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BDNF polymorphism rs6265 and hippocampal structure and memory performance in healthy control subjects

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) is highly expressed in the hippocampus of many species, including humans. The single-nucleotide polymorphism rs6265 on the BDNF gene is thought to alter activity-dependent secretion of the protein, and previous research suggests that the Met allele is associated with smaller hippocampal volumes and poorer memory performance in human populations. For this study, we genotyped 154 healthy human subjects for the Val66Met polymorphism. The effects of genotype upon hippocampal volume, as assessed using high resolution magnetic resonance imaging and high-dimensional brain mapping, and upon memory performance, as assessed using a battery of neuropsychological tests, were determined. We found that genotype had no significant effect on hippocampal structure, nor did it have a significant effect on memory performance, covarying for age. Age, however, was significantly related to changes in whole brain volume and performance on memory tasks. We concluded that in a large cohort of healthy human subjects, the Met allele of rs6265 is not associated with hippocampal structure or memory performance.

1. Introduction

Neurotrophins are thought to be involved in both the proliferation of neurons and in the maintenance of synapses. Animal studies have shown that neurotrophins are also necessary for neuronal survival (Lewin and Barde, 1996) in both the peripheral nervous system and central nervous system (CNS). The mammalian brain contains four principal neurotrophic proteins: nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and brain-derived neurotrophic factor (BDNF) (Reichardt, 2006).

Neuronal secretion of BDNF during embryogenesis and early postnatal life is required for strengthening neurons and neural connections. More specifically, this neurotrophin has been shown to regulate the branching of cortical dendrites (Dijkhuizen and Ghosh, 2005). *In vitro* exposure to BDNF enhances survival of neuronal precursor cells derived from the forebrain subependymal zone of the adult rat. In fact, it is the only neurotrophin to do so (Kirschenbaum and Goldman, 1995). Finally, in both adult rodents and adult humans, BDNF is intensely expressed in the hippocampus (Murer et al., 2001), a key substrate for memory as shown through lesion studies in both animals (Alvarado and Rudy, 1995; Pascalis and Bachevalier, 1999) and humans (Zola-Morgan et al., 1986; Milner, 2005; Bright et al., 2006; Gold and Squire, 2006). Consistent with the hypothesis that BDNF expression has an important influence on learning and memory, heterozygous BDNF knockout mice show significant deficits in spatial memory as compared to wild-type mice (Bartoletti et al., 2002), and others have found induction of BDNF expression during periods of hippocampus-dependent learning (Hall et al., 2000).

BDNF is secreted via two different pathways. It is secreted constitutively at a low basal level and also, in a regulated manner based on the level of synaptic activity. Current scientific thinking is that constitutive secretion maintains basal synaptic function during development of the CNS (Wang et al., 1998). In keeping with this hypothesis, the amount of constitutively secreted BDNF has been related to the capacity for a synapse to undergo long-term potentiation (LTP) in the adult mammalian brain (Poo, 2001). In turn, synaptic activity is thought to trigger activity-dependent secretion of BDNF. Activity-dependent BDNF secretion has been observed to improve the strength of the synapse, perhaps as a means of modulating specific synaptic connections (Lu, 2003). Interestingly, pairing BDNF administration with neuronal activity at rat CA1 and dentate gyrus synapses strengthens LTP (Lu, 2003).

The human BDNF gene on chromosome 11 at position 13 (11p13) contains numerous polymorphisms. The Val66Met polymorphism of BDNF (rs6265) produces a valine to methionine substitution at codon 66 in the BDNF amino acid sequence, and is thought to disrupt activity-dependent BDNF secretion (Egan et al., 2003). In studies of

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small human cohorts, carriers of the BDNF Met allele have been shown to have small hippocampal volumes compared to Val homozygotes (Szeszko et al., 2005; Bueller et al., 2006; Frodl et al., 2007). Also, individuals with the Met allele as opposed to the Val allele have shown poorer performance on tests of memory (Egan et al., 2003; Dempster, 2005; Goldberg et al., 2008). However, in other studies, no link between the Val66Met polymorphism and memory has been found (Strauss et al., 2004; Oroszi et al., 2006). Small sample sizes in such studies may have contributed to inconsistent findings, making it also difficult to evaluate potential interactions between the influence of the Val66Met polymorphism on brain structure, memory, age, and gender. Also, the small human cohorts used in such studies have not always been carefully screened for neuropsychiatric disorders that have been associated with changes in hippocampal structure and function (e.g., mood disorders, schizophrenia, and dementia). Indeed, several groups have found an association between the Met allele and the various neuropathologies including schizophrenia (Ho et al., 2006; Numata et al., 2006), and drug abuse (Cheng et al., 2005). Still others have shown through work with rodents that the Val66Met polymorphism may serve as a genetic risk factor for the development of anxiety and depressive disorders in people (Chen et al., 2006).

The purpose of this study was to attempt to replicate previously published findings of a relationship between the Val66Met BDNF polymorphism and hippocampal structure and memory in a large sample of human subjects, who were carefully screened to eliminate individuals with any evidence for neuropsychiatric illness. We hoped to replicate previous findings of a relationship between the Val66Met polymorphism and: (1) hippocampal volume and (2) performance on memory-related neuropsychological tests. We investigated these relationships in a large pool of healthy subjects of both genders and across a broad age range.

2. Methods

2.1. Subjects

The data included in this study were drawn from populations of healthy comparison subjects in two databases: one related to the study of brain structure and cognition in middle-aged adult subjects with and without schizophrenia, and a second related to the study of brain structure and cognition in elderly subjects with and without probable Alzheimer's disease. These databases were selected for the present study because of the special effort to exclude subjects from the comparison groups that showed any evidence of neuropsychiatric illness.

The recruitment criteria for the adult subjects in the first database were previously described in Delawalla et al. (2006). To summarize, 88 healthy subjects were recruited through the Conte Center for the Neuroscience of Mental Disorders (CCNMD) at Washington University in St. Louis. The absence of schizophrenia or any other psychiatric disorder, including mood disorders, was determined by the consensus between a psychiatrist who conducted an unstructured mental status examination and a Master's level clinician who used the Structured Interview for the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (SCID-IV) (First et al., 2002). This group of subjects had an approximately even distribution of males versus females with a mean age of 39 years, S.D. = 20.6. The majority (83%) of these subjects were Caucasian, the remainder being African American. The key exclusion criterion for the participants drawn from this database and included in the present study was a lifetime history of any Axis I psychiatric disorder or having a first-degree relative with a psychotic disorder. Participants were also excluded if they (1) met DSM-IV criteria for substance abuse or dependence within the past month; (2) had a clinically unstable or severe medical disorder, or a medical disorder that would confound the assessment of psychiatric diagnosis or render research participation dangerous; (3) had head injury (past or present) with documented neurological sequelae or resulting in loss of consciousness; and (4) met DSM-IV criteria for mental retardation (mild or greater in severity).

The recruitment criteria for the second set of healthy subjects were described in Wang et al. (2006). In brief, these subjects were enrolled in longitudinal studies of healthy aging and dementia at the Alzheimer Disease Research Center (ADRC) at Washington University School of Medicine. The absence of dementia and other neuropsychiatric disorders, including mood disorders, was determined in these subjects by a clinical neurologist who conducted a semi-structured interview using the Clinical Dementia Rating scale (CDR) (Morris, 1993). We included 69 healthy comparison subjects from this database with approximately 30% of our subjects being male and 70% being female. Again, the majority (90%) of these subjects were Caucasian, with the remainder being African American. The mean age of this group was 79 years; S.D. = 7.7. Table 1 summarizes our demographic data for each of our two groups of interest: Val/Val homozygotes and Met carriers.

2.2. Structural MRI collection and processing

Magnetic resonance (MR) scans were collected from all subjects in both datasets using the same Magnetom SP-4000 1.5 T Siemens imaging system, equipped with a standard head coil, and an identical turbo-FLASH sequence (TR = 20 ms, TE = 5.4 ms, flip angle = 30-, 1-mm section thickness, 180 slices, 256-mm field of view, matrix = 256–256, number of acquisitions = 1, scanning time = 13.5 min) that acquired three-dimensional datasets with 1 mm^3 isotropic resolution across the entire cranium (Venkatesan and Haacke, 1997; Wang et al., 2006). Our procedure for collecting and processing our scans was described in detail (Csernansky et al., 2004). MR datasets were reformatted using Analyze™ software (Analyze-AVW, 2004), and signed 16-bit MR datasets were compressed to unsigned 8-bit MR datasets by linearly rescaling voxel intensities such that voxels with intensity levels at two standard deviations above the mean of white matter (corpus callosum) were mapped to 255, and voxels with intensity levels at two standard deviations below the mean of cerebrospinal fluid (CSF; lateral ventricle) were mapped to 0. The white matter and CSF means and standard deviations were obtained by sampling voxels from these respective regions. Ultimately, of our 154 genotyped subjects, we had 129 subjects with usable scans.

2.3. Neuroanatomical measurements

Our methods for measuring the volume and surface conformation of the hippocampus in populations of subjects from young to older adulthood have been previously detailed in several publications (Haller et al., 1997; Csernansky et al., 2002; Wang et al., 2006). To summarize, the anatomic boundaries of the left and right hippocampus were first defined in a single subject using manual outlining; this scan was collected from an individual who was not otherwise included in the analysis and referred to as the template scan. In the template scan and in all remaining (i.e., target) MR scans, landmarks were placed at the anterior, posterior, superior, inferior, and lateral brain boundaries and at points where the anterior and posterior commissures intersected the midsagittal plane. Points at the anterior and posterior boundaries of the hippocampus were also demarcated, defining an anterior/posterior axis. Five equally distanced slices were then selected along this axis, and four landmarks (superior, lateral, inferior, and medial) surrounding the hippocampus were placed in each slice.

Transformation of the template scan onto the target MR scans occurred in two steps (Miller et al., 1997). First, it was coarsely aligned to each target scan by using the previously placed landmarks, and then a high-dimensional transformation was applied to achieve an optimal voxel-by-voxel match. During the transformation, the movement and deformation of template voxels were constrained by assigning them the physical properties of a fluid. We have previously shown that the reliability of this process, including landmark placement and both steps of the template transformation, is equivalent or superior to manual outlining by experts for defining the neuroanatomical boundaries of the hippocampus (Haller et al., 1997); estimates of the overlap of hippocampal surface contours produced by the semi-automated procedure outlined above versus manual segmentation exceed 80% which is comparable to the accuracy of repeated attempts at manual outlining of the hippocampal surface by the same expert.

Although BDNF is densely expressed throughout the hippocampus, it has a differential level of expression across the hippocampal subfields, with the highest level of expression in the CA3 and CA4 subfields as well as the dentate gyrus (Murer et al., 1999). Therefore, to more carefully assess the relationship between genotype and specific anatomical components of the hippocampus, we divided the surface of the hippocampus in the template into three zones of approximately equal area corresponding to the underlying subfields of the hippocampus (see Wang et al., 2006 for details). We have previously reported that subjects with neuropsychiatric conditions could be discriminated from controls by examining the pattern of hippocampal surface variation (Csernansky et al., 2002 and Wang et al., 2003). We then went on to show through the mapping of hippocampal zones approximating the subfields of the hippocampus that specific inward deformations in the surface of the hippocampus could be used to distinguish subjects with mild dementia from those without (Wang et al., 2006). These zones were termed the lateral zone (LZ), proximal to the CA1 subfield, the superior zone (SZ), proximal to the combined CA2, CA3, CA4 subfields and the dentate gyrus and the inferior-medial zone (IMZ), proximal to the subiculum (see Fig. 1 in Csernansky et al., 2005). The reliability of this procedure has been previously shown (Wang et al., 2006). Briefly, comparing the manual outline of the three hippocampal surface zones in the right hemisphere of 10 randomly selected subjects with the surface zones as mapped from the template yielded the following intra-class correlation coefficients of the areas of the three surface zones: LZ −0.97; IMZ−0.97; SZ−0.90. We hypothesized that the strongest relationship would be observed between genotype and variation in the SZ.

Table 1
Demographic data for homozygotes of the Val allele and for Met carriers (1 homozygote; 41 heterozygotes).

Genotype $n = 129$	Age (mean, S.D.)	Race (Caucasian/African-American)	Gender (m/f)
Val/Val homozygotes $n = 87$	51.3, 25.8	77/10	36/51
Met carriers $n = 42$	45.2, 23.9	39/3	23/19

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In summary, four neuroanatomical variables were generated for our analyses: 1) combined left and right hippocampal volume, 2) combined left and right LZ displacement, 3) combined left and right SZ displacement, and 4) combined left and right IMZ displacement.

As described in Csernansky et al. (2004), total cerebral volumes (excluding the brainstem and cerebellum) were derived by using a landmark-based elastic transformation (Miller et al., 1997) of the template scan. During these transformations, the entire template scan was globally registered with each target scan using scalar points at the boundaries of the brain and in the midline.

2.4. Neuropsychological testing

Four memory-related neuropsychological tasks were administered to all subjects derived from the two datasets; i.e., Category Fluency task for animals (Goodglass and Kaplan, 1983), Wechsler Memory Scale (WMS) Logical Memory I (Wechsler, 1987), WMS Digit Span Forwards (Wechsler, 1987), and WMS Digit Span Backwards (Wechsler, 1987). Since slightly different versions of the WMS logical memory test were administered to the ADRC and the CCNMD subject groups, the scores from this task were normalized across the subjects in each of the groups through z-scoring before being analyzed. The reference group for the younger CCNMD subjects was the reference group from the WMS-III manual made up of subjects with an age range of 20–34 years. For the older ADRC subjects, the reference group was the 18–39-years-old group from the normative database described in Crook et al., 1990. For the other memory-related tests, raw scores were used in all analyses.

2.5. Genotyping

The procedure used to genotype the subjects recruited by the ADRC was similar to that outlined in Li et al. (2005). To summarize, single-nucleotide polymorphism (SNP) genotyping was performed by allele-specific real-time polymerase chain reaction (PCR) in individual samples. For the genotyping of the CCNMD subjects, we used a procedure similar to that outlined by Willis-Owen et al. (2005). Using a Sequenom™ system, PCRs were carried out in 10-μL reaction volumes using specific primers. Each reaction included 2 μL DNA template (2 ng/μL), 0.5 μL primers (1 μm), 0.04 μL Titanium Taq (Clontech) (BD Biosciences, San Jose, CA, USA), 1 μL Titanium Taq buffer (BD Biosciences, San Jose, CA, USA), 1 μL deoxyribonucleotide triphosphates (dNTPs) (2 mmol/L), 0.4 μL magnesium chloride ($MgCl_2$) (25 mmol/L), and 5.06 μL Milli-Q H_2O. The reaction was carried out as follows: 95 °C for 1 min (1 cycle), 95 °C for 30 s, 60 °C for 30 s, 68 °C for 1 min (45 cycles), and 68 °C for 3 min (1 cycle). These products were then subject to a shrimp alkaline phosphatase (SAP) digest for removal of nonincorporated dNTPs and a final extension reaction via use of another specific primer. Extension products were cleaned and spotted onto 384 SpectroCHIPs, which were read on a mass spectrometer.

Genotyping revealed 1 Met/Met subjects, 41 Met/Val subjects and 87 Val/Val subjects in the combined group of subjects. For the purposes of all subsequent analyses, we grouped the Met/Val heterozygotes and the Met/Met homozygotes.

2.6. Data analysis

Separate general linear models were applied to an individual in which analyses of covariance (ANCOVAs) were used to assess the effects of genotype on performance scores from the four memory-related tests, with age and genotype by age interactions as covariates. Age has been previously correlated with both performance on memory-related tests (Friedman et al., 2007) and measures of hippocampal structure (Driscoll et al., 2003). We were especially interested in the possibility of genotype by age interactions as a means of assessing whether genotype differentially affected the rate of age-related decline in cognitive performance, hippocampal volume or hippocampal zone deformation. In our dataset, we found no significant effect of gender on any of the dependent measures, and therefore, gender was not included as a covariate. Finally, hippocampal volume was corrected for whole brain volume by dividing hippocampal volume by whole brain volume (i.e., corrected hippocampal volume). A summary of our volumetric and cognitive data is presented for both genotype groups in Table 2.

Table 2
Volumetric and cognitive means and standard deviations for homozygotes of the Val allele and Met carriers.

Memory task	Degrees of freedom	*F* value	*P* value
Logical memory immediate recall ($n=142$)	141	0.14	0.71
Digit span: forward ($n=124$)	123	1.18	0.28
Digit span: backward ($n=124$)	123	2.04	0.16
Category fluency ($n=145$)	144	0.00	1.00

3. Results

There was no significant effect of genotype on whole brain volume (Fig. 1) or on corrected left or right hippocampal volumes (Fig. 2). Also, genotype had no significant effect on any measure of hippocampal surface structure (any of the three zones, see Section 2) (Table 3) or on any measure of cognitive performance (Table 4).

Age had a significant effect on whole brain volume ($F=19.3$, $df=128$, $P<0.0001$), corrected left hippocampal volume ($F=4.0$, $df=128$, $P=0.05$), but not on corrected right hippocampal volume ($F=0.51$, $df=128$, $P=0.48$). Because of this finding, we performed a repeated measures mixed model procedure, and included hemisphere as a main effect in our analyses of hippocampal zones. Additionally, we added hemisphere*genotype and age*hemisphere as interactions of interest too. We found the following significant results: age had a significant effect on the combined left and right LZ ($F=19.73$, $df=217$, $P<0.001$) of the hippocampus. We also found on the SZ a significant hemisphere effect ($F=49.82$, $df=217$, $P<0.001$), and a significant effect of hemisphere by age interaction ($F=26.88$, $df=217$, $P<0.001$). Finally, age was significantly related to performance on the verbal fluency task ($F=4.10$ and $P=0.04$), and performance on the WMS Digit Span Forward task ($F=5.35$ and $P=0.02$), but not on the other two tasks. After applying the Bonferonni correction for multiple comparisons, the only results to survive were the effect of age on whole brain volume, the effect of age on combined LZ, and the effects of hemisphere and hemisphere by age interactions on the SZ.

We found no age by genotype interactions on any of our measures of brain structure or cognitive performance, and therefore the rates of change for our measures over time were not affected by genotype.

In a post-hoc analysis, in order to determine if: 1) there was an effect of whole brain volume on hippocampal volume, and 2) if including whole brain volume as a covariate altered the relationship between hippocampal volume and age, we included it as a covariate in analyses of both uncorrected left and right hippocampal volumes. We found that whole brain volume was significantly related to both left ($F=27.2$, $df=128$, $P<0.0001$) and right ($F=24.93$, $df=128$, $P<0.0001$) hippocampal volumes. Additionally, including whole brain volume as a covariate in our analyses strengthened the relationship between left hippocampal volume and age ($F=11.74$, $df=128$, $P<0.001$), and rendered the relationship between right hippocampal volume and age significant ($F=4.84$, $df=128$, $P<0.02$). The only significant result of including whole brain volume as a covariate that did not survive the Bonferroni correction for multiple comparisons was the relationship between right hippocampal volume

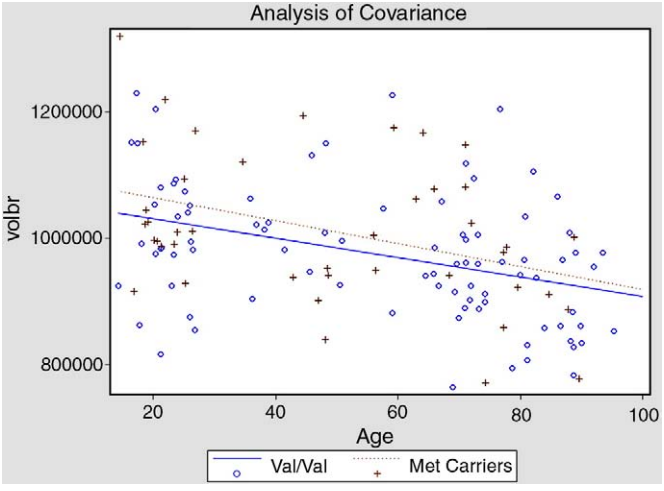

Fig. 1. Effect of Val66Met polymorphism of BDNF on relationship between whole cerebral brain volume (volbr, mm^3) and age (years), $F=0.79$, $P=0.375$; $n=129$.

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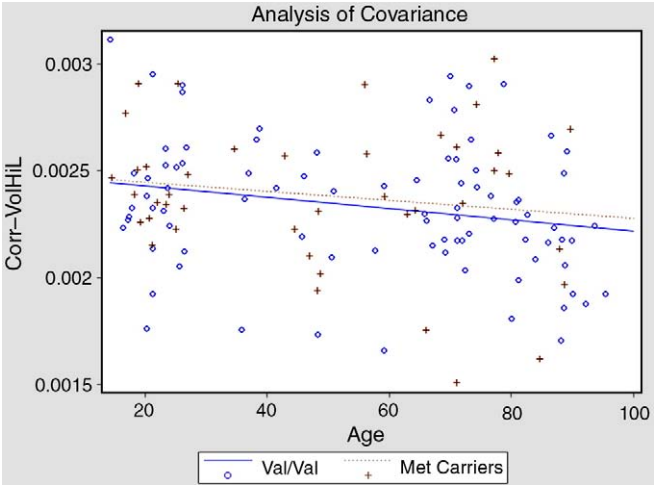

Fig. 2. Relationship of hippocampal volume (mm^3), corrected for whole brain volume (mm^3), Corr_VolHiL, and age (years) was unaffected by the Val66Met polymorphism, $F = 0.00$, $P = 0.956$; $n = 129$.

and age. Including whole brain volume as a covariate did not alter our findings on the lack of an effect of genotype on hippocampal volume.

Based upon a retrospective power analysis, our sample sizes would have permitted us to detect an effect of genotype on our dependent measures for an effect size between 0.27 and 0.31 with 90% power and an alpha value of 0.05. These effect sizes suggest that we would be unable to detect only a very small effect of genotype on our measures.

4. Discussion

In a large cohort of human subjects, carefully screened for the presence of neuropsychiatric illness, and representative of both genders and a broad range of ages, we failed to find a relationship between the Val66Met polymorphism and measures of hippocampal structure or performance on memory-related tests. These results differ significantly from previously published findings for hippocampal structure in relation to the Val66Met polymorphism (Szeszko et al., 2005; Bueller et al., 2006; Frodl et al., 2007; Chepenik et al., 2009). It should be noted though that Bueller et al. reported their finding of an 11% reduction in volume of the hippocampus of Met carrying subjects based on a total sample of only 36. Also, the findings of Szesko et al., were not significant for their healthy control group. Their overall finding of a relationship between the Val66Met genotype and hippocampal volume was derived primarily from the population of patients with schizophrenia including in the study cohort. Also, we failed to find relationships between the Val66Met polymorphism and performance on memory-related tasks. Unlike the results of prior studies, possession of the Met allele was not associated with poorer memory performance. Notably, one group that reported such an effect (Dempster et al., 2005), based their findings on only a single test of memory, the WMS Logical Memory Delayed Task, which was not included in this study.

Because our subjects were a mixture of two racial groups in which allele frequencies for the Val66Met genotype have been observed to differ, our ability to detect the predicted relationships may have been weakened. However, even after covarying for race, we found that genotype still did not have a significant effect on measures of brain structure (Table 5) or cognitive performance. Another feature of our study that may have undermined our ability to detect a significant effect of the Val66Met genotype is that we grouped our single Met/Met homozygote with the more numerous Val/Met heterozygotes. In one prior study in which the Val66Met genotype was found to have an effect on memory performance (Egan et al., 2003), 6 Met/Met homozygotes were compared to 91 Val/Val homozygotes and 36 heterozygotes for Val/Met. However, the positive result of this study was largely based on the comparison of the 6 Met/Met homozygotes, and consistent with our results, a significant difference was not found between the Val/Met group and the Val/Val group.

In summary, we failed to find a significant effect of the Val66Met BDNF polymorphism on hippocampal structure and performance on a group of memory-related cognitive tasks in a large group of healthy human subjects. We selected the cohort of subjects for this study from the comparison cohorts of large ongoing studies of neuropsychiatric disorders because of the care used in these studies to ensure that the comparison cohort did not include subjects with any evidence of neuropsychiatric disorders in which abnormalities of hippocampal structure and function have been described (Csernansky et al., 2002; Posener et al., 2003; Wang et al., 2006). Thus, while the results of our study suggest that the Val66met BDNF polymorphism may have little relevance for normative hippocampal structure and function, it does not preclude an important role for the polymorphism in groups of individuals with specific neuropsychiatric disorders.

Table 3
Genotype effect on deformation of each of three left–right combined hippocampal zones.

Hippocampal zone ($n = 112$)	Degrees of freedom	F value	P value
Lateral zone left–right combined	217	1.54	0.22
Superior zone left–right combined	217	0.01	0.92
Inferior-medial zone left–right combined	217	0.44	0.51

Table 4
Statistics for genotype effect on cognitive tasks.

Memory task	Degrees of freedom	F value	P value
Logical memory immediate recall ($n = 142$)	141	0.14	0.71
Digit span: forward ($n = 124$)	123	1.18	0.28
Digit span: backward ($n = 124$)	123	2.04	0.16
Category fluency ($n = 145$)	144	0.00	1.00

Table 5
Statistics for genotype effect on left–right combined hippocampal zone deformation after covarying for race.

Hippocampal zone ($n = 111$)	Degrees of freedom	F value	P value
Lateral zone left–right combined	213	0.49	0.48
Superior zone left–right combined	213	0.06	0.81
Inferior-medial zone left–right combined	213	0.00	0.99

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