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Brain-derived neurotrophic factor genotype is associated with brain gray and white matter tissue volumes recovery in abstinent alcohol-dependent individuals

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Abstract

Neuroimaging studies have linked the methionine (Met) allele of the brain-derived neurotrophic factor (*BDNF*) gene to abnormal regional brain volumes in several psychiatric and neurodegenerative diseases. However, no neuroimaging studies assessed the effects of this allele on brain morphology in alcohol use disorders and its demonstrated change during abstinence from alcohol. Here we assessed the effects of the *BDNF* Val66Met (rs6265) polymorphism on regional brain tissue volumes and their recovery during short-term abstinence in treatment-seeking alcohol-dependent individuals. 3D T1 weighted magnetic resonance images from 62 individuals were acquired at 1.5 T at one week of abstinence from alcohol; 41 of the participants were rescanned at 5 weeks of abstinence. The images were segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid and parcellated into regional volumes. The *BDNF* genotype was determined from blood samples using the TaqMan technique. Alcohol-dependent Val (Valine)/Met heterozygotes and Val homozygotes had similar regional brain volumes at either time point. However, Val homozygotes had significant GM volume increases, while Val/Met heterozygotes increased predominantly in WM volumes over the scan interval. Longitudinal increases in GM but not WM volumes were related to improvements in neurocognitive measures during abstinence. The findings suggest that functionally significant brain tissue volume recovery during abstinence from alcohol is influenced by *BDNF* genotype.

Keywords

Alcohol-dependence; brain structure; brain-derived neurotrophic factor; intracranial volume; magnetic resonance imaging; neurocognition

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin that is widely expressed in the mammalian brain with particularly high levels in the hippocampus and the cerebral cortex (Hofer *et al*. 1990; Phillips *et al*. 1990; Wetmore *et al*. 1990). BDNF plays an important role in the long-term survival, differentiation, growth and maintenance of neurons (Ernfors *et al*. 1995; Murer *et al*. 2001). It also promotes myelination in both the peripheral and central nervous systems (Cellerino *et al*. 1997; Vondran *et al*. 2010; Xiao *et al*. 2010). The methionine (Met) allele of the *BDNF* Val66Met (rs6265) polymorphism is associated with impaired intracellular trafficking and activity-dependent secretion of BDNF (Chen *et al*. 2004; Egan *et al*. 2003). The Met allele is also associated with poorer verbal memory (Egan *et al*. 2003; Hariri *et al*. 2003), processing speed (Miyajima *et al*. 2008; Raz *et al*. 2009) and general intelligence (Tsai *et al*. 2004) in controls and individuals with various neuropsychiatric conditions.

In voxel-based morphometric magnetic resonance imaging (MRI) studies of healthy controls, *BDNF* Met allele carriers showed smaller hippocampi, prefrontal, parietal and occipital lobes, as well as smaller frontal and temporal gyri compared with valine (Val) homozygotes (Eker *et al*. 2005; Pezawas *et al*. 2004). The Met carriers compared with Val homozygotes also had higher rates of gray matter (GM) volume reductions in the dorsolateral prefrontal cortex and the dorsal premotor cortex as a function of normal aging (Nemoto *et al*. 2006); *BDNF* Met carriers also showed smaller volumes of specific brain regions in schizophrenia (Takahashi *et al*. 2008), major depression (Frodl *et al*. 2007), Alzheimer's disease (Hashimoto *et al*. 2009) and mild cognitive impairment (Forlenza *et al*. 2010); and Met carriers with schizophrenia had larger time-related reductions of frontal GM volumes than Val homozygotes (Ho *et al*. 2007). Furthermore, hypomyelination of developing axons of the spinal cord, the optic nerve and the corpus callosum was reported in *BDNF* knock-out/deficient mice (Cellerino *et al*. 1997; Vondran *et al*. 2010; Xiao *et al*. 2010). Together, these findings indicate that the *BDNF* Met allele is related to abnormalities in brain morphology and function in normal and in various neuropsychiatric conditions. Similar to psychiatric and neurodegenerative diseases, alcohol use disorders [i.e. abuse or dependence according to the diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV) criteria] are also associated with abnormalities in brain structure and function. Neuroimaging studies of those with an alcohol use disorder have consistently showed GM and/or white matter (WM) reductions (particularly in the frontal and temporal regions) and enlarged ventricles and sulci (Bühler and Mann 2011), which at least partially normalize within the first month of sustained abstinence from alcohol (Gazdzinski *et al*. 2005; Pfefferbaum *et al*. 1995). Although the Val66Met *BDNF* polymorphism has been associated with brain morphological abnormalities in individuals with neurodegenerative or psychiatric disorders, no MRI studies have assessed the potential effect of this polymorphism on brain morphology in those with an alcohol use disorder. Therefore, the primary goal of this study was to assess the influence of the *BDNF* Val66Met polymorphism on MRI-derived regional brain tissue volumes and their longitudinal changes in alcohol-dependent individuals (ALC) during the first few weeks of abstinence from alcohol. We also assessed the functional relevance of any longitudinal brain volume changes. Specifically, we hypothesized that:

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1. At 1 week and 5 weeks of abstinence, *BDNF* Val homozygotes have significantly larger GM and WM volumes in the frontal, parietal and temporal lobes than Val/Met heterozygotes.
2. Val homozygotes during abstinence from alcohol recover faster from lobar GM and WM volume reductions than Val/Met heterozygotes, particularly in the frontal lobes.
3. Longitudinal increases of tissue volumes relate positively to improvements on measures of working memory, visuospatial scanning speed and incidental learning, auditory-verbal and visuospatial learning.

Materials and methods

Participants

Sixty-two (53 males and 9 females) ALC participated in this study. They were part of a larger longitudinal cohort recruited from the San Francisco VA Medical Center Substance Abuse Day Hospital and the San Francisco Kaiser Permanente Chemical Dependence Recovery Programs for research assessing the effect of chronic alcohol consumption and comorbid cigarette smoking on neurobiology and neurocognition. Baseline time point 1 (TP1) scans were performed at 6.0 ± 3.3 days of abstinence from alcohol and follow-up scans time point 2 (TP2) at 34.0 ± 9.4 days of abstinence. Of the 62 participants, 41 were scanned at both TPs (longitudinal sample). All ALC participants had MRI and *BDNF* genotype data and met DSM-IV criteria for alcohol-dependence. Male participants consumed more than 150 alcoholic drinks (one drink contains 13.6 g of pure ethanol) per month for at least 8 years and females more than 80 drinks per month for at least 6 years prior to the enrollment. The ALC participants were in outpatient treatment and were screened daily for alcohol consumption and substance use to ensure that sobriety was maintained. Briefly, participants were excluded for a history of abuse or dependence on other substances but nicotine, within the past 5 years and for biomedical or psychiatric disorders (such as schizophrenia, bipolar and other psychotic disorders) that are known to affect brain biology or neurocognition. Hepatitis C, type-2 diabetes, hypertension, unipolar mood disorder (major depression and/or substance-induced mood disorder) were permitted given their high prevalence in alcohol use disorder (Hasin *et al*. 2007; Mertens *et al*. 2003, 2005; Parekh and Klag, 2001; Stinson *et al*. 2005). For a comprehensive list of inclusion and exclusion criteria, see e.g. Durazzo *et al*. (2004).

Seventeen non-smoking light drinkers (LD) were also studied as age-matched controls (47.9 ± 7.0 years). They were part of the same longitudinal research study as the ALC participants and had MRI data at baseline and about 7 months later. They were recruited from the local community and had no history of medical, psychiatric or substance use conditions known to influence MRI outcome measures, and drank on average 19 ± 15 alcoholic drinks per month over lifetime.

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Clinical assessment

All ALC participants completed the structured clinical interview for DSM-IV Axis I Disorder Patient Edition, Version 2.0 (First *et al*. 1998) at baseline and standardized questionnaires for alcohol withdrawal [CIWA-Ar (Addiction Research Foundation Clinical Institute of Withdrawal Assessment for Alcohol); Sullivan *et al*. 1989], depression (Beck Depression Inventory; Beck 1978) and anxiety symptomatologies (State-Trait Anxiety Inventory, Y-2; Spielberger *et al*. 1977) within 1 day of the baseline and follow-up MRI studies. Alcohol consumption over lifetime was assessed with the lifetime drinking history (LDH) (Skinner and Sheu 1982; Sobell and Sobell 1990; Sobell *et al*. 1988). From the LDH, we estimated the age at onset of heavy drinking (heavy drinking is defined as consuming >100 alcoholic drinks per month), the average number of alcoholic drinks consumed per month over 1, 3 and 8 years before enrollment and over lifetime.

To evaluate the nutritional status and alcohol-related or other hepatocellular injury, we obtained laboratory tests for serum albumin, pre-albumin, alanine aminotransferase, aspartate aminotransferase and γ-glutamyltransferase, typically within 2 days of each MRI scan.

Table 1 shows demographics, alcohol consumption and clinical variables for both genotype groups of ALC derived from the longitudinal sample of 41 ALC at TP1. The variables were numerically similar to those of the entire sample of 62 ALC.

A brief neurocognitive battery was administered to ALC at both TPs: the battery assessed aural working memory [Wechsler Adult Intelligence Scale-III (WAIS-III) Digit Span], visuomotor scanning speed and incidental learning (WAIS-III Digit Symbol; Wechsler 1997), auditory-verbal (Delis *et al*. 2000) and visuospatial learning and memory (Brief Visual memory Test-Revised; Benedict 1997; Durazzo *et al*. 2007).

MRI data acquisition and processing

The MRI images for both ALC and LD were acquired on a 1.5-T system (Vision, Siemens Medical Systems, Iselin, NJ, USA), using a T1-weighted magnetization prepared rapid acquisition gradient echo sequence (TR/TI/TE = 9/300/4 milliseconds, 1 × 1 mm^2 in-plane resolution, 1.5 mm slabs) oriented orthogonal to the long axis of the hippocampus and an oblique-axial T2-weighted spin-echo imaging sequence (TR/TE = 2500/80 milliseconds, 1 × 1 mm^2 in-plane resolution, 3 mm slice thickness).

Three-tissue intensity based segmentation (based on the expectation-maximization segmentation method of Van Leemput *et al*. (1999) was applied to the T1-weighted images to assign a set of probabilities of WM, GM and cerebrospinal fluid to each MRI voxel. The T2-weighted images were used to remove non-brain tissue voxels from the segmented images. Absolute volumes for WM and GM tissues of the major lobes and subcortical regions were then parcellated by overlaying the tissue maps on a reference atlas as previously described (Studholme *et al*. 2001, 2003). Total absolute cortical GM and WM volumes were calculated by summing the respective GM and WM values from the frontal, parietal, temporal and occipital lobes. To account for individual variability in intracranial

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volume (ICV), regional tissue volumes for each participant were standardized to their corresponding ICV.

Genotyping

Genomic DNA was isolated from whole blood samples of ALC. The *BDNF* single nucleotide polymorphism (SNP) rs6265 was assayed using TaqMan genotyping assays from Applied Biosystems (Foster City, CA, USA). The SNP assays were performed using a reaction volume of 15 μl, which consisted of 7.5 μl of TaqMan 2× universal master mix, 0.38 μl of 20× TaqMan pre-designed SNP genotyping assay, 6.14 μl of nuclease-free water and 1 μl genomic DNA. After PCR amplification as per manufacturer's recommendations, SNP genotypes were determined by allelic discrimination using the ABI-7500 instrument (Applied Biosystems, Foster City, CA, USA). The entire ALC sample comprised of 68% Val/Val homozygotes (Val homozygotes), 32% Val/Met heterozygotes and 0% Met homozygotes; the longitudinal ALC sample had 63% Val homozygotes and 37% Val/Met heterozygotes. These distributions were in Hardy–Weinberg equilibrium ($\chi^2 = 0.39$, $P > 0.05$).

Data analyses

Cross-sectional—Multivariate analysis of variance (MANCOVA) assessed differences between ALC *BDNF* Val homozygotes and Val/Met heterozygotes on age, education, drinking severity, days of abstinence, anxiety, depression, smoking severity and basic clinical laboratory measures. The MANCOVA (with age as a covariate) was used to assess cross-sectional differences in brain tissue volumes between *BDNF* genotypes at each TP.

Longitudinal—To ensure that changes in brain tissue volumes of ALC during abstinence were indeed abstinence related, the longitudinal data of the 17 LD were assessed for any changes in tissue volumes using paired *t*-tests. Differences between ALC Val homozygotes and Val/Met heterozygotes on regional volume changes from TP1 to TP2 for individual brain regions were assessed with linear mixed modeling. The age range of the participants was large (28–65 years), so age was used as a covariate in all analyses. In addition, average monthly alcohol consumption over lifetime and smoking status were used as covariates in all longitudinal analyses, as both have been shown to be associated with greater age-related brain tissue volume reductions (Durazzo *et al*. 2010; Ge *et al*. 2002; Good *et al*. 2001; Pfefferbaum *et al*. 1992). In secondary analyses, substance, biomedical and psychiatric comorbidities (including mood disorders) were individually entered as covariates (together with age and smoking status) to evaluate the potential influence of these factors on longitudinal volume change. The left and right hemispheres showed highly similar regional change patterns, so regional volumes represent the sum of both hemispheres.

Correction for multiple comparisons—Although we had *a priori* predictions, α-level (0.05) for main effects, interactions, pairwise *t*-tests and within subject *t*-tests (two-tailed for all *t*-tests) for regional cortical GM and lobar WM volumes were adjusted for multiple comparisons based on the eight lobar regions (i.e. GM and WM volume for the four lobes) and the average inter-correlations among the volumes for all ALC participants combined across TPs ($r = 0.70$) (Sankoh *et al*. 1997). Thus, the adjusted α-level for statistical tests for

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individual lobar GM and WM regions was $P \leq 0.027$. α-level (0.05) for main effects, interactions and within subject *t*-tests (two-tailed) for subcortical GM and WM volumes were adjusted for multiple comparisons based on the five measures (i.e. GM volumes of thalamus, lenticular nuclei and caudate, whole volumes of brainstem and cerebellum) and the average inter-correlations among these measures for all ALC participants combined across TPs ($r = 0.33$) (Sankoh *et al*. 1997). Thus, the adjusted α-level for statistical tests for subcortical regional volumes was $P \leq 0.017$.

Associations of changes in regional volumes and neurocognition: The associations between changes in regional brain volumes and neurocognitive test performance across the TP1–TP2 scan interval in the combined longitudinal sample (i.e. Val homozygotes and Val/Met heterozygotes) and separately for each genotype were examined with linear mixed modeling; volume change in the individual regions and age were used as predictors of change for each neurocognitive test. All regional volumes that significantly predicted change of the individual neurocognitive tests at $P < 0.05$ were considered statistically significant. These analyses were not corrected for multiplicity in order to explore any potentially meaningful structure–function relationships across neurocognitive measures.

Results

Participants

The ALC participants were predominantly White (84.0%, including 5% Hispanic), followed by African-American (11.0%). Native Americans, Pacific Islanders and Asians each contributed less than 2% to the sample. The Val homozygotes and Val/Met heterozygotes did not differ significantly on age, cigarette smoking severity or any clinical measure (Table 1). Also, there were no significant differences in the prevalence of cigarette smoking between the genotypic groups ($\chi^2 = 1.62$, $P > 0.10$) and in serum albumin, prealbumin, liver enzyme levels and γ-glutamyltransferase, hemoglobin levels and other clinical laboratory measures. The distributions of all psychiatric comorbidities (including mood disorders) were equivalent between Val homozygotes and Val/Met heterozygotes. There was no significant difference in the frequency of antidepressant medication usage between Val homozygotes (19%) and Val/Met heterozygotes (15%).

Cross-sectional analyses

Table 2 shows mean tissue volumes of ALC by genotype at baseline (TP1) and follow-up (TP2); MANCOVA showed no effect for genotype on ICV- and age-corrected measures of regional cortical GM, regional lobar WM, total cortical GM, total lobar WM or subcortical tissue volumes of ALC at either TP1 or TP2. Val homozygotes had a 6.5% larger ICV than heterozygotes (1544 ± 153 ml vs. 1445 ± 88 ml; $P = 0.027$); ALC as a group had similar ICV as the LD control group (1519 ± 138 ml and 1505 ± 144 ml, respectively).

Longitudinal Analyses

MRI volume changes in ALC as a function of genotype—Table 2 lists relative changes of regional tissue volumes between scans in ALC by genotype and the results of paired *t*-tests for each region.

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Interactions for lobar GM and WM: Significant genotype-by-time interactions were observed for frontal GM ($F_{(1,34)} = 5.37$, $P = 0.027$) and frontal WM ($F_{(1,34)} = 5.97$, $P = 0.020$). Figure 1 shows ICV-normalized frontal GM and WM volume changes between the TPs (i.e. TP2 ICV-normalized tissue volume – TP1 ICV-normalized tissue volume) for both *BDNF* genotype groups. Interaction trends were seen for total cortical GM ($F_{(1,34)} = 4.06$, $P = 0.051$) and total lobar WM ($F_{(1,36)} = 4.07$, $P = 0.051$). No genotype-by-time interactions were observed for subcortical regions.

For frontal GM, Val homozygotes showed a significant volume increase over the scan interval ($P < 0.001$), while Val/Met heterozygotes showed no significant volume change ($P = 0.25$). The opposite pattern was observed for frontal WM, with no significant volume change in Val homozygotes ($P = 0.97$) and a significant volume increase for Val/Met heterozygotes ($P = 0.020$). The Val homozygotes and Val/Met heterozygotes showed similarly different patterns of change for total cortical GM and total WM over the 4-week scan interval.

Main effects for lobar GM and WM: Significant main effects for TP were observed for parietal GM ($F_{(1,34)} = 14.93$, $P < 0.001$), parietal WM ($F_{(1,34)} = 10.98$, $P = 0.002$), temporal GM ($F_{(1,34)} = 10.66$, $P = 0.003$) and temporal WM ($F_{(1,34)} = 6.86$, $P = 0.011$). No main effects were found for the occipital GM or WM. In the parietal GM, Val homozygotes showed a significant volume increase ($P = 0.002$) over the TP1–TP2 interval, while Val/Met heterozygotes exhibited no significant volume change ($P = 0.16$); this indicated that the main effect for parietal GM was primarily driven by the volume increase in Val homozygotes. Both Val homozygotes ($P = 0.020$) and Val/Met heterozygotes ($P = 0.021$) showed volume increases in the temporal GM. Val/Met heterozygotes showed trends for increases in parietal ($P = 0.061$) and temporal WM ($P = 0.062$), while Val homozygotes exhibited no volume changes in the parietal and temporal WM (both $P > 0.94$); this indicated that the main effects for parietal and temporal WM were largely driven by volume increases in Val/Met heterozygotes.

Alcohol consumption measures, medical, substance and psychiatric comorbidities (including mood disorders) as well as antidepressant usage did not significantly predict GM or WM volume changes in any lobar region.

Main effects for subcortical regions: Main effects for TP were also observed for GM volume changes of the thalamus ($F_{(1,24)} = 9.14$, $P = 0.006$) and caudate ($F_{(1,24)} = 8.01$, $P = 0.004$) and for total cerebellar volume ($F_{(1,32)} = 15.27$, $P < 0.001$). For thalamic GM, Val homozygotes showed significant volume increases ($P = 0.008$), while thalamic GM volume in Val/Met heterozygotes did not change significantly ($P = 0.22$). The caudate GM volumes of Val homozygotes decreased significantly ($P = 0.013$), while Val/Met heterozygotes showed no significant change ($P = 0.16$). Total cerebellar volume showed no change in Val homozygotes ($P = 0.71$), but tended to increase in Val/Met heterozygotes ($P = 0.034$). No main effects were observed for GM of the lenticular nuclei or brainstem volume over the TP1–TP2 scan interval. Finally, as for lobar regions, subcortical volume changes between time points were not associated with alcohol consumption, medical, substance and psychiatric comorbidities or antidepressant usage.

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MRI volume changes in LD—The paired *t*-test analyses of our longitudinal MRI data of LD showed no significant differences in regional or total lobar GM and WM volumes over the 7-month scan interval. Figure 2 shows exemplary plots of the means (and SE) of several regional volume changes. Volume changes of the other analyzed regions are not shown, but showed similar stability over 7 months. This data suggests that the significant volume changes in ALC over 4 weeks of abstinence relate to structural recovery, which is associated with *BDNF* genotype.

Associations between changes of regional brain volumes and neurocognitive measures in ALC

Combining the ALC participants across genotypes, volume increases in several cortical and subcortical GM regions were associated with improving neurocognitive performance, primarily on measures of visuospatial learning, auditory working memory and processing speed (Table 3). Changes of regional WM volumes were not significantly related to changes in performance on any neurocognitive measure. These patterns were not significantly different between *BDNF* Val homozygotes and Val/Met heterozygotes.

Discussion

In this quantitative MRI study assessing the effects of the *BDNF* Val66Met (rs6265) polymorphism on brain tissue volume changes in abstinent ALC, Val homozygotes and Val/Met heterozygotes differed on the degree of regional GM and WM volume recovery over 1 month of abstinence from alcohol. Total cortical GM volume increased significantly in Val homozygotes, whereas total lobar WM volume increased significantly in Val/Met heterozygotes. Specifically, Val homozygotes showed significant increases in GM volumes of all but the occipital lobes, while showing no longitudinal changes in any lobar WM volume. Val/Met heterozygotes showed significant increase of frontal WM and trends for increased WM in the parietal and temporal lobes. For the subcortical volumes, thalamic GM increased only in Val homozygotes, whereas total volumes of the cerebellum and the brainstem increased only in Val/Met heterozygotes. Temporal GM volume increased significantly in both genotypes, the lenticular nuclei showed no significant change in either genotype, and the caudate showed a significant decrease in Val homozygotes.

Overall, our results indicate two disparate patterns of brain tissue volume recovery for the *BDNF* Val homozygotes and Val/Met heterozygotes in this alcohol-dependent cohort: Val/Val was associated with significant volume increases restricted to cortical GM, while Val/Met was primarily related to volume increases in lobar WM. Despite the genotype-specific longitudinal changes, there were no significant cross-sectional regional volume differences between Val homozygotes and Val/Met heterozygotes at baseline or follow-up. However, the alcohol-dependent Val homozygotes had significantly larger ICV compared with their Val/Met heterozygous counterparts. Cole *et al*. (2011) reported smaller ICV for Met carriers compared with Val homozygotes with major depression, but this difference was not apparent in healthy controls (Bueller *et al*. 2006; Cole *et al*. 2011), despite high heritability of ICV (Glahn *et al*. 2007). The lack of *BDNF* genotyping for our control participants in this study and the small control sample did not allow assessing the effects of

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BDNF Val66Met polymorphism on ICV in controls. Our light-drinking controls showed no regional GM or WM volume changes over 7 months, and none of our alcohol consumption measures were associated with brain tissue volume change over the 4-week-interval in ALC; together, this data suggest that the observed ALC volume changes were indeed related to structural changes over approximately 1 month of abstinence, and several of these regional changes were mediated by *BDNF* genotype.

Given that BDNF is associated with neuronal survival, neuronal growth and synaptic plasticity in the adult brain (Lu 2003), our observation of significant longitudinal recovery of cortical and subcortical GM in only Val homozygous ALC over 1 month of abstinence is congruent with the action of BDNF binding with tyrosine kinase (Trk) B receptors in the cortex. Both BDNF and TrkB show high expression in the cortex, and BDNF-mediated activation of TrkB is associated with dendritic growth, axonal outgrowth and synaptic plasticity (Bath and Lee 2006). As the Met allele is associated with suppressed secretion of BDNF and its extracellular trafficking (Egan *et al*. 2003; Chen *et al*. 2004), the reportedly higher concentrations of BDNF in blood of Val homozygotes relative to Met allele carriers (Egan *et al*. 2003; Duncan *et al*. 2009) appears consistent with our observation of greater cortical GM recovery in Val homozygotes. In mammals, optimal neuronal growth and survival depends on the concentration of growth and neurotrophic factors, such as BDNF (Davies 2000; Hetman and Gozdz 2004).

On the other hand, the significant lobar WM volume increases observed in Val/Met heterozygotes seem counterintuitive, because BDNF is associated with promotion of central nervous system (CNS) myelination. Specifically, *BDNF* knock-out mice (a single Val allele deleted) exhibited hypo-myelination in the optic nerve, hippocampus, cerebellum and cortex after 21 days of delivery (Cellerino *et al*. 1997), suggesting the necessity of BDNF for normal CNS myelination. This observation was confirmed by two other studies in the developing brain of mice models (Vondran *et al*. 2010; Xiao *et al*. 2010). Given that BDNF has been described as a promyelination neurotrophin in the developing brain (Cellerino *et al*. 1997; Vondran *et al*. 2010; Xiao *et al*. 2010), we predicted our Val homozygous ALC would show increases in regional WM volumes; instead, the Val/Met heterozygotes showed greater WM volume increases during short-term abstinence than the Val homozygotes. These discrepancies may be related to differences in BDNF activity during brain development and brain repair of the adult brain after removal of a chronic insult (here alcohol).

In the combined ALC group (i.e. Val homozygotes + Val/Met heterozygotes), longitudinal changes in regional cortical and subcortical GM volumes during abstinence were positively associated with improvement in neurocognition. Most notably, increases in all cortical and subcortical GM regions were related to improving visuospatial learning. Additionally, changes in measures of working memory related positively to frontal and parietal GM volume increases; and improvement in measures of processing speed related positively to parietal GM volume increases. On the other hand, longitudinal increases in WM volumes, which were observed mainly in the Val/Met heterozygotes, were not associated with improvement in any neurocognitive measure. This may suggest that the WM increases observed primarily in Val/Met heterozygotes were of little functional significance in this cohort. However, the complexity of the cortical–cortical and cortical–subcortical WM

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interconnectivity is not well captured by our lobar WM volumetric measures (Pfefferbaum and Sullivan 2005); volumetrics cannot determine if remyelination, a likely contributor to the WM volume increases (Harper 2009; Sullivan and Pfefferbaum 2005), is the primary contributing factor to the WM increases demonstrated here by Val/Met heterozygotes. More regionally specific measures of WM microstructural integrity provided by diffusion weighted imaging measures (i.e. fractional anisotropy, radial and longitudinal diffusivity) may serve as better proxies of neurocognitive function (Chiang *et al*. 2011; Pfefferbaum *et al*. 2000; Pfefferbaum *et al*. 2005; Pfefferbaum and Sullivan 2005) and may assist in determining if the WM volume increases are indeed related to myelination.

We expected that the observed differential recovery rates of regional GM and WM volumes between the two genotypes (which were statistically equivalent at TP1) would result in cross-sectional group differences at 1 month of abstinence. However, we observed statistically similar regional brain volumes between genotypic groups at the 1-month follow-up (i.e. TP2). The 1-month scan interval may not have been of sufficient duration to allow the observed differences in recovery rates between the groups to result in significant volume differences at TP2. If Val homozygotes and Val/Met heterozygotes continue to show different rates of change between regional GM and WM tissue volumes, significant cross-sectional differences may become apparent with extended abstinence.

This study has limitations which may affect the generalizability of our findings. The sample, although of adequate size for neuroimaging studies, was relatively small for genetic research. However, our analysis focused on only one specific common polymorphism with a demonstrated influence on human brain morphology. The majority of the participants were males, which precluded assessment of potential sex effects on regional tissue volumes. Also, we did not have a control sample with *BDNF* genotypes for comparisons of genotype-specific regional brain volumes. Furthermore, we did not evaluate our alcohol-dependent participants for DSM-IV Axis II disorders, such as antisocial personality disorders (Grant *et al*. 2004; Pridmore *et al*. 2005), and we did not measure potential group differences in nutrition, exercise and other genetic predispositions.

In summary, the *BDNF* Val66Met (rs6265) polymorphism was significantly related to the recovery of regional GM and WM tissue volumes within the first 5 weeks of sobriety, suggesting genetic influences on brain tissue changes during abstinence from alcohol in this alcohol-dependent cohort. Significant associations of cortical and sub-cortical GM volume increases (observed predominantly in Val homozygotes) with improved performance on multiple neurocognitive abilities during short-term abstinence highlight the functional relevance of genotype-specific brain tissue recovery during abstinence from alcohol. To better understand the mechanisms contributing to long-term morphological changes in alcohol use disorders, it is necessary to determine the influence of the *BDNF* Val66Met polymorphism, (and other polymorphisms associated brain morphology (e.g. superoxide dismutase; Srivastava *et al*. 2010) on changes in regional brain volumes and other morphometrics (e.g. cortical thickness) with extended abstinence from alcohol. Although the mechanisms by which *BDNF* genotype and protein mediate brain tissue recovery in abstinent ALC are unknown, these neuroimaging findings highlight the need to consider

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genetic factors such as *BDNF* in the treatment of alcohol use disorders, as gene variants may influence the pattern of brain tissue recovery and associated neurocognitive improvements.

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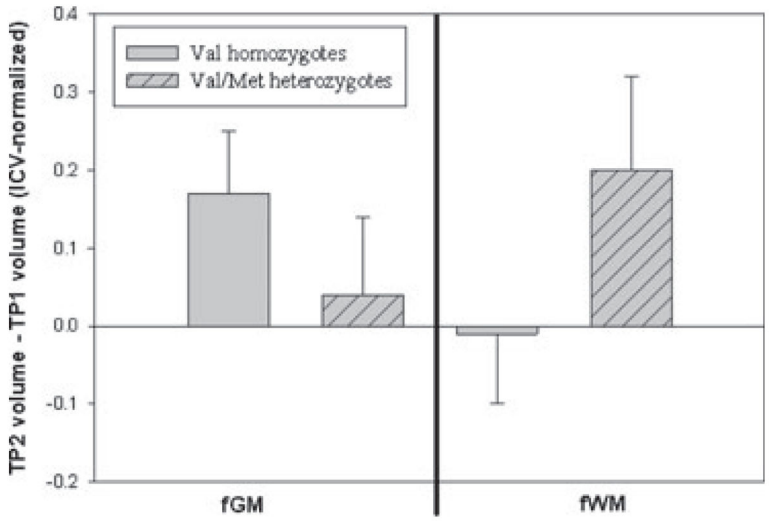

Figure 1.

Frontal gray and white matter volume changes in Val homozygotes and Val/Met heterozygotes between TP1 and TP2 (i.e. ICV-normalized TP2 volume – ICV-normalized TP1 volume).

Val homozygotes increased significantly in gray matter ($P < 0.001$) but not in white matter, while Val/Met heterozygotes increased significantly in white matter ($P = 0.020$) but not gray matter.

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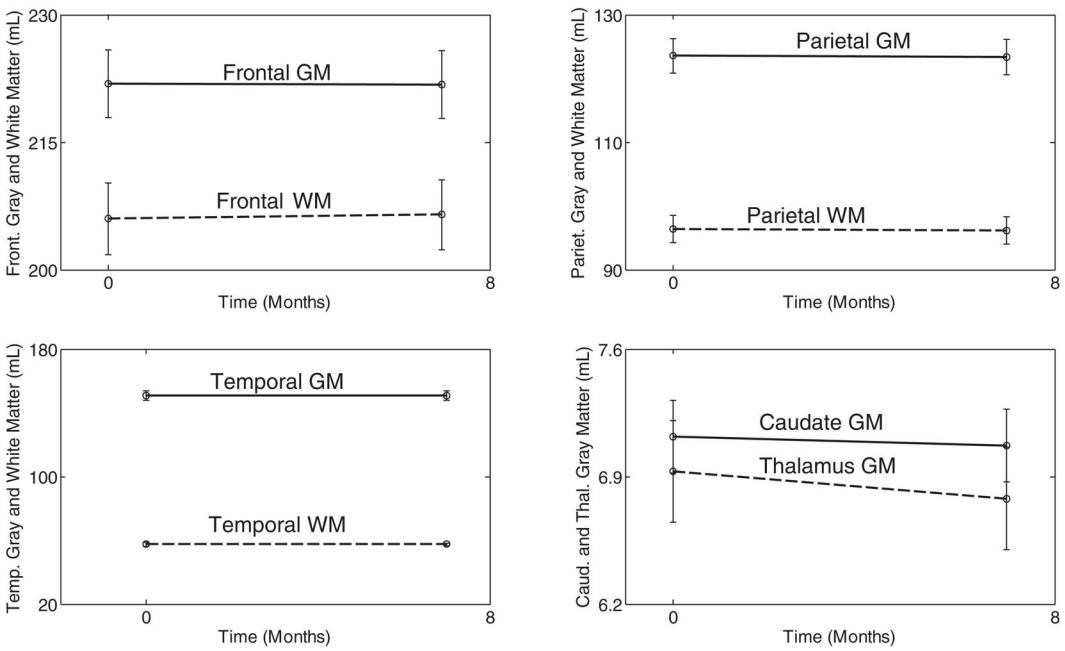

Figure 2.

Longitudinal volume changes of regional brain tissues in non-smoking light-drinking controls.

Top: gray and white matter in frontal (left) and parietal lobes (right); bottom: gray matter in subcortex and caudate (left) and in thalamus and lenticular nuclei (right).

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Table 1

Demographics, frequency of smoking, alcohol consumption and blood variables of the longitudinal ALC sample at TP1

Variable	Combined genotypes	Val homozygotes	Val/Met heterozygotes
N (females)	41 (5)	26 (3)	15 (2)
Age (years)	50.8 ± 10.6	51.3 ± 9.4	49.9 ± 12.6
Education (years)	14.3 ± 2.4	14.3 ± 2.5	14.3 ± 2.4
8 years average (drinks per month)	295 ± 140	308 ± 155	274 ± 112
3 years average (drinks per month)	344 ± 162	363 ± 185	310 ± 109
1-year average (drinks per month)	363 ± 181	379 ± 214	321 ± 96
Lifetime average (drinks per month)	196 ± 100	204 ± 111	183 ± 81
Age of onset of heavy drinking (years)	27 ± 10	26 ± 9	27 ± 12
Months of heavy drinking	239 ± 102	240 ± 97	239 ± 114
TP1 (days)	6.5 ± 3.3	6.5 ± 2.5	6.5 ± 3.1
Smokers (%)	50	43	65
Pre-albumin (g/dl)	27.5 ± 8.8	27.0 ± 10.9	30.0 ± 10.3
Albumin (g/dl)	4.6 ± 0.3	4.4 ± 0.5	4.1 ± 0.3
AST (IU)	39.8 ± 26.7	36.6 ± 16.0	37.0 ± 17.1
SGPT-ALT (IU)	40.8 ± 21.2	42.9 ± 23.1	47.7 ± 22.3
WBC	6.6 ± 1.7	6.7 ± 2.2	7.2 ± 1.5
RBC	4.4 ± 0.4	4.4 ± 0.5	4.4 ± 0.2
Hemoglobin	14.2 ± 1.3	14.3 ± 1.5	14.3 ± 1.0
Hematocrit	41.2 ± 3.8	41.1 ± 4.4	41.9 ± 2.2
GGT (IU)	75.8 ± 56.3	77.2 ± 63.1	101.3 ± 57.8
AMNART	114.6 ± 8.2	115.1 ± 7.0	116.8 ± 12.9
BDI	12.4 ± 9.0	11.8 ± 9.2	12.6 ± 10.0
STAI Y-2	45.9 ± 9.5	46.3 ± 11.4	42.2 ± 9.7
CIWA	1.4 ± 2.2	2.0 ± 2.8	1.5 ± 1.8
BMI	27.6 ± 5.6	28.2 ± 4.8	27.0 ± 5.4

Months heavy drinking denotes number of months of >100 alcoholic drinks per month.

AMNART, American National Adult Reading Test; AST, aspartate aminotransferase; BDI, Beck Depression Inventory; BMI, body mass index; CIWA, clinical institute withdrawal assessment; GGT, γ-glutamyltransferase, RBC, red blood cells; STAI Y-2, State-trait Anxiety Inventory – State; WBC, white blood cells; local normal range = 7–64; Lifetime average, number of alcoholic drinks per month over lifetime; Prealbumin, local normal range = 18–45.

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Table 2

Age-adjusted mean tissue volumes for Val homozygotes and Val/Met heterozygotes at baseline (TP1) and follow-up (TP2), percent tissue volume change, and *P*-values of paired *t*-tests. The time point specific measures are mean ± SD and expressed in percent of ICV

Region	Genotype	TP1 (mean ± SD)	TP2 (mean ± SD)	% Change	*P*-value
Frontal GM	Val/Met	14.24 ± 0.57	14.28 ± 0.64	0.30	NS
	Val/Val	14.19 ± 0.53	14.36 ± 0.65	1.20	<0.001
Parietal GM	Val/Met	7.87 ± 0.40	7.90 ± 0.46	0.38	NS
	Val/Val	7.84 ± 0.37	7.92 ± 0.46	1.02	0.002
Temporal GM	Val/Met	9.74 ± 0.41	9.80 ± 0.47	0.60	0.021
	Val/Val	9.74 ± 0.38	9.81 ± 0.47	0.70	0.020
Occipital GM	Val/Met	3.69 ± 0.30	3.71 ± 0.34	0.54	NS
	Val/Val	3.63 ± 0.28	3.64 ± 0.34	0.28	NS
Total cortical GM	Val/Met	35.55 ± 1.22	35.69 ± 1.40	0.39	0.12
	Val/Val	35.40 ± 1.13	35.72 ± 1.40	0.90	<0.001
Frontal WM	Val/Met	13.44 ± 0.69	13.64 ± 0.78	1.49	0.020
	Val/Val	13.90 ± 0.65	13.89 ± 0.79	−0.07	NS
Parietal WM	Val/Met	6.32 ± 0.39	6.39 ± 0.44	1.11	0.061
	Val/Val	6.32 ± 0.37	6.33 ± 0.45	0.16	NS
Temporal WM	Val/Met	3.74 ± 0.20	3.80 ± 0.22	1.60	0.062
	Val/Val	3.77 ± 0.19	3.75 ± 0.2	−0.53	NS
Occipital WM	Val/Met	2.27 ± 0.19	2.28 ± 0.21	0.44	NS
	Val/Val	2.31 ± 0.18	2.30 ± 0.22	−0.43	NS
Total lobar WM	Val/Met	25.78 ± 1.14	26.09 ± 1.28	1.18	0.018
	Val/Val	26.22 ± 1.11	26.21 ± 1.32	0.00	NS
Caudate GM	Val/Met	0.48 ± 0.04	0.47 ± 0.04	−2.08	NS
	Val/Val	0.47 ± 0.04	0.46 ± 0.04	−2.13	0.013
Lenticular GM	Val/Met	0.44 ± 0.05	0.43 ± 0.06	−2.27	NS
	Val/Val	0.43 ± 0.05	0.44 ± 0.06	2.33	NS
Thalamic GM	Val/Met	0.37 ± 0.07	0.38 ± 0.08	2.70	NS
	Val/Val	0.37 ± 0.06	0.38 ± 0.08	2.70	0.008
Cerebellum	Val/Met	8.18 ± 0.62	8.24 ± 0.71	0.73	0.034

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Region	Genotype	TP1 (mean ± SD)	TP2 (mean ± SD)	% Change	*P*-value
	Val/Val	7.97 ± 0.60	8.00 ± 0.74	0.37	NS
Brainstem	Val/Met	1.83 ± 0.13	1.85 ± 0.14	1.09	0.022
	Val/Val	1.80 ± 0.12	1.81 ± 0.15	0.56	NS

$P > 0.12$ listed as not significant (NS).

'% change' defined as ((TP2 measure − TP1 measure)/TP1 measure) × 100.

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Table 3

Associations of longitudinal changes in neurocognitive measures with longitudinal changes in brain tissue volumes in ALC; both genotypes combined

Neurocognitive measure	Region	Parameter estimate (Slope ± SE)	*P*-value
BVMT-R learning	Frontal GM	0.05 ± 0.02	0.043
WAIS-III digit span		0.04 ± 0.02	0.032
WAIS-III digit symbol		0.26 ± 0.11	0.019
WAIS-III symbol search		0.06 ± 0.03	0.021
BVMT-R learning	Parietal GM	0.10 ± 0.04	0.019
WAIS-III digit span		0.06 ± 0.03	0.032
WAIS-III digit symbol		0.32 ± 0.10	0.002
WAIS-III symbol search		0.11 ± 0.05	0.017
BVMT-R learning	Temporal GM	0.09 ± 0.04	0.010
	Occipital GM	0.14 ± 0.07	0.041
	Caudate GM	1.09 ± 0.52	0.038
	Lenticular GM	1.42 ± 0.44	0.002
	Thalamic GM	1.03 ± 0.42	0.002
	Cerebellum	0.10 ± 0.05	0.002
	Total cortical GM	0.024 ± 0.01	0.002

BVMT-R, Brief Visuo-spatial Memory Test-Revised.