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Childhood Stress, Serotonin Transporter Gene and Brain Structures in Major Depression

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The underlying neurobiology of major depression (MD) is likely to represent an interaction between genetic susceptibility and environmental factors such as stress. We investigated, in a multimodal high-resolution magnetic resonance imaging (MRI) genetic study, whether reduced hippocampal volumes and other brain alterations are associated with the tri-allelic polymorphism of the serotonin transporter and childhood stress in patients with MD and healthy subjects. Patients with MD and healthy participants were investigated using high-resolution MRI and genotyping for serotonin transporter polymorphism in the promoter region of the serotonin transporter gene (*SLC6A4*, *5-HTTLPR*). Region of interest analysis of the hippocampus, whole-brain voxel-based morphometry (VBM), and assessment of childhood stress were carried out. Patients carrying the risk S-allele developed smaller hippocampal volumes when they had a history of emotional neglect compared with patients who only had one risk factor (environmental or genetic). In patients, childhood stress also predicted further hippocampal white matter alterations independently from the genotype. Moreover, the left prefrontal cortex was smaller in patients, whereby childhood stress resulted in larger prefrontal volumes in those subjects carrying the non-risk L-allele, suggesting preventive effects. The findings indicate that subjects with both environmental and genetic risk factors are susceptible to stress-related hippocampal changes. Structural brain changes due to stress represent part of the mechanism by which the illness risk and outcome might be genetically mediated.
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INTRODUCTION

Major depression (MD) is one of the most common human diseases, with a lifetime prevalence of 16% and a 12-month prevalence of 6.6% (Kessler *et al*, 2003). A recent meta-analysis on 14 250 participants showed that stressful life events have a potent association with the risk of developing depression (Risch *et al*, 2009). The underlying neurobiology of MD can be understood as the interaction of genetic susceptibility and environmental factors. On the basis of the meta-analysis, the serotonin transporter promoter polymorphism (*5-HTTLPR*) does not additionally seem to enhance the risk for MD in subjects with critical life events (Risch *et al*, 2009). However, it may still be a matter of debate whether early-life stress and the 5-HTTLPR may interact as shown by Caspi *et al* (2003). Moreover, an increased risk for MD was also detected in maltreated children homozygous for the short (S)-allele (Kaufman *et al*, 2006). Early-life neglect and abuse produce long-lasting emotional problems (Heim and Nemeroff, 2001). Experimental animal studies have shown stress-related neuroplastic changes in the brain that reverse under effective antidepressant treatment (McClung and Nestler, 2008). In line with this hypothesis, neuroimaging studies have shown that the hippocampus is about 4–5% smaller in patients with MD than in healthy controls, and that reduced hippocampal volumes are consistently found in MD (Campbell *et al*, 2004; Frodl *et al*, 2008d; Videbech and Ravnkilde, 2004). These hippocampal alterations are clinically relevant, because subjects who enter remission have larger hippocampal volumes than those who do not achieve remission (for review see MacQueen, 2009). In addition, pediatric patients with familial MDD showed decreased hippocampal volumes, indicating that reduced hippocampal volume may be present at very early stages and may be suggestive of a risk factor for developing MDD (MacMaster *et al*, 2008). In addition, volumes of other brain regions such as the prefrontal cortex, orbitofrontal cortex, gyrus cinguli, and the basal ganglia had been found to be reduced in patients with MD compared with healthy controls (Frodl *et al*, 2008d).

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Three studies reported that these hippocampal volume reductions and basal ganglia abnormalities are influenced by *5-HTTLPR* in patients with depression (Frodl *et al*, 2008d). The diallelic L/L genotype of the *5-HTTLPR* was associated with smaller hippocampal volumes in 40 patients with MD and was not associated in 40 healthy controls (Frodl *et al*, 2004b). Moreover, 63 patients with a late-onset geriatric depression who were homozygous for the L-allele of the *5-HTTLPR* exhibited smaller hippocampal volumes than those with other genotypes, whereas a significant association between the S-allele and smaller hippocampal volumes was observed in 72 patients with an early age of onset (Taylor *et al*, 2005). Another study did not find a significant association between *5-HTTLPR* and hippocampal or amygdala volumes in 45 patients with MD, whereas the short allele of *5-HTTLPR* was associated with smaller caudate nucleus volumes (Hickie *et al*, 2007).

However, so far the interaction between environmental factors, genetic susceptibility, and brain changes has not been investigated in patients with MD. The aim of this study was to investigate whether reduced hippocampal and prefrontal volumes in patients with MD are associated with *5-HTTLPR* and childhood stress either independently or interactively.

Therefore, we indexed brain volume using magnetic resonance imaging (MRI), voxel-based morphometry (VBM), a well-established method, as well as manual tracing of the hippocampus. We used the well-validated childhood trauma questionnaire (CTQ), which assesses trauma in terms of emotional neglect, emotional abuse, physical abuse, sexual abuse, and physical neglect. We genotyped the *5-HTTLPR* as previously described (Frodl *et al*, 2008b). To test our hypothesis, we undertook both regression analysis and repeated measures ANOVA.

MATERIALS AND METHODS

Participants

A total of 24 patients (aged 18–65 years) being treated for their first time as inpatients for MD at the Department of Psychiatry and Psychotherapy of the Ludwig-Maximilians-University, Munich, were investigated with structural MRI and childhood stress. Psychiatric diagnoses were made according to DSM-IV criteria and the structured clinical interview for DSM-IV (SCID) (First *et al*, 1997, 2001; Wittchen *et al*, 1997), and were determined by a consensus of at least two psychiatrists. Clinical variables were documented using the 21-item Hamilton Depression Rating Scale (HDRS).

Patients were compared with 27 healthy control subjects from the local community who were matched for age (aged 18–65 years) and gender. Demographic variables and exclusion criteria were assessed using a standardized questionnaire. Neither the healthy controls nor their first-degree relatives had a history of neurological or mental illness. Age, gender, handedness, and weight were similar in patients and controls. Patients drank less alcohol at the last time before investigation compared with healthy controls, because they were admitted to hospital (Table 1). At the time of MRI scanning, patients received the following medication: seven patients received serotonin reuptake inhibitors, seven received tricyclic antidepressants, three mirtazapine, four venlafaxine, two reboxetine, and one patient received no medication. Age, gender, weight, and alcohol consumption did not differ in healthy controls with the S-allele of *5-HTTLPR* and those with the LL genotype. These variables as well as age of onset, cumulative illness duration, and HDRS did not differ in patients with the S-allele of *5-HTTLPR* and those with the LL genotype.

Table 1 Demographic and Clinical Data of Healthy Controls and Patients with an Episode of Major Depression

	Patients ($n = 24$) Mean ± SD	Controls ($n = 27$) Mean ± SD	t-Test[a]/ χ^2-test[b] *p*-value
Age (years)	43.8 ± 11.9	41.9 ± 13.2	0.58[a]
Female/male*	18/9	13/11	0.36[b]
Weight (kg)	65.0 ± 12.5	71.1 ± 15.0	0.12[a]
Age of onset (years)	38.3 ± 9.9		
Cumulative illness duration (months)	31.3 ± 39.3		
HDRS—baseline	24.1 ± 6.2		

Mean and standard deviations (±) are given. *No significant differences were found between patients and controls, as measured with the *t*-test or χ^2-test.

A structured interview was used to assess medical history, trauma, and other exclusion criteria. Exclusion criteria for all subjects were previous head injury with loss of consciousness, earlier treatment with hydrocortisone, a history of alcohol or substance abuse, and neurological diseases. Comorbidity with other mental illnesses, for example, bipolar disorders or personality disorders, was also an exclusion criterion. None of the subjects had ever been treated with electroconvulsive therapy. Handedness was determined by the Edinburgh inventory (Oldfield, 1971).

Written informed consent was obtained from patients and controls after they had been given a detailed description of the study. The study was designed and performed in accordance with the ethical standards laid down in the Declaration of Helsinki and was approved by the local ethical committee.

MRI Procedures

Magnetic resonance imaging images were obtained (1.5 Tesla Magnetom Vision, Siemens, Erlangen, Germany) using a coronal T2- and proton density-weighted Dual-Echo-Sequence (TR 3710 msec/TE 22/90 msec; total acquisition time: 9 min, number of acquisitions: 1; field of view (FOV) 230 mm; matrix 240 × 256, slice thickness: 3 mm) and a 3D-MPRAGE sequence (TR/TE 11.6 msec/4.9 msec; total acquisition time: 9 min, number of acquisitions: 1; FOV 230 mm; matrix 512 × 512, slice thickness: 1.5 mm). The commercial software package Analyze was used (ANALYZE, Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) for further image processing, with size reduction from 16 to 8 bit and transformation to a uniform matrix of 256 × 256 on 192 slices of 1.0-mm slice thickness. All data sets were realigned and resampled three

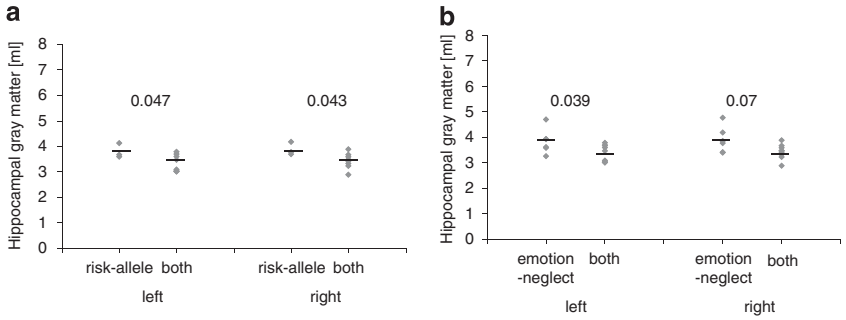

Figure 1 Patients carrying the risk S-allele developed smaller hippocampal volumes when they had suffered emotional neglect ($N = 9$) compared with those who had only one risk allele ($N = 6$ for emotional neglect and $N = 3$ for risk S-allele carrier). (a) Carriers of both risk factor versus carriers of only the genetic risk factor—$F = 5.2$, df = 1,13, $p = 0.039$. (b) Carriers of both risk factors versus carriers of only the environmental risk factor—$F = 5.1$, df = 1,10, $p = 0.048$). *P*-values for the left and right hippocampal volume comparisons are also indicated.

dimensionally in the anterior commissure to posterior commissure (AC–PC) line, according to the coordinates of Talairach, using the software program BRAINS (Brain Research: Analysis of Images, Networks and Systems; developed by Andreasen *et al* (1992). The program BRAINS allowed the regions of interest (ROIs) to be controlled on sagittal and transverse sections simultaneously, and to be segmented to enable calculation of the intracranial content (ICC) and the gray and white matter (WM) volume (ccm^3) within the defined ROI. Software and hardware was not changed during the study.

Definition of the Hippocampal Formation

For a detailed description of the hippocampal borders, see Frodl *et al* (2002b). The description is illustrated in Figure 1. The evaluation staff (ER) was blind to subject status. The hippocampus was outlined manually using a mouse-driven cursor.

To determine inter-rater reliability, 10 brains were randomly chosen and ROIs determined by two raters (TF, ER) independently. The intraclass correlation for both the inter-rater reliability (hippocampus: $r_{ICC} = 0.97$) and the intra-rater reliability (hippocampus: $r_{ICC} = 0.96$) was high (Frodl *et al*, 2002a, 2002b). Morphometric data were normally distributed.

Voxel-Based Morphometry

After manually reorienting and centering the images on the anterior commissure, data pre-processing was performed using the SPM5 software package (Wellcome Department of Cognitive Neurology, London, UK) running under MATLAB 6.5 (The MathWorks, Natick, MA).

The present study employed the VBM5 toolbox, which uses and extends the new unified segmentation approach implemented in SPM5 (Ashburner and Friston, 2005). Unified segmentation provides a generative model of VBM pre-processing that integrates tissue classification, image registration, and MRI inhomogeneous bias correction. Thus, the model avoids the 'circularity problem' of the optimized VBM procedure, as the initial image registration does not require an initial tissue segmentation and vice versa (Good *et al*, 2001). The VBM5 toolbox extends the unified segmentation model, as it increases the quality of segmentation by applying a Hidden Markov Field model on the segmented tissue maps (Cuadra *et al*, 2005).

The final tissue maps of gray matter (GM), WM, and cerebrospinal fluid were modulated with the deformation fields obtained by normalization to standard space to analyze volume differences between study populations. Finally, the modulated GM partitions were smoothed with a 12-mm FWHM Gaussian kernel and used for statistical analysis.

An analysis of covariance (ANCOVA) was designed to investigate focal gray matter volume (GMV) differences between the patients with MD and healthy controls, as well as between genotypes. Age and gender were entered as covariates of no interest in the statistical design. First, GM volume differences (increases/decreases) between patients with MD and healthy controls were assessed at the whole-brain level using T contrasts ($p < 0.05$, FWE corrected).

To analyze the interaction between childhood stress as well as cumulative illness duration and genotype on the structural alterations, we extracted the eigenvariate from the significant resulting differences in GM volume and further used these data for statistical analysis.

Childhood Stress

The CTQ (Bernstein *et al*, 1994; Bernstein *et al*, 2003) was used to assess childhood stress. This questionnaire is a self-report instrument that assesses five types of childhood maltreatment, namely, emotional, physical, and sexual abuse, and emotional and physical neglect. Subjects rate items about childhood experiences (defined as before the age of 18 years) on five-point Likert-type scales anchored by 'never true' and 'very often true.' Reliability and validity of the CTQ have been established, including measures of convergent and discriminate validity from structured interviews, stability over time, and corroboration (Bernstein *et al*, 1994; Bernstein *et al*, 2003).

Genetics

The DNA was extracted from a 5 ml blood sample using the QIAamp Blood Isolation Kit (QIAGEN GmbH, Hilden, Germany) according to the supplier's instructions. We used the tri-allelic polymorphism *SNP-rs25531* because the L-allele can be subtyped into La- and Lg-alleles, the latter

of which is thought to be similar to the S-allele in terms of reuptake efficiency (Hu *et al*, 2005). Genotyping was carried out by applying the PCR amplification in a final volume of 25 µl consisting of 50 ng DNA, 1 µmol/l of each primer, 200 µM deoxynucleotide triphosphate, 100 µM 7-deazaguanosine triphosphate, 5% dimethyl sulfoxide, 10 mM Tris hydrochloride (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, and 2.5 U of DNA polymerase (AmpliTaq Gold; PerkinElmer, Langen, Germany). The PCR products were separated on a 3% agarose gel (FMC NuSieve 3 : 1; Biozym Diagnostic GmbH, Oldendorf, Germany) and visualized by ethidium bromide staining.

Statistical Analyses

All statistical tests were considered to be significant if $p<0.05$. Morphometric measurements in both groups were normally distributed (using Kolmogorov–Smirnov test) and their variance was homogenous (using Levine's test). The genotypes of patients and controls were in Hardy–Weinberg equilibrium.

Hippocampal volumes were subjected to ANCOVA to assess the main and interaction effects of the within-subjects factor, hemisphere (left, right), and the between-subjects factors, diagnosis (depression, control) and gender (male, female), using total brain volume as the covariate. The whole-brain analysis for differences was carried out as described above.

To test our hypothesis that childhood stress and the *5-HTTLPR* polymorphism are independently and interactively associated with hippocampal volume, we undertook both regression analysis and repeated measures ANOVA. Patients showed significantly increased values compared with healthy controls for emotional neglect ($t=-2.1$, $df=1,49$, $p=0.040$) and physical neglect ($t=-2.5$, $df=1,49$, $p=0.019$); hence, we included these two items in the analysis as follows:

Hippocampal volumes were subjected to an ANOVA to assess the main and interaction effects of the within-subject factor, hemisphere (left, right), and the between-subject factors, diagnosis (depression, control), childhood stress (emotional neglect and physical neglect, high/low according to median split), and *5-HTTLPR* (S-allele carriers, ll homozygous). The same design, without the factor hemisphere, was used for the extracted left prefrontal lobe volumes.

Linear multiple regression analyses were then used to examine whether childhood stress (emotional neglect or physical neglect) and *5-HTTLPR* independently predicted hippocampal or prefrontal volumes in patients or controls. These analyses had two steps: (1) *5-HTTLPR* (S-allele carriers, ll homozygous) was regressed on hippocampal volumes. (2) Emotional neglect or physical neglect was regressed on the remaining hippocampal variance.

RESULTS

Association between Stress, *5-HTTLPR*, and Hippocampus

There was no difference in hippocampal GMVs between patients and healthy controls and no significant hemisphere (left, right) effect. However, for patients, when subjects were grouped on the basis of (a) *5-HTTLPR* polymorphism and (b) high versus low emotional neglect (based on a split halves cutoff) in the repeated measures ANOVA, an interaction between these variables was observed for hippocampal gray matter ($F=7.1$, $df=1,20$, $p=0.015$). Patients who both carried the S-allele and had a positive history for emotional neglect developed smaller hippocampal volumes than patients with only one of these risk factors (Figure 1a and b). Hippocampal WM was significantly smaller in patients than in controls ($F=16.3$, $df=1,46$, $p<0.001$). There was a significant interaction between diagnosis and *5-HTTLPR* ($F=8.2$, $df=1,46$, $p=0.006$), but no significant interaction between *5-HTTLPR* and emotional neglect in patients ($p>0.05$).

The regression analysis revealed that in the patient group, *5-HTTLPR* polymorphism explained 33% of variance in hippocampal WM volume ($r^2_{change}=0.33$; $F_{change}=10.8$, $df=1,22$, $p=0.003$; 95% CI: 0.04–1.6). After the effects of genotype were accounted for, emotional neglect independently accounted for a further 24% of variance in WM volume ($F_{change}=11.4$, $df=1,21$, $p=0.003$, Figure 2a and b). Together, these two variables, that is, *5-HTTLPR* and emotional neglect, alone explained 57% of variance in hippocampal WM. In healthy volunteers, neither stress nor *5-HTTLPR* polymorphism explained a significant proportion of variance in hippocampal volume ($p>0.05$). The regression analysis did not show independent effects of *5-HTTLPR* or childhood stress on hippocampal gray matter.

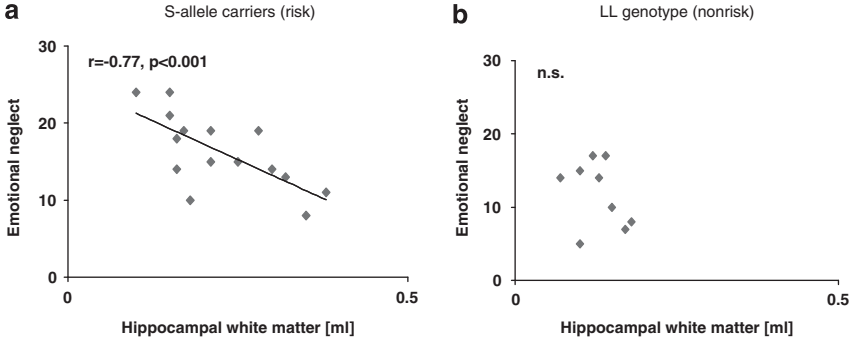

Figure 2 Childhood stress goes along with hippocampal reductions in genetically vulnerable patients carrying at least one short allele of the 5-HTTLPR. Scatterplots of total hippocampal volumes (ml) and childhood stress. (a) S-allele carriers show significant negative correlations between hippocampal white matter and emotional neglect. (b) Childhood stress shows no significant association with hippocampal volumes in patients with the LL genotype.

Association between Stress, *5-HTTLPR*, and Prefrontal Cortex

A significant cluster for smaller GMVs in patients compared with healthy controls was detected in the left prefrontal cortex with extensions to the left orbitofrontal and to the pre-/postcentral cortices in the VBM ($p<0.05$, FWE corrected, Figure 3). There was a significant interaction between emotional neglect and *5-HTTLPR* toward dorsolateral prefrontal volumes (DLPFC) ($F=3.8$, $df=1,50$, $p=0.05$). *Post hoc* analysis indicated larger DLPFC in subjects homozygous for the L-allele, irrespective of being healthy or depressed, who had a history of emotional neglect compared with those without a history of emotional neglect ($F=4.9$, $df=1,15$, $p=0.04$).

DISCUSSION

Evidence for the importance of epigenetic processes derives from the finding that the S-allele of the *5-HTTLPR* affects susceptibility to hippocampal changes in the case of critical

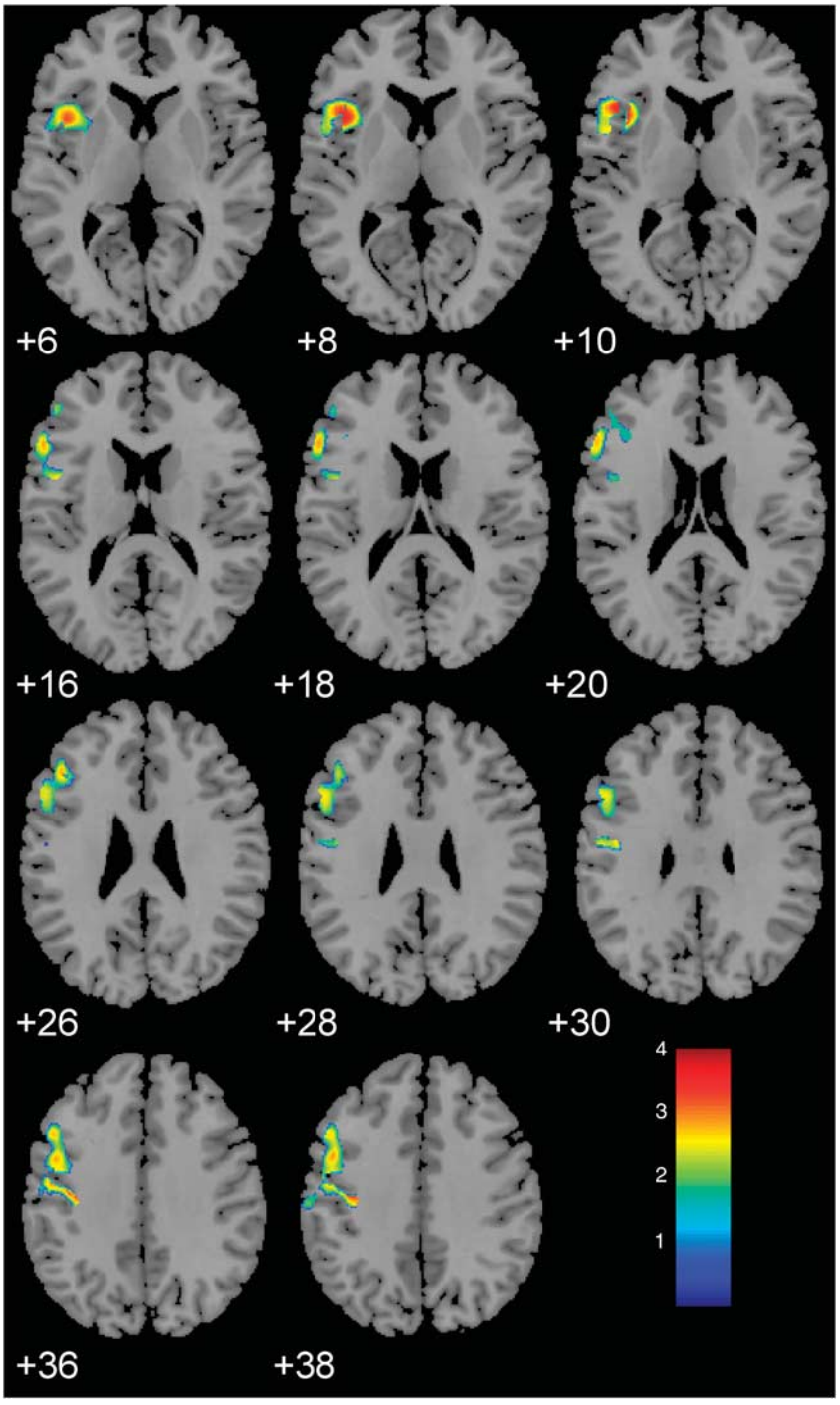

Figure 3 Voxel-based morphometry resulted a in significant smaller cluster for gray matter volumes within the left orbitofrontal–dorsolateral prefrontal cortex for patients compared with healthy controls (FWE corrected, $p<0.05$).

stressful childhood events in patients with MD. This is important because it weaves disparate threads together into a coherent hypothesis of how susceptibility to depression might arise when the environment interacts with genetic factors to change neuroanatomy.

The gene–environmental interaction affected even the gray matter of the hippocampal formation, suggesting a disturbance of this structure in patients with MD who carry the genetic and the environmental risk factors compared with those, who only have one of these risk factors. Both of these two risk factors resulted independently in significant changes of the hippocampal WM, which seems to be more sensitive to subtle changes. The hippocampal WM mainly reflects the outgoing fibers of the fimbria to the additional structures of the limbic system. Therefore, these WM changes may be the result of an axonal reduction due to cell loss or to primary neuroplastic changes, which might favor cognitive as well as emotional disturbances during depression (McClung and Nestler, 2008).

Evidence for the importance of epigenetic processes also derives from a study in healthy controls using MRI, genotyping, and self-reported life stress. It could be shown that life stress affected, as a function of serotonin transporter genotype, the structural features of the gray matter, as well as functional connectivity of the amygdala and hippocampus with a wide network of other regions (Canli *et al*, 2006). Another recent study in healthy controls could show that Met-allele carriers of the *BDNF* polymorphism have smaller hippocampal volumes when they have elevations in stress as well as when they show more neuroticism (Joffe *et al*, 2009). In a recent study of 89 healthy participants, significant interactions between BDNF genotype and early-life stress were detected on hippocampal and amygdala volumes, heart rate, and working memory. Using structural equation modeling (SEM), the explicit pathways were investigated through which *BDNF* genotype and early-life stress interact to produce effects on brain structure, body arousal, emotional stability, and in turn predict alterations in symptoms and cognition. SEM suggested that the combination of Met carrier status and exposure to early-life stress predicted reduced HC volumes, and, in turn, associated lateral prefrontal cortex volumes and higher depression (Gatt *et al*, 2009).

All these studies support the hypothesis that early-life stress and in addition interactive genetic variants contribute to smaller hippocampal volumes and, in turn, may then increase the risk to MD. These smaller hippocampal volumes, or of course the underlying neurobiological processes (for review see Dranovsky and Hen, 2006; Duman, 2004; Sapolsky, 2000) of these smaller hippocampal volumes, are clinically relevant. Hence, longitudinal studies showed an association between smaller hippocampal volumes and a worse clinical outcome (Frodl *et al*, 2008a; Frodl *et al*, 2004a). Moreover, patients with geriatric depression and small right hippocampal volumes were less likely to achieve remission compared with those with larger hippocampal volumes (Hsieh *et al*, 2002), and women who responded to 8 weeks of fluoxetine had larger right HC volumes than non-responders (Vakili *et al*, 2000). Smaller HC volumes also predicted low rates of remission in patients with no past treatment history, suggesting that the association between HC volumes and short-term clinical response was not simply a function of past treatment responsiveness (MacQueen *et al*, 2008).

Whereas *5-HTTLPR* modulated the effects of childhood stress on hippocampal volumes, indicating that the hippocampus is a stress-sensitive brain region, this was different for the DLPFC. Subjects with more emotional neglect had larger prefrontal cortex volumes when they carried the non-risk allele. This effect was seen in the whole sample and was irrespective of diagnosis, suggesting that compensatory or preventive strategies could result in increased cortex volumes during development. Furthermore, this underlines the role of the serotonergic system in neuroplasticity and brain development, as discussed in the next section. The L-allele might have protective effects in subjects who had emotional neglect, in line with a study showing increased DLPFC in monkeys raised only together with monkeys in their age compared with monkeys raised in their families (Spinelli *et al*, 2009). In turn, this might correspond to the better response of those patients, who carry the L-allele, to antidepressant treatment (Serretti *et al*, 2007).

To bridge the gap between childhood stress, reduced structural brain volumes, and serotonin transporter polymorphisms, it is interesting to speculate, how specifically subjects might be vulnerable to develop smaller brain structures. With extreme or chronic stress, there are volumetric decreases in the hippocampal formation, with an increased vulnerability to metabolic insults, and even death of the CA3 region (Sapolsky, 2003). High levels of glucocorticoids (Woolley *et al*, 1990) or behavioral stress (Magarinos *et al*, 1996; Watanabe *et al*, 1992) result in atrophy and retraction of the apical dendrites of hippocampal pyramidal cells. Prolonged stress and increased levels of glucocorticoids also disrupt hippocampal neurogenesis (Dranovsky and Hen, 2006; Duman, 2004). Interestingly, the hippocampus is vulnerable to stress, particularly during the early developmental period (Teicher *et al*, 2003). In addition to its role as a neurotransmitter, serotonin acts as a trophic factor modulating developmental processes, such as neuronal division, differentiation, migration, synaptogenesis (Gaspar *et al*, 2003), early CNS development (Lauder, 1993), and adult neurogenesis (Gould, 1999). Considering the functional role of the serotonin transporter polymorphism—the S-allele of the *HTTLPR* shows decreased 5-HTT expression and decreased 5-HT-reuptake *in vitro* and *in vivo* (Bengel *et al*, 1998; Heinz *et al*, 2000; Lesch *et al*, 1996)—recent experimental studies on the interaction between serotonin and stress may be helpful for bridging the gap to the underlying mechanisms. Interestingly, early parental deprivation in marmoset monkeys, in the absence of subsequent stressors, already has a long-term effect on the hippocampal expression of genes implicated in synaptic function and plasticity, such as reductions of GAP-43 and serotonin 1A receptor expressions, which are comparable with findings in mood disorder. However, the stressor did not change the hippocampal volume (Law *et al*, 2009), supporting the view that hippocampal atrophy in mood disorder is not related to the early developmental component of pathogenesis, but is a correlate or consequence of the disorder (Czeh and Lucassen, 2007), or that other factors such as genetic factors might be necessary (Frodl *et al*, 2008c). The latter view is

supported by another recent study. Serotonin transporter knockout rats had lower basal Arc mRNA levels, which is a marker for synaptic plasticity, in the hippocampus and prefrontal cortex and showed altered stress responsiveness compared with wild-type rats. Hence, an acute swim stress test significantly upregulated the levels of Arc mRNA in the hippocampus and prefrontal cortex, as well as of Zif-268, in the frontal cortex, only in mutant SERT(+/−) and SERT(−/−) rats (Molteni *et al*, 2009). For explanation, Arc is an immediate early gene that might bridge neuronal activity with structural remodeling and functional changes, and it is also thought to have a role in activity-dependent synaptic modifications (Bramham *et al*, 2008); Zif-268, also known as early growth response gene 1 (Egr-1), is tightly linked to neuronal plasticity (Knapska and Kaczmarek, 2004). Therefore, we might speculate that risk S-allele carriers show an enhanced stress response and increased neuroplastic changes due to early-life stress. This, in turn, might affect neuronal development and result in smaller hippocampal and prefrontal cortex volumes, as seen in the present study in humans.

A limitation of the present study is the relatively small sample size to conduct subgroup analysis with respect to lifetime illness course. It also has to be mentioned that abuse, in particular, sexual abuse, remains underreported, for example, in healthy individuals (McBeth *et al*, 2001). A bias caused by the acute depressive episode is unlikely, because we assessed the questionnaire after patients improved and were discharged from the hospital. The findings have to be confirmed with a larger sample in a prospective study.

For conclusion, the structural hippocampal brain changes due to stress represent part of the mechanism by which the illness risk to develop MD may be genetically mediated and, therefore, underline the importance of stress–gene–brain interactions in MD. These findings may also underline the need for psychotherapy in the treatment of MD and to find antidepressants that enhance neuroplasticity and result in a normalization of neuronal alterations.

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DISCLOSURE

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