCV as dependent variable, and genotype (5HTTLPR diallelic S carrier vs. L/L, 5HTTLPR triallelic S or L_G carrier vs. L_A/L_A and BDNF Met carrier vs. Val/Val) and hemisphere as the between-subject factors. To examine the influence of ICV, each *ANOVA* was run twice, using raw and then normalized [(raw HCV/ICV) × 1000] scores.

Epistasis groups derived from combining both 5HTTLPR and BDNF Val66Met genotypes resulted in four groups per sample (healthy: L/L-Val/Val, L/L-Met, S-Val/Val and S-Met. MDD: L_A/L_A-Val/Val, L_A/L_A-Met, S or L_G-Val/Val and S or L_G-Met). Multivariate ANOVAS were used for each sample. Hippocampal volume was the dependent variable, with hemisphere and epistasis group the between-subject factors to test for a gene–gene interaction. As with the single genotype analysis, separate analyses were conducted for both raw and normalized HCV.

Three-dimensional shape analysis

Analysis of hippocampal shape followed the surface mesh modelling procedures developed by Thompson *et al.* (2004). This method generates parametric surface meshes from the manually derived hippocampal outlines with 15 000 uniform surface points, before calculating a radial distance measure between each surface point and the medial core. Statistical differences can be tested at each hippocampal surface point between groups and the resulting *P*-values mapped onto an average hippocampal shape. An analysis of covariance (ANCOVA) was run with group (5HTTLPR or BDNF Val66Met genotype) as the independent variable and ICV included as a covariate. To account for the multiple tests conducted, permutation tests were run, whereby the genotypes were randomly assigned to the set of subjects (while preserving the total numbers of subjects carrying each genotype) 100 000 times. This procedure compares the real maps with the permuted maps and generates an empirically corrected *P*-value for each hippocampal map (Thompson *et al.* 2003).

Results

Demographic analysis

All demographic and volumetric data were normally distributed (Shapiro–Wilk, $P > 0.1$). In the healthy sample, analysis of demographic measures by 5HTTLPR or BDNF Val66Met genotype indicated that there were no significant differences in age, sex and IQ (Table 2). In the MDD group, there were no significant differences in 5HTTLPR or BDNF Val66Met genotype subgroups in age, sex and BDI (Table 3). However, MDD patients carrying the 5HTTLPR S allele were more likely to be currently taking antidepressants, based on the diallelic classification ($\chi^2 = 5.79$, $P = 0.016$), although not with the triallelic classification ($\chi^2 = 2.62$, $P = 0.11$). There was no significant difference in BDI score between those taking antidepressants and those currently unmedicated ($P = 0.27$). With the BDNF Val66Met genotype, Val/Val MDD patients had significantly higher IQ scores than Met allele carriers (full-scale IQ, $t = 2.23$, $P = 0.03$). Thus, the analysis of BDNF Val66Met and HCV was performed in a subset of IQ-matched subjects with the exclusion of 5 Val/Val participants: 32 Met carriers and 47 Val/Val with no significant difference in full-scale IQ ($t = -1.51$, $P = 0.11$).

Hippocampal volume

In the healthy sample, age was negatively correlated with left ($r = -0.24$, $P = 0.01$) and right ($r = -0.27$, $P < 0.01$) raw HCV and with ICV ($r = -0.34$, $P < 0.01$), but not with normalized HCV. In MDD patients, there were no significant relationships between age and brain volumes, aside from a trend for the right raw HCV ($r = -0.20$, $P = 0.07$). As expected, males had significantly greater ICV than females among both the healthy participants ($t = 5.15$, $P < 0.01$) and MDD patients ($t = 6.68$, $P < 0.01$). Left normalized HCV in healthy participants ($t = -2.40$, $P = 0.02$) and bilateral normalized HCV in MDD were significantly greater in males (left, $t = -2.49$, $P = 0.02$ and right, $t = -3.00$, $P < 0.01$). Intelligent quotient was positively correlated with raw left ($r = 0.29$, $P = 0.01$) and right ($r = 0.26$, $P = 0.02$) HCV and ICV ($r = 0.24$, $P = 0.03$) in MDD patients, but not healthy individuals. Normalized HCV

was not correlated with IQ in either sample. Beck Depression Inventory score and medication status were not significantly associated with either HCV or ICV (Tables S1 and S2).

In the healthy group (Table 2), there was no significant effect of 5HTTLPR genotype for either the raw ($F=0.07$, $P=0.79$) or normalized HCV ($F=0.53$, $P=0.47$) or ICV ($P=0.40$). For the BDNF Val66Met groups, there was also no main effect of genotype on the raw ($F=0.51$, $P=0.60$) or normalized HCV ($F=0.21$, $P=0.81$) or an effect on ICV ($P=0.85$). Given the effect sizes from previous research (Bueller *et al.* 2006), the current analysis had 97.1% power to detect an effect of BDNF Val66Met (Cohen's f : left = 0.37 and right = 0.34). There was no precedent to derive effect size estimates for the 5HTTLPR analysis, but the design had the sensitivity to detect an effect of $f=0.34$ at 95% power and an effect of $f=0.27$ at a lower power threshold of 80%. In all models, the within-subject effect of hemisphere was significant, whereby the right HCV was greater than left (raw volumes, $F=21.96$, $P<0.001$ and normalized volumes, $F=25.42$, $P<0.001$), irrespective of genotype.

In the MDD group (Table 3), there was no significant effect of 5HTTLPR genotype on HCV for diallelic (raw, $F=0.003$, $P=0.96$ and normalized, $F=0.72$, $P=0.40$) or triallelic 5HTTLPR genotypes (raw, $F=0.30$, $P=0.59$ and normalized, $F=0.001$, $P=0.90$). Taking effect size estimates from the study by Frodl *et al.* (2008a) who found significant volume reductions in HCV in L/L homozygotes with MDD (Cohen's $f=0.33$), we had 88.4% power to correctly reject the null hypothesis. There was no 5HTTLPR effect on ICV (diallelic, $P=0.45$ and triallelic, $P=0.57$). As there was a potentially confounding relationship between the S allele and medication use under the diallelic classification, we also tested for the influence of 5HTTLPR genotype in a subgroup of unmedicated patients, but found no significant effects (raw, $F=2.68$, $P=0.11$ and normalized, $F=0.02$, $P=0.89$).

In the IQ-matched BDNF Val66Met groups, there was a significant difference in raw ($F=6.23$, $P=0.014$) but not normalized in HCV ($F=0.01$, $P=0.87$). When analysing all MDD participants, there was a significant effect of BDNF Val66Met genotype on raw HCV ($F=8.4$, $P=0.004$) but not on normalized HCV ($F=0.07$, $P=0.79$). Using the effect size of BDNF on HCV from Frodl *et al.* (2007) analysis ($f=0.26$), our analysis had 64.3% power to detect a similar effect. Val/Val genotype was associated with significantly greater ICV ($t=2.29$, $P=0.025$). There were no differences between medicated and unmedicated MDD patients in either left (raw, $P=0.42$ and normalized, $P=0.93$) or right HCV (raw, $P=0.34$ and normalized, $P=0.98$). Right HCV was significantly greater than left HCV, across genotypes, for both raw ($F=44.67$, $P<0.001$) and normalized scores ($F=42.22$, $P<0.001$).

The full epistatic model testing for an interaction between genotypes in HCV showed no significant effects in either healthy individuals (raw HCV: $F=0.62$, $P=0.43$ and normalized HCV: $F=0.81$, $P=0.37$) or in MDD patients (raw HCV: $F=0.69$, $P=0.56$ and normalized HCV: $F=0.35$, $P=0.79$). There was no direct precedent from which epistatic effects size estimates could be generated, although the healthy individual sample has previously been used to show an interaction between 5HTTLPR and BDNF Val66Met (Pezawas *et al.* 2008), albeit in a different brain region.

Hippocampal shape

For all the hippocampal shape analyses, permutation correction for multiple comparisons indicated that none of the alterations depicted in the 3D maps were empirically significant ($P>0.1$) (Figures S2 and S3).

Discussion

This study found no significant influence of 5HTTLPR or BDNF Val66Met genotype on HCV or shape-related morphological measures in healthy individuals or in recurrent MDD patients. Despite the use of a sophisticated analysis method, sensitive to local hippocampal surface deformations, no differences were found between S allele carriers and L/L homozygotes in healthy individuals or in MDD patients, in whom we were able to test both the diallelic and triallelic classification of 5HTTLPR, nor were any differences in hippocampal surface shape detected between BDNF Met allele carriers and Val/Val homozygotes in either sample.

Our analysis showed that MDD patients carrying the BDNF Val had higher IQ than Met carriers, IQ was correlated with HCV and ICV and BDNF Val carriers had higher ICV. However, when controlling for ICV, neither BDNF genotype nor hippocampal were significantly related to IQ. This indicates that the driving factor behind these results was the positive correlation between IQ and ICV (Andreasen *et al.* 1993). The increased ICV in BDNF Val carriers (Toro *et al.* 2009) requires further investigation to establish whether this is meaningful or merely a false-positive. We also report a negative correlation between age, HCV and ICV, although there was no association between age and normalized HCV, so this relationship may also be a false-positive.

The absence of significant 5HTTLPR effects is in accordance with previous findings in these same healthy individuals (Pezawas *et al.* 2005) and in other healthy control groups in MDD research (Eker *et al.* 2011; Frodl *et al.* 2004, 2008b; Hickie *et al.* 2007; Taylor *et al.* 2005). No effect of 5HTTLPR genotype in MDD patients, either diallelic or triallelic, concurs with Hickie *et al.* (2007) but not with other reports (Eker *et al.* 2011; Frodl *et al.* 2004, 2008b; Taylor *et al.* 2005). As this study consists of similar methods and participants as Frodl *et al.* (2004), albeit with our larger sample, the negative outcome cannot be easily attributed to statistical power. Taylor *et al.* (2005) recruited older adults and divided the group by age of illness onset (before and after 50 years), finding that reduced HCV associated with L/L genotype in late-onset patients and S/S genotype in early onset patients. The latter finding was supported by Eker *et al.* (2011) report in younger patients. With a mean age of 49 years, the majority of our MDD sample would have fallen into the early onset category, yet no effect of 5HTTLPR genotype was evident. The effects of onset age on HCV and morphology, have been previously shown (Ballmaier *et al.* 2008) and should be accounted for, particularly in older adult samples.

Regarding BDNF Val66Met, volumetric analysis in healthy individuals found no evidence of an effect once ICV was accounted for, despite directly replicating the methods of Bueller *et al.* (2006) with a much greater sample size of 109 participants compared with their 36. Interestingly, Bueller *et al.* (2006) report this effect in both raw and normalized data, however, this may well be a false-positive as it seems that the design was substantially underpowered (Flint *et al.* 2010). Our results are more akin to those of Karnik *et al.* (2010) who found no difference in HCV between healthy Val/Val homozygotes and Met carriers. Furthermore, we did not detect any genetic effects in either sample when using our more sensitive hippocampal shape mapping methodology. Therefore, our results concur with reports that find no evidence for an influence of BDNF Val66Met genotype on MRI-derived HCV in MDD patients (Benjamin *et al.* 2010; Jessen *et al.* 2009) and do not support studies that report a significant effect (Frodl *et al.* 2007; Gonul *et al.* 2010; Kanellopoulos *et al.* 2011). These positive reports use similar methodologies to the current approach and again our analysis employed a greater number of MDD patients: 84 compared with 60 (Frodl *et al.* 2007), 33 (Gonul *et al.* 2010) and 33 (Kanellopoulos *et al.* 2011), respectively. The main difference between these studies is in the type of sample used: younger adults (Gonul *et al.*

2010), middle-aged hospital in-patients (Frodl *et al.* 2007) and older adults (Kanellopoulos *et al.* 2011), while this study consisted of middle-aged out-patients with recurrent depression. It is possible that the effects of BDNF Val66Met genotype, if present, are evident in a greater severity of illness as reflected by an early onset (Gonul *et al.* 2010), in-patient status (Frodl *et al.* 2007) or with increasing duration of illness in older adults (Kanellopoulos *et al.* 2011).

A proportion of the MDD patients were taking antidepressant medication. Genotype is believed to influence the effects of antidepressants (Lesch & Gutknecht 2005), and antidepressant medication is likely to influence the hippocampus, but not necessarily at a gross anatomic level (Vythilingam *et al.* 2004). We did not detect HCV differences between medicated and unmedicated patients and while there were differences in medication use based on 5HTTLPR genotype, there were no genotypic effects in unmedicated patients. On this basis, it is unlikely that any medication effects would have altered the outcome of the analysis. Studies examining 5HTTLPR or BDNF Val66Met genotype and HCV vary in how to account for medication effect: including a washout-period (Kanellopoulos *et al.* 2011), medication-naïve participants (Eker *et al.* 2011) or mixed samples of both medicated and unmedicated patients (Frodl *et al.* 2007; Hickie *et al.* 2007), while others do not factor in medication effects (Taylor *et al.* 2005). There is no clear pattern differentiating positive and negative studies, suggesting that the use of antidepressants within MDD samples does not systematically influence the relationship between genotype and HCV. Despite the fact that all MDD patients in the study had suffered at least two moderate or severe depressive episodes, there was considerable variation in current illness. Potentially, the relatively mild current state may have lessened any effects, which may be more evident in those more acutely ill. However, we did not observe any relationships between BDI score and HCV, so the mild symptom severity of our sample may not have significantly influenced the results.

Limitations include the use of independent participant cohorts drawn from different populations, precluding a direct comparison between groups. Also, structural MRI at 1.5T as an approach to imaging the hippocampus provides less resolution than at a higher field strength, but scanning at 1.5T is still commonly and successfully used for manual hippocampal segmentation studies (Frodl *et al.* 2007; Taylor *et al.* 2005) and we were able to produce reliable results using a robust and well-validated methodology (Thompson *et al.* 2004). Another drawback is that we were unable to examine internal hippocampal subregions such as the dentate gyrus. This means that genotypic differences specific to the hippocampal subregions may have gone undetected, something that could be rectified with improved resolution.

Genetic effects on complex phenotypes, such as clinical disorders and regional brain morphometrics, are likely to be the result of multiple genes acting at different points in development and in concert with other genetic and environmental factors. Therefore, single candidate gene association designs are not necessarily a full reflection of the underlying genetic processes. We did not observe epistasis between 5HTTLPR and BDNF Val66Met on HCV in healthy individuals or in depression. In the same cohort of healthy individuals, reductions in VBM-derived anterior cingulate and amygdala volumes were associated with the 5HTTLPR S allele in BDNF Val/Val homozygotes, with 5HTTLPR effect found in BDNF Met allele carriers (Pezawas *et al.* 2008). Our negative results indicate that such an interaction may be regionally specific and is unlikely to influence HCV directly. Differential epistatic effects on the neighbouring amygdala and hippocampus are interesting in light of previously highlighted volumetric differences in MDD (Campbell *et al.* 2004) and may have implications for functional differentiation within the limbic system, however, caution should be taken when directly comparing manual and VBM results. Furthermore, it should be acknowledged that HCV is exquisitely sensitive to potentially confounding environmental

factors in patients, including medication, exercise, endogenous steroid levels and cumulative life-stress exposure as well as diet.

Future research could target other genes associated with MDD or the hippocampus such as *COMT*, *DISC1* or *NR3C1* (see Scharinger *et al.* 2010 for review). An expansion of this effort includes whole-gene investigations whereby associations with markers from across a gene of interest are tested. Such an approach has implicated *GSK3 β* in structural neuroanatomical differences (Inkster *et al.* 2009). Polygenic or genomic (Potkin *et al.* 2009) investigations may also show relationships between genes and regional brain structures in depression.

Conclusions

The influence of 5HTTLPR and BDNF Val66Met genotypes on the hippocampus was investigated in healthy individuals and patients with recurrent MDD. We did not observe an effect of these polymorphisms, either independently or epistatically, on HCV or shape. Effects of these genotypes may be specific to other regional cerebral structures and may have complex interactions with environmental or other genetic factors.

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Table 1

Demographic data for healthy, unaffected and MDD patient samples

	Healthy individuals	MDD patients
<i>N</i>	111	84
Age, years	33.00 (9.23)	48.82 (8.93)
Sex (male/female)	55/56	27/57
Full-scale IQ	108.00 (8.45)	117.18 (11.34)
BDI	n.a	15.25
Medication	None	59 antidepressants/25 unmedicated
5HTTLPR diallelic		
Allele frequency	S = 0.43, L = 0.57	S = 0.48, L = 0.52
Genotype frequency	S/S = 0.16, S/L = 0.53, L/L = 0.31	S/S = 0.31, S/L = 0.345, L/L = 0.345
5HTTLPR triallelic		
Allele frequency	n.a	S = 0.49, L _G = 0.07, L _A = 0.44
Genotype frequency	n.a	S/S = 0.32, S/L _G = 0.06
		L _G /L _G = 0.01, S/L _A = 0.29
		L _A /L _G = 0.05, L _A /L _A = 0.27
BDNF Val66Met		
Allele frequency	Val = 0.79, Met = 0.21	Val = 0.80, Met = 0.20
Genotype frequency	Val/Val = 0.62, Val/Met = 0.34, Met/Met = 0.04	Val/Val = 0.62, Val/Met = 0.34, Met/Met = 0.04
Epistasis		
Genotype frequency	L/L, Val/Val = 0.18	L _A /L _A , Val/Val = 0.18
	L/L, Met = 0.12	L _A /L _A , Met = 0.09
	S, Val/Val = 0.44	S or L _G , Val/Val = 0.44
	S, Met = 0.26	S or L _G , Met = 0.29

Age and full-scale IQ are presented in mean (standard deviation) format. n.a = Not applicable.

Table 2

Volumetric data by genotype – healthy sample

Healthy	All	5HTTLPR diallelic		BDNF val66met	
		S carriers	L/L	Met carriers	Val/Val
N	111	77	34	41	68
Age	33.00 (9.23)	32.87 (9.1)	33.2 (9.9)	33.17 (9.4)	32.85 (9.3)
Sex (male/female)	55/56	38/39	17/17	22/19	32/36
IQ	108.00 (8.45)	108.08 (8.7)	107.3 (7.9)	108.4 (8.6)	107.93 (8.2)
Raw HCV (mm ³)					
Left	1708.69 (252.55)	1706.66 (252.48)	1713.30 (256.44)	1720.47 (270.13)	1706.90 (244.25)
Right	2090.35 (257.42)	2086.20 (258.30)	2099.74 (259.01)	2092.58 (266.32)	2091.05 (253.57)
Normalized HCV					
Left	1.18 (0.19)	1.18 (0.18)	1.17 (0.16)	1.19 (0.19)	1.18 (0.17)
Right	1.44 (0.18)	1.44 (0.19)	1.43 (0.17)	1.45 (0.18)	1.44 (0.18)
ICV (mm ³)	1 478 467 (125 728)	1 452 002 (174 525)	1 476 197 (182 065)	1 455 041 (174 496)	1 462 648 (180 465)

Comparisons within genotype were not significantly different ($P > 0.05$).

Table 3

Volumetric data by genotype – MDD sample

MDD	5HTTLPR diallelic			5HTTLPR triallelic			BDNF val66met	
	All	S carriers	L/L	S or L _G carriers	L _A /L _A	Met carriers	Val/Val	
<i>N</i>	84	53	28	58	21	32	47	
Age	48.82 (8.93)	49.6 (8.3)	47.18 (9.9)	49.12 (8.6)	48.5 (10.1)	50.09 (10.2)	47.70 (8.0)	
Sex (male/female)	27/57	17/36	9/19	19/39	6/15	6/23	16/31	
IQ	117.18 (11.3)	117.21 (11.8)	117.21 (11.3)	117.21 (11.5)	117.19 (12.3)	113.56 (12.5)	116.63 (11.0)	
BDI	15.25 (11.3)	15.23 (11.2)	16.54 (11.7)	14.9 (10.9)	16.95 (12.7)	17.38 (13.4)	14.3 (10.0)	
Medicated/unmedicated	59/25	42/11	15/13	44/14	12/9	25/7	31/16	
Raw HCV (mm ³)								
Left	1671.50 (237.93)	1676.01 (254.58)	1674.92 (218.29)	1672.89 (247.96)	1659.15 (214.30)	1613.34 (281.74)	1693.89 (204.3)	
Right	1928.47 (248.21)	1931.92 (263.31)	1937.45 (228.42)	1933.46 (257.31)	1899.77 (206.96)	1850.73 (268.14)	1965.93 (228.5)	
Normalized HCV								
Left	1.13 (0.16)	1.14 (0.18)	1.12 (0.13)	1.13 (0.18)	1.13 (0.13)	1.13 (0.19)	1.13 (0.16)	
Right	1.30 (0.18)	1.31 (0.19)	1.29 (0.14)	1.31 (0.19)	1.30 (0.14)	1.30 (0.19)	1.32 (0.18)	
ICV (mm ³)	1 486 882 (150 096)	1 480 222 (147 488)	1 507 214 (156 562)	1 490 172 (158 016)	1 468 301 (119 160)	1 435 285 (148 783)*	1 513 238 (147 989)*	

* Denotes significant differences within genotype. All other comparisons within genotype were not significantly different ($P > 0.05$).