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# Expression of glucocorticoid inducible genes is associated with reductions in cornu ammonis and dentate gyrus volumes in patients with major depressive disorder

THOMAS FRODL,[a,b] ANGELA CARBALLEDO,[a] EVA-MARIA FREY,[b] VERONICA O'KEANE,[a] NORBERT SKOKAUSKAS,[a,c] DERREK MORRIS,[a] MICHAEL GILL,[a] MARTINA MARY HUGHES,[a] ANDREW HARKIN,[a] AND THOMAS CONNOR[a]

[a]*Trinity College Dublin;* [b]*University of Regensburg; and* [c]*Norwegian University of Science and Technology*

**Abstract**

Alterations of the glucocorticoid system and of hippocampal volumes have consistently been reported in patients with major depressive disorders (MDD). The aim of the present study was to investigate whether the messenger RNA (mRNA) expression of glucocorticoid inducible genes is associated with changes in the cornu ammonis (CA) and dentate gyrus subfields. Forty-three patients with MDD and 43 healthy controls were recruited and investigated with high resolution magnetic resonance imaging. Hippocampal subfields were measured using freesurfer. Measurement of whole blood mRNA expression of glucocorticoid inducible genes serum and glucocorticoid-regulated kinase 1 (*SGK1*), FK506 binding protein 5 (*FKBP5*), and glucocorticoid induced leucine zipper (*GILZ*) was performed. Patients with MDD had significantly smaller volumes of CA1, CA2/3, CA4/DG, and subiculum compared to healthy controls. In the regression analysis, the factor diagnosis had a significant moderating effect on the association of *SGK1* and hippocampal volumes. Patients with low expression of *SGK1* had significantly smaller CA2/3 and CA4/DG volumes compared to patients with high expression of *SGK1* mRNA and to healthy controls with low/high expression of *SGK1*, respectively. Therefore, a lack of mRNA expression of glucocorticoid inducible genes in patients with MDD that seems to correspond to a blunted cortisol response is associated with smaller hippocampal CA and dentate gyrus volumes. *SGK1* seems to be particularly relevant for stress-related mental disorders.

Major depressive disorder (MDD) is among the most prevalent and burdensome of all psychiatric illnesses (Goetzel, Hawkins, Ozminkowski, & Wang, 2003; Murray & Lopez, 1996). Evidence has suggested that aberrant neuronal plasticity or neural remodeling play a significant role in the pathophysiology of MDD. Recent experimental studies reveal that certain aspects of depression result from maladaptive, stress-induced neuroplastic changes in specific neural circuits (Krishnan & Nestler, 2008). Stress, including psychological, emotional, or psychosocial stress, is associated with structural changes to the hippocampus (Chaney et al., 2014; Frodl & O'Keane, 2013). Multiple studies have demonstrated that patients with MDD hypersecrete cortisol (Vreeburg et al., 2009), have impaired glucocorticoid receptor (GR) functioning (Pace & Miller, 2009), and have reduced hippocampal volumes (MacQueen & Frodl, 2011).

In cases when stress hits the individual in sensitive developmental time windows it may have enduring effects on the neural and neuroendocrine systems (Danese & McEwen, 2012). This is understandable because the human brain shows significant age-related changes at least until young adulthood (Giedd & Rapoport, 2010). Thus, the time when stress occurs in addition to the duration and severity of stress might be very important. Early stress seems to result in overactivity of the stress hormone system, but later due to ongoing stress the cortisol response diminishes and results in blunted responses (Frodl & O'Keane, 2013; Trickett, Noll, Susman, Shenk, & Putnam, 2010).

In humans early life adversity was found to be associated with both hypothalamus–adrenal–pituitary (HPA) axis abnormalities as well as with hippocampal volume changes (Frodl & O'Keane, 2013). Chronic stress was found to be associated with smaller cortisol response to the dexamethasone suppression test, suggesting the development of glucocorticoid resistance (Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Raison & Miller, 2003). In the hippocampus, chronic stress exposure causes a plastic remodeling with volumetric reductions of the hippocampus (Sapolsky, Krey, & McEwen, 1986).

Understanding the development of these hippocampal and HPA axis abnormalities is important. An integrative attempt is necessary and needs to incorporate multiple levels of analysis of intermediate phenotypes. Then it might be possible to investigate disorders in a more dynamic fashion that reflects social, environmental, genetic, and neurobiological aspects

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Address correspondence and reprint requests to: Thomas Frodl, Department of Psychiatry and Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland; E-mail: frodlt@tcd.ie.

(Cicchetti & Toth, 2009). The first steps of a systemic neurosciences approach are to integrate brain imaging and molecular methods in order to understand the functional interplay between systems.

An association between higher cortisol levels and smaller hippocampal volumes has been supported from studies in healthy controls and samples with psychiatric disorders with a continuous measure of the cortisol levels over 1 day, rather than measures taken at one time of the day only (Frodl & O'Keane, 2013). Only one study in MDD investigated the association between 24-hr urinary cortisol and the hippocampus; it failed to find a significant result in the patient's group, but it was seen in the healthy comparison group (Vythilingam et al., 2004). Although some studies with smaller sample sizes of between 20 to 40 patients did not show such an association between cortisol measures post-dexamethasone (DST) and the hippocampal volume (Frodl & O'Keane, 2013), a study in a larger sample of patients with arteriosclerosis detected associations between hippocampal volume and DST cortisol (Knoops, Gerritsen, van der Graaf, Mali, & Geerlings, 2010).

Nearly all of these studies measured the whole hippocampal volume. However, hippocampal cell layers are precisely arranged in subfields of gyrus dentates and cornu ammonis (CA1–3; Jones & McHugh, 2011). These hippocampal subregions, like the dentate gyrus (DG) and the CA, have different functions and seem to have different stress sensitivities. Stress has been found to suppress neurogenesis and cause atrophy of the CA subfields in animal studies (McEwen & Magarinos, 2001), which are mostly present in hippocampal head and tail. In line with this, we recently found that childhood maltreatment was associated with smaller hippocampal head volumes in subjects at risk for MDD (Carballedo, 2012). Moreover, smaller volumes in the CA2/3-DG subfield of the hippocampus were linked to depressive symptoms and were associated with hyperreactivity of cortisol secretion during the day in multiple sclerosis patients (Gold et al., 2010), suggesting region-specific effects of stress and daily cortisol levels at least in subjects vulnerable for depression. In a study exploring hippocampal subfield volumes with high resolution magnetic resonance imaging (MRI), 9 unmedicated patients with MDD had lower DG volumes than 27 control subjects or 11 medicated patients with MDD and lower CA1–3 volumes than 27 control subjects (Huang et al., 2013).

There is thus a rationale for selectively exploring these subfields. Moreover, it might be interesting to simultaneously examine measures of cortisol functioning that might be linked to brain changes. In this context, the glucocorticoid inducible genes, such as the glucocorticoid inducible leucin zipper (*GILZ*), serum and glucocorticoid-inducible kinase-1 (*SGK1*), and FK506 binding protein 5 (*FKBP5*), are target genes activated by GR activation; their expression has been shown to be disrupted in the hippocampus as a result of hypercortisolemia (Sarabdjitsingh et al., 2010). A previous study on messenger RNA (mRNA) expression of *SGK1* found that *SGK1* mRNA is increased in the peripheral blood of drug-free depressed patients and in the hippocampus of rats after unpredictable chronic mild stress and prenatal stress. The GR target gene, *SGK1*, mediates the cortisol-induced decrease in proliferation and neuronal differentiation of human hippocampal progenitor cells, by acting both downstream of and upstream of GR activation (Anacker et al., 2013). A recent study demonstrated a relationship between downregulation of *GILZ* and an enhanced inflammatory profile in microglia isolated from mice subjected to a chronic stress regimen (Wohleb et al., 2011). Single nucleotide polymorphisms in *FKBP5* were found to be associated with response to antidepressants and the recurrence of depressive episodes (Binder et al., 2004). In a previous study on the sample under investigation, we had shown that patients with MDD, who had less expression of the glucocorticoid-inducible genes *GILZ* or *SGK1* had smaller total hippocampal volumes (Frodl et al., 2012).

The goal of this study was to extend this previous finding and to investigate whether there is a significant association among glucocorticoid inducible genes *SGK1*, *GILZ*, and *FKBP5* and hippocampal subfield volumes. As mentioned above, *SGK1*, *GILZ*, and *FKBP5* were hypothesized from experimental studies to play a role for MDD. Moreover, an aim was to explore whether these associations are specific to the CA subfields that were found to be influenced by experimental stress in previous studies. Another aim was to confirm in a larger sample changes in the hippocampal subfields DG and CA, in particular, region CA3 in patients with MDD compared to healthy controls.

## Methods

### *Participants*

The study included 43 adult patients with MDD from the mental health services of the Adelaide and Meath Hospital, incorporating the National Children's Hospital, Dublin, or St. James's Hospital, Dublin. The diagnosis of these patients with MDD was a clinical diagnosis based on DSM-IV criteria and confirmed by an independent psychiatrist using the Structured Clinical Interview for DSM Disorders. Forty-three healthy subjects (HC) from the local community were recruited, and the groups were balanced for age and gender (Table 1). Patients were prescribed monotherapy with antidepressants. Treatment with antipsychotics or mood stabilizers were exclusion criteria. Other exclusion criteria were ages of $<18$ or $>65$ years, a history of neurological or comorbid psychiatric disorders (Axis I or Axis II), other severe medical illness, or head injury or severe substance abuse in their lifetime history. Demographic variables, as well as inclusion and exclusion criteria, were documented using a standardized questionnaire and through a structured interview by a psychiatrist.

Written informed consent was obtained from all participants after being given detailed description of the study, which was designed and performed in accordance with the ethical standards laid out by the Declaration of Helsinki and was approved by the Research Ethics Committee of St. James and the Adelaide and Meath Hospitals, Dublin.

**Table 1.** *Demographic and clinical data*

| | Patients ($N = 43$) | Controls ($N = 43$) | *df* | Diagnosis Effect |
|---|---|---|---|---|
| Age | 41.2 (10.2) | 37.3 (13.0) | 84 | $T = 1.6, p = .12$ |
| Sex, female/male | 26/17 | 26/17 | 84 | $\chi = 0.17, p = .68$ |
| Height | 171.3 (8.4) | 171.9 (10.4) | 84 | $T = –0.27, p = .78$ |
| Weight | 74.8 (15.1) | 70.4 (16.2) | 84 | $T = 1.3, p = .19$ |
| Medication (none/SSRI/dual acting AD) | 12/15/16 | | | |
| Age of onset | 25.9 (12.4) | | | |
| Cumulative illness duration | 9.1 (9.9) | | | |
| Days treated | 2268.5 (3285.8) | | | |
| Days depressed and not treated | 1426.1 (2576.1) | | | |
| Hamilton depression score | 28.9 (5.9) | 2.3 (2.2) | 84 | $T = 27.7, p < .001$ |
| Beck Depression | 33.0 (11.1) | 1.9 (2.3) | 84 | $T = 18.0, p < .001$ |

*Note:* SSRI, Selective serotonin reuptake inhibitor; AD, antidepressant.

### *Rating instruments*

Self- and observer-rated scales were also filled out for all participants included in the study. The rating scales that were used were the following: the Childhood Trauma Questionnaire (CTQ) is a standardized self-report instrument that assesses five types of childhood trauma; the Hamilton Rating Scale for Depression (Hamilton, 1969) is an observer-rating instrument to assess current depression severity; the Beck's Depression Inventory (Beck, Steer, Ball, & Ranieri, 1996) is a self-rating instrument to indicate depression severity; and the Structured Clinical Interview for DSM-IV personality questionnaire (Spitzer, Williams, Gibbon, & First, 1992) is a self-rating and observer-rating instrument to assess personality disorders. Specifically, the CTQ (Bernstein et al., 1994) was used to assess childhood maltreatment: emotional, physical, and sexual abuse, and emotional and physical neglect. Reliability and validity of the CTQ have been established, including measures of convergent and discriminative validity from structured interviews, stability over time, and corroboration (Bernstein et al., 2003). Based on this previous research, a threshold was used in order to categorize adversity when a participant had scores greater than the cutoff score in at least one of the subscales of physical abuse (≥10) and/or emotional abuse (≥12) and/or sexual abuse (≥8) and/or emotional neglect (≥15) and/or physical neglect (≥10).

### *MRI data acquisition*

Magnetic resonance images were obtained with a Philips Achieva MRI scanner (Philips Medical System, Netherland B.V., Best, The Netherlands) operating at 3 Tesla. A sagittal Time 1 three-dimensional turbo field echo was used to scan all participants (repetition time user defined at 8.5 ms; echo time user defined at 3.9 ms; total acquisition time of 7 min; field of view of foot to head, 256 mm; anterior to posterior, 256 mm; right to left, 160 mm; 256 × 256 matrix). The slice thickness was 1 mm, and the voxel size was 1 × 1 × 1 mm.

### *Definition of hippocampus*

Hippocampal subfield volumes were assessed fully automatically with the software FreeSurfer. Freesurfer uses a Bayesian modeling approach, in which an explicit computational model of an MRI image around the hippocampal area is generated, and subsequently this model is used to obtain automated segmentations for the hippocampal subfields. The algorithm uses models built from manual segmentations of the hippocampus and applies these to the MRI data (http://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldSegmentation). The original development of this algorithm and detailed description can be found in Van Leemput et al. (2009).

Here we use the main hippocampal subfields belonging to the CA and the DG (CA1, CA2/3, and CA4/DG), as well as the subiculum and presubiculum (Figure 1). Manual quality control was done on each subject to ensure that the automated routines captured the hippocampus adequately. Moreover, data were checked for outliers using boxplots and histograms.

It was shown that automatically calculated volumes of CA2/3 and CA4/DG are strongly correlated with those volumes derived from manual delineation, with correlation coefficients of .91 ($P \leq .0002$) and .83 ($P \leq .0028$), respectively. Thus, the reliability of the subiculum ($r = .66$, $p = .06$) was weaker and that of the presubiculum was not significant (Van Leemput et al., 2009). Total intracranial volume was also measured using Freesurfer.

### *Measurement of expression of GR and glucocorticoid-inducible genes*

*Blood sampling.* A blood sample (2.5 ml) was drawn into a PAXgene blood RNA tube (Qiagen UK) and used for whole blood RNA isolation. The PAXgene tube was stored at –80 °C until RNA extraction was performed.

*Real-time polymerase chain reaction (PCR) analysis of mRNA expression of FKBP5, GILZ, and SGK1 in whole blood samples.* RNA isolation was performed using a

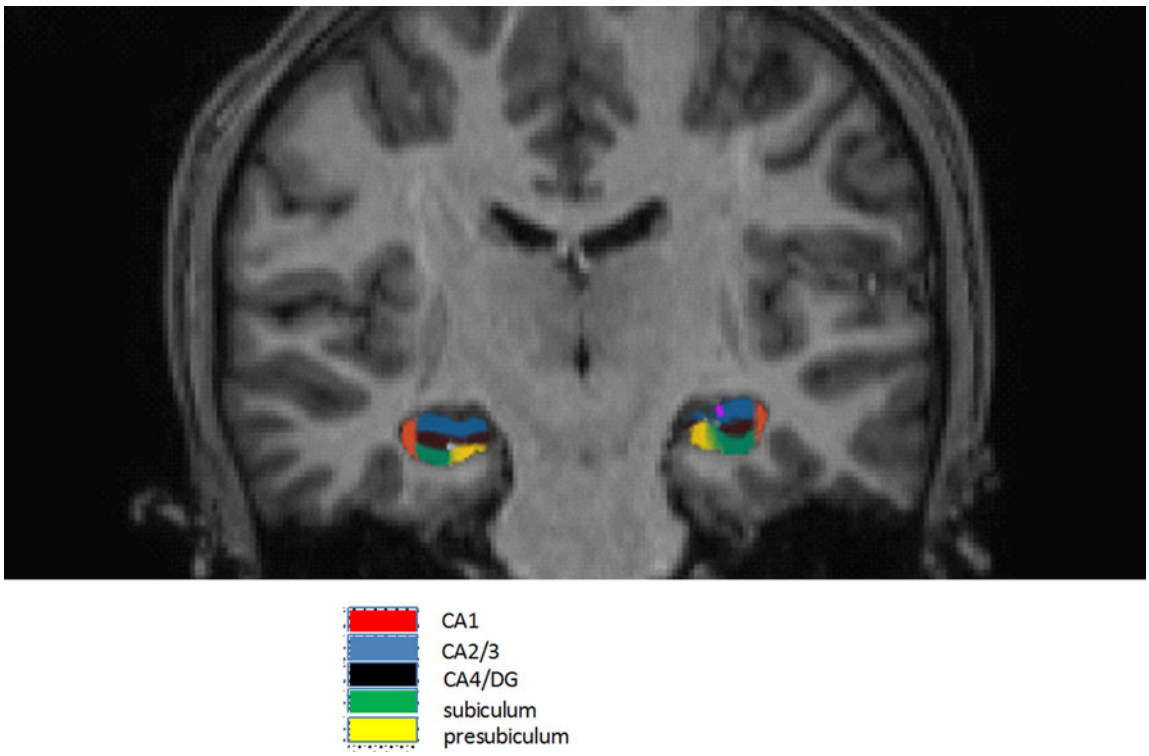


**Figure 1.** (Color online) Example for hippocampal subfield delineation. Shown are cornu ammonis (CA) subfields CA1, CA2/3, CA4/dentate gyrus (DG), subiculum, and presubiculum. The program Freesurfer automatically assessed volumes of subfields, which are then manually viewed and checked for quality.

PAXgene blood RNA kit (Qiagen) and was followed by DNAase treatment to remove contaminating genomic DNA as previously described (Chai, Vassilakos, Lee, Wright, & Young, 2005). Following RNA quantification and equalization, complementary DNA was synthesized using a cDNA archive kit (Applied Biosystems UK). Gene expression analysis was conducted using real-time PCR employing Taqman® Gene Expression Assays (assay IDs: *FKBP5*, Hs01561006_m1; *GILZ*, Hs00608272_m1; *SGK1*, Hs00178612_m1; Applied Biosystems UK), and *GAPDH* served as endogenous control. PCR was performed using ABI's universal cycling conditions on the StepOnePlus™ Real-Time PCR system.

### *Statistics*

All statistical analysis were considered to be significant if $p < .05$. Differences in demographic variables were tested using the Student *t* test, the chi-square test for gender distribution, and the Mann–Whitney *U* test for differences in nonparametric clinical variables. Morphometric measurements in both groups were normally distributed (using the Kolmogorov–Smirnov test), and their variances were homogenous (using the Levene test).

The mRNA expressions of glucocorticoid-inducible genes were subjected to an analysis of covariance in order to analyze main effects of diagnostic group (MDD, HC), gender (female, male), and childhood adversity (yes, no) using age as a covariate. Multiple regression analyses were carried out to analyze the association between the independent factors diagnostic group (MDD, HC), gender (male, female), childhood adversity, age, total intracranial volumes, and mRNA expression of *SGK1* (*GILZ*, *FKBP5*, respectively) on hippocampal volumes. Because there were three subsequent analyses carried out, we also considered multiple testing and indicate where results survive Bonferroni correction with a threshold of $0.05/3 = 0.017$. This analysis was also performed for subfield volumes CA1, CA2–3, DG, subiculum, and presubiculum. Thus, we performed five subsequent analyses on hippocampal subfields, which however are correlated to each other. Statistical correction for multiple testing was therefore done with a false discovery rate (FDR).

Moreover, stratifying patients and controls by *SGK1* (high/low expression based on the median) resulted in four groups: analysis of variance with hippocampal subfields as dependent variable and group as factor was carried out also applying FDR correction. This was followed by Dunnett-T3 post hoc subgroup comparisons assuming different variances between groups.

## Results

With regard to mRNA expression of *SGK1*, *GILZ*, or *FKBP5*, no significant main effect of diagnostic group, childhood

**Table 2.** *Parameters of the investigated regression model: gender, age, MDD diagnosis, childhood trauma, mRNA expression of SGK1, the interaction term of SGK1 × Diagnosis, and hippocampal volume as outcome variable*

| Statistical Model | Multiple *R* | Overall *p* | Stand. Beta | *t* | *P* Stand. Beta |
|---|---|---|---|---|---|
| Predictors | .51 | .001 | | | |
| Gender | | | −0.07 | −0.52 | .61 |
| Age | | | 0.09 | 0.76 | .44 |
| MDD diagnosis | | | −0.88 | −2.9 | .005 |
| Childhood trauma | | | −0.06 | −0.51 | .61 |
| mRNA-*SGK1* | | | 0.014 | 0.10 | .92 |
| ICV | | | 0.35 | 2.7 | .009 |
| Diagnosis × mRNA-*SGK1* | | | 0.75 | 2.43 | .017 |

*Note:* MDD, Major depressive disorder; mRNA, messenger RNA; *SGK1*, serum and glucocorticoid-regulated kinase 1 gene; ICV, total intracranial volume.

adversity, or sex was detected, and no significant interaction between these factors was observed.

A diagnosis of MDD was associated with smaller hippocampal volumes. Age, gender, mRNA expression of *SGK1*, and childhood adversity were not significantly associated. Moreover, the regression analysis showed that diagnosis had a moderating effect on the association of *SGK1* and hippocampal volumes (Table 2). These results survived Bonferroni correction for *SGK1* when considering that *GILZ* and *FKBP5* were also analyzed. The association between mRNA expression of *SGK1* and hippocampal volumes was seen in patients with MDD (Figure 2).

In regard to the hippocampal subfields, these effects were most significant for CA2/3, overall effect: $F$ (6/79) = 11.8, $p < .001$, $p_{FDR} < .005$; diagnosis effect: $p = .002$, $p_{FDR} = .01$; Diagnosis × *SGK1* interaction: $p = .015$, $p_{FDR} = .08$; and CA4/DG, overall effect: $F$ (6/79) = 9.0, $p < .001$, $p_{FDR} < .005$; diagnosis effect: $p = .006$, $p_{FDR} = .024$; and Diagnosis × *SGK1* interaction: $p = .025$, $p_{FDR} = .10$. The model for CA1 was significant, $F$ (6/79) = 11.8, $p < .001$, $p_{FDR} < .005$, with a trend toward significance for the factor diagnosis ($p = .027$, $p_{FDR} = .081$), but no significant *SGK1* × Diagnosis interaction ($p = .073$, $p_{FDR} = .22$). Similarly, the subiculum showed a trend toward a significant association of diagnosis, $p = .043$, $p_{FDR} = .086$; overall model: $F$ (6/79) = 9.6, $p < .001$, $p_{FDR} < .005$, but no interaction between diagnosis and *SGK1*. For the presubiculum, no significant diagnosis or Diagnosis × *SGK1* interaction was detected. Significant diagnosis effects are presented in Figure 3.

The results of the regression analysis were similar, but not as strong, when using *GILZ* mRNA expression. Interactions on an uncorrected level between diagnosis and *GILZ* mRNA expression were found on CA2/3 (β = 0.72, $p = .05$, $p_{FDR} = .20$), on CA4/DG (β = 0.77, $p = .048$, $p_{FDR} = .24$), and on subiculum (β = 0.73, $p = .05$, $p_{FDR} = .15$), but not on presubiculum and CA1. However, these did not survive FDR cor-

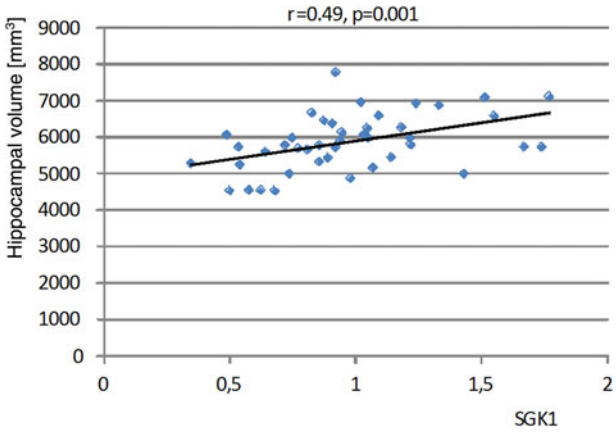


**Figure 2.** (Color online) Association between hippocampal volumes and serum and glucocorticoid-regulated kinase 1 gene (*SGK1*). A significant positive correlation was observed between hippocampal volumes and *SGK1* in patients. Thus, patients with lowest *SGK1* messenger RNA expression were those with the smallest hippocampal volumes.

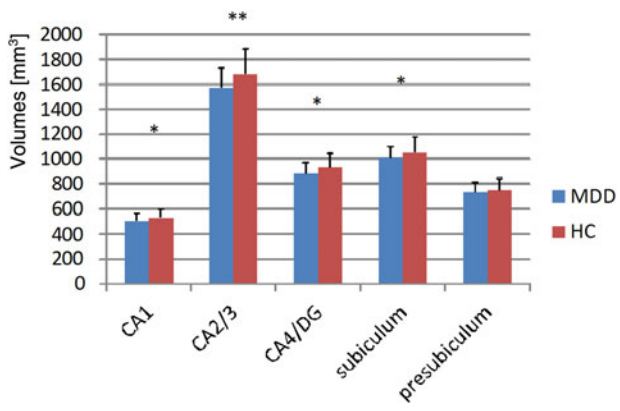


**Figure 3.** (Color online) Comparison of hippocampal subfields between patients and healthy controls. Cornu ammonis (CA) subfields CA1, CA2/3, CA4/dentate gyrus (DG), and subiculum volumes are significantly smaller in patients with major depressive disorder (MDD) compared to healthy controls (HC). Bars above columns indicate standard deviations.

rection. There were neither significant associations between mRNA expression of *FKBP5* and hippocampal volumes or subfield volumes, nor any significant interaction between diagnosis and *FKBP5* mRNA expression.

Stratifying patients and controls by mRNA expression of *SGK1* revealed significant analysis of variance effects for CA1, (F 3/82) = 3.6, $p = .017$, $p_{FDR} = .05$; CA2/3 (F 3/82) = 4.7, $p = .005$, $p_{FDR} = .025$; CA4/DG (F 3/82) = 3.9, $p = .012$, $p_{FDR} = .048$; and subiculum, (F 3/82) = 3.6, $p = .018$, $p_{FDR} = .036$. Post-hoc Dunnett T3 testing for multiple subgroup comparisons found that patients with low expression of *SGK1* had significantly smaller volumes of CA2/3 compared to patients with high *SGK1* expression ($d = 1242$, $p = .05$), HC with low *SGK1* expression ($d = 1901$, $p = .013$), and HC with high *SGK1* expression ($d = 1454$, $p = .022$; Figure 3). Patients with low expression of *SGK1* had significantly smaller volumes of CA4/DG compared to HC with low *SGK1* expression ($d = 940$, $p = .029$) and HC with high *SGK1* expression ($d = 741$, $p = .047$), and showed a trend toward significance compared to patients with high *SGK1* expression ($d = 659$, $p = .089$). Patients with low expression of *SGK1* had significantly smaller volumes of CA1 compared to HC with low *SGK1* expression ($d = 555$, $p = .013$).

Unmedicated patients ($N = 12$) with MDD did not differ compared to medicated patients ($N = 31$) with regard to mRNA expression of glucocorticoid-inducible genes or hippocampal subfield volumes.

## Discussion

In the present study we confirmed that CA and DG volumes are significantly smaller in patients with MDD compared to a healthy control group. Smaller hippocampal volumes have consistently been reported in patients with MDD compared to healthy controls (Campbell, Marriott, Nahmias, & MacQueen, 2004; Frodl et al., 2002; MacQueen & Frodl, 2011). The results are also in line with the study from Huang et al. (2013), who reported smaller DG and CA (CA1–3) volumes in patients with MDD compared to control subjects. Reduced hippocampal volume is not a specific finding in depression and has been demonstrated in other psychiatric disorders. Hippocampal volume reductions can be seen in schizophrenia (Meisenzahl et al., 2010), in posttraumatic stress disorders (Schmahl et al., 2009), and in preclinical stages of Alzheimer disease (Hampel et al., 2008; Jack et al., 1999). Thus, finding novel blood biomarkers with clinical relevance for diagnosis or therapy prediction in psychiatric diseases that are associated with hippocampal volume changes has wider implications than just in the area of depression.

In a previous study on mRNA expression of *SGK1*, it was found that *SGK1* mRNA is increased in the peripheral blood of drug-free depressed patients and in the hippocampus of rats after unpredictable chronic mild stress and prenatal stress (Anacker et al., 2013). In the present study, we did not find any significant differences for the measured glucocorticoid-inducible genes between patients with MDD and HC, and there was no significant effect of childhood adversity. Exploring whether medication could have an effect here, we demonstrated that there was no difference between unmedicated patients and patients currently taking antidepressants.

Smaller CA and DG volumes in patients with MDD were found in those patients who had reduced expression of *SGK1* or *GILZ* mRNA, respectively, as a marker of reduced activation of the glucocorticoid system compared to those with higher *GILZ* mRNA expression. In the patients, *GILZ* and *SGK1* mRNA was positively correlated with these hippocampal subfield volumes, indicating that a subgroup of patients with MDD showing both reduced mRNA expression of glucocorticoid-inducible genes and reduced hippocampal volumes exists. The GR target gene, *SGK1*, mediates the cortisol-induced decrease in proliferation and neuronal differentiation of human hippocampal progenitor cells, by acting both downstream and upstream of GR activation (Anacker et al., 2013), and thus might be involved in hippocampal neurogenesis. An association between lower mRNA expression of *SGK1* or *GILZ* with smaller hippocampal volumes might be contradictory to the idea that increased cortisol levels may result in decreased hippocampal volumes. However, it has to be taken into account that a blunted cortisol response was found for patients with MDD (Suzuki, Belden, Spitznagel, Dietrich, & Luby, 2013) as a sign of deregulation of the HPA axis that might result in reduced expression of glucocorticoid-inducible genes as well. There is some evidence that stress early in life results in increased cortisol response at the beginning but that later due to ongoing stress the cortisol response diminishes and results in blunted responses (Frodl & O'Keane, 2013; Trickett et al., 2010). At the same time, early life stress is associated with reduced hippocampal volume development (Frodl & O'Keane, 2013), and these parallel processes might explain the association between reduced mRNA expression of *SGK1* and hippocampal volumes in the current study.

No significant associations were detected between hippocampal subfields and mRNA expression of *FKBP5*. Although mRNA expression and certain variants of a gene are not necessarily associated, this lack of finding should be discussed in terms of recent results about an association with genetic variants. A previous study showed an effect of polymorphisms of *FKBP5* on posterior cingulum diffusivity that links the hippocampus with cortical brain regions in a sample of 82 traumatized females. Compared with individuals without this allele, individuals who carried two "risk" alleles for this *FKBP5* single nucleotide polymorphism demonstrated significantly lower fractional anisotropy in the left posterior cingulum, even after statistically controlling for variance associated with age, trauma exposure, and posttraumatic stress disorder symptoms (Fani et al., 2013). Moreover, in a study with 120 school children, the interaction of genetic profile scores of *FKBP5* and early life stress predicted left hippocampal and left amygdala volume changes (Pagliaccio et al., 2013).

The association between mRNA expression of *SGK1* and *GILZ* with hippocampal subfields were found to be strongest in the DG, lower but still significant in the subfield CA2/3, and lowest in the subfield CA1. Whether this might have to do with the hippocampal information circuit among the entorhinal cortex, DG, and CA3 to CA1 regions can only be speculative at this stage. These subfields may be involved differently in encoding, consolidation, and recall (Jones & McHugh, 2011). In addition, it is important here that neurogenesis happens within the DG. Because the CA3 and CA1 subfields were also found to be associated with mRNA expression, this association is driven by at least additional factors other than neurogenesis. A limitation here might be that these subfield measures were automatic measures oriented on the radiological information that may not be in line with the exact histological determination of subfields.

It is noteworthy that a recent study in mice showed that repeated social defeat stress reduced mRNA expression of the glucocorticoid-responsive genes, including *GILZ*, in microglia (Wohleb et al., 2011), indicating the involvement of *GILZ* in stress-related diseases like depression or anxiety disorders. Moreover, it has been suggested that glucocorticoid resistance may contribute to the inflammatory profile observed in MDD (Zunszain, Anacker, Cattaneo, Carvalho, & Pariante, 2011). Nevertheless, the glucocorticoid system plays a central role in the pathophysiology of MDD. There is mounting evidence that specific neuronal circuits, particularly in the developing brain, are damaged by environmental stress inducing changes in the HPA axis and inflammatory pathways (Krishnan & Nestler, 2008). Stress-related hypercortisolemia leads to central downregulation of GRs (Krishnan & Nestler, 2010) and to glucorticoid resistance in peripheral immune cells (Zunszain et al., 2011).

There is much broader research on the association between brain structure and the glucocorticoid system. The first study that assessed the association between hippocampal volumes and cortisol measures was published in 1998 by Lupien et al. This longitudinal study conducted over 5 years in older adults has demonstrated a correlation over time between cortisol levels and hippocampal volume changes. The total hippocampal volume of six subjects with increasing or high cortisol levels was significantly reduced by 14% in comparison to that of five subjects with decreasing/moderate cortisol levels, and the degree of hippocampal atrophy correlated strongly with both the degree of cortisol elevation over time and current basal cortisol levels (Lupien et al., 1998). Subsequent studies then used different measures of the HPA axis including the DST, cortisol-awakening response, stress tests, and basal as well as diurnal cortisol measures. A review of this literature indicated that most consistent associations were found between increased levels of cortisol over the day and reduced hippocampal volumes. Just measuring cortisol in the morning at awakening seems to not be a stable index of cortisol functioning as linked to brain structures. However, the cortisol-awakening response with several measures after awakening and cortisol responses after stress tests might have a better potential to reflect the physiology of the system, possibly reflecting that the hippocampus might have some regulatory influences (Frodl & O'Keane, 2013).

It is important to note that there were some limitations present in this study. As mentioned in the Methods Section, the automatic delineation of the subiculum and presubiculum was not found to be strongly in line with manual segmentations. Thus, the results about these two latter subfields and in particular the lack of significant effects with regard to the presubiculum have to be taken with caution. The significant finding seen for the group differences in CA2/3 and DG volumes might be promising that the automatic method is valid and captures the hippocampal changes, because we confirmed the results from a previous manual tracing study. A limitation might further be that two-thirds of our patients were currently on antidepressant medication, and the other third came to our service medication free and were scanned before a treatment was initiated. However, the results did not change when medication status was used as a covariate in the analysis, and hippocampal volumes did not differ between those with and without antidepressant medication. The acute effect that antidepressants might have on brain structure has not yet been shown consistently (Vermetten, Vythilingham, Southwick, Charney, & Bremner, 2003; Vythilingam et al., 2004), and this is a matter for future studies. Interpretation of the findings would have been enhanced by measures of cortisol output. To overcome the issue of multiple testing, we used multiple regression analysis with FDR and Bonferroni correction and omnibus analysis of covariance designs with post hoc tests. In addition, the sample size with 43 patients and 43 controls was reasonable for an imaging study looking at objective correlations with blood markers. For more in-depth interactive analyses among diagnostic groups, glucocorticoid profile, and childhood maltreatment, a larger group size would have been preferable. From the current cross-sectional research, it is not possible to conclude on the direction of effects between MDD, mRNA expression of *SGK1*, and structural changes in brain. There seem to be effects from both directions, because it is known from longitudinal studies that structural changes may render subjects more vulnerable to develop depression and that also a more severe illness course results in structural volume declines (Frodl, Jager, et al., 2008; Frodl, Koutsouleris, et al., 2008).

In summary, a reduced expression of glucocorticoid inducible genes S*GK1* and *GILZ* was associated with reduced hippocampal subfield volumes, in particular CA2/3 and CA4/DG in MDD. Thus *SGK1* and *GILZ* seem to be particularly relevant for stress-related mental disorders. These findings might have potential for identifying blood biomarkers associated with hippocampal changes relevant for psychiatric diseases. Messenger RNA expression of *SGK1* from the peripheral blood system associated with hippocampal volumes thus might include characteristic information of the glucocorticoid system. Whether a blunted cortisol response or glucocorticoid

resistance might be indicated by reduced mRNA expression of *SGK1* and whether *SGK1* represents trait characteristics needs further investigation. Further studies also need to explore the possible clinical usefulness of such a blood biomarker (e.g., for diagnosis or prediction of therapy response or even for risk and resilience).

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