Neurobiology of Disease

# Additive Gene–Environment Effects on Hippocampal Structure in Healthy Humans

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Hippocampal volume loss has been related to chronic stress as well as genetic factors. Although genetic and environmental variables affecting hippocampal volume have extensively been studied and related to mental illness, limited evidence is available with respect to  $G \times E$  interactions on hippocampal volume. The present MRI study investigated interaction effects on hippocampal volume between three well-studied functional genetic variants (COMT Val158Met, BDNF Val66Met, 5-HTTLPR) associated with hippocampal volume and a measure of environmental adversity (life events questionnaire) in a large sample of healthy humans (n = 153). All three variants showed significant interactions with environmental adversity with respect to hippocampal volume. Observed effects were additive by nature and driven by both recent as well as early life events. A consecutive analysis of hippocampal subfields revealed a spatially distinct profile for each genetic variant suggesting a specific role of 5-HTTLPR for the subiculum, BDNF Val66Met for CA4/dentate gyrus, and COMT Val158Met for CA2/3 volume changes. The present study underscores the importance of  $G \times E$  interactions as determinants of hippocampal volume, which is crucial for the neurobiological understanding of stress-related conditions, such as mood disorders or post-traumatic stress disorder (PTSD).

Key words: COMT; SLC6A4; BDNF; MRI; hippocampus; stress

# Introduction

Hippocampal neuroplasticity is critical for cognitive plasticity, novelty learning, and individuality in humans and animals (Garthe et al., 2009; Sahay et al., 2011; Freund et al., 2013; Spal-

Received July 23, 2013; revised May 14, 2014; accepted May 22, 2014.

Author contributions: U.R., C.W., B.B., H.H.S., D.D.P., J.-C.D., N.P.-R., E.M., H.E., and L.P. designed research; U.R., B.M.M., K.D., L.B., A.B., D.M., A.P., C.S., J.H., G.P., H.H., T.P., C.W., N.P.-R., E.M., H.E., and L.P. performed research; U.R., B.M.M., K.D., A.B., D.M., and K.K. analyzed data; U.R., B.M.M., K.D., L.B., A.B., J.H., D.D.P., S.K., N.P.-R., E.M., and L.P. wrote the paper.

This work was supported by the Special Research Project SFB-35 (F3514-B11 and F3506-B11) of the Austrian Science Fund, the Oesterreichische Nationalbank (OeNB 11903), and the Institute for the Study of Affective Neuroscience. U.R. was further supported by an award of the Theodor Körner Fonds. J.-C.D. was supported by the LABEX ANR-11-LABEX-0042 of Université de Lyon, within the program "Investissements d'Avenir" (ANR-11-IDEX-0007) operated by the French National Research Agency. We thank Kristin Anacker, Department of Psychology, Dresden University of Technology, Germany, for sharing expertise on the life events questionnaire; Pia Auersperg, Anastasia Gudakovskaja, Viktoria Köller, Elisabeth Kühtreiber, Franziska Mayr, Helge Oswald, Lisa Ott, Sophia Petschnak, Matthias Pilgerstorfer, Sebastian Ribar, and Marie Janina Schwidde for support of study participant recruitment and evaluation; Christian Kases, Wolfgang Huf, and Roland Boubela for technical advice; and the anonymous reviewers for their suggestion to analyze effects of multiple genetic variants and other thoughtful comments.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.3113-13.2014

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ding et al., 2013) and further moderates the adaptation to environmental changes, a process that is intimately linked to hypothalamus-pituitary-adrenal (HPA) axis function (Snyder et al., 2011). Apart from providing negative feedback to the HPA axis, the hippocampus is also a major target of cortisol, which is highlighted by cortisol-mediated hippocampal volume loss after chronic stress exposure (Sapolsky et al., 1990; McEwen, 2001; Brown et al., 2004). The clinical importance of hippocampal volume loss has been shown by numerous studies investigating stress-related and heritable neuropsychiatric disorders, such as major depressive disorder (MDD), post-traumatic stress disorder (PTSD), and schizophrenia (Nelson et al., 1998; MacQueen and Frodl, 2011; Kühn and Gallinat, 2013). In addition to environmental adversity (Gianaros et al., 2007; Dannlowski et al., 2012), genetic variation is known to affect hippocampal volume (MacQueen and Frodl, 2011). Interestingly, several genetic variants known for their direct effects on hippocampal volume have been related to stress susceptibility suggesting the possibility of gene-environment (G  $\times$  E) interactions at the neural level (Caspi and Moffitt, 2006). This applies specifically to three genetic variants impacting serotonin, dopamine, and neurotrophin signaling. 5-HTTLPR, a functional promoter polymorphism of the serotonin transporter gene (SLC6A4), modulates the relationship between environmental adversity and depression

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risk (Caspi et al., 2003) resulting in increased cortisol signaling and amygdala response to stressors in S carriers (Hariri et al., 2002; Canli et al., 2006; Miller et al., 2013). Similarly, BDNF Val66Met, a single nucleotide polymorphism (SNP) located in the brain-derived neurotrophic factor gene (BDNF), has been associated with stress susceptibility (Gatt et al., 2009; Alexander et al., 2010). Further, COMT Val158Met, a functional SNP located in the catechol-O-methyltransferase gene (COMT), has been implicated in HPA axis hyper-reactivity (Armbruster et al., 2012), altered  $\mu$ -opioid neurotransmitter responses to pain stressors (Zubieta et al., 2003), and increased limbic reactivity (Smolka et al., 2005). In line with their effects on the stress system and brain function, these variants have repeatedly been shown to directly affect hippocampal volume (Pezawas et al., 2004; Frodl et al., 2008; Honea et al., 2009), although others failed to show these effects (Dutt et al., 2009; Cole et al., 2011; Molendijk et al., 2012). Such inconsistency is not surprising given the complex interplay between various genes and environmental factors that determine hippocampal volume. The nonconsideration of  $G \times E$  interactions might be the leading reason for such inconclusive results, especially given that the functional role of the mentioned variants in stress reactivity provides a strong argument for their candidacy in  $G \times E$ research (Moffitt et al., 2005).



**Figure 1.** Type and timeline of LEs as reported by the sample (n = 153). LEs are sorted by number of occurrence.

To elucidate potential G  $\times$  E interactions of these variants on the volume of the hippocampus and its subfields, which considerably vary with respect to their stress sensitivity (McEwen, 2001; Teicher et al., 2012), we conducted an MRI study in a large sample of healthy individuals.

# Materials and Methods

Subjects. Healthy subjects were recruited by online advertisements, announcements on bulletin boards, and word of mouth at two study sites (Division of Biological Psychiatry, Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria, and Department of Psychology, Dresden University of Technology, Dresden, Germany). All assessments were performed following the same standard procedure, which has been approved by the local ethics committee according to the Declaration of Helsinki (2008): Only right-handed native German speakers of European ancestry aged between 18 and 45 years were invited to participate in this study. Before inclusion, study protocol procedures have been fully explained to study participants before obtaining written informed consent. All participants were further financially compensated for their expenditure of time. At the screening day, subjects underwent a thorough physical examination, including electrocardiography, blood pressure measurement, and routine laboratory testing. Moreover, subjects underwent the Structured Clinical Interview for DSM-IV Axis I disorders (APA, 2000) to ascertain absence of any past or present psychiatric diagnosis except nicotine dependence. The final sample consisted of 153 subjects (21 from the Dresden study site) after exclusion of three subjects due to segmentation failure (female, age 31 years, COMT Val158Met: Met/Met, 5-HTTLPR: S<sub>A</sub>/L<sub>A</sub>, BDNF Val66Met: Val/Val; female, age 19 years, COMT Val158Met: Val/Met, 5-HTTLPR: LA/

 $L_A$ ,BDNF Val66Met: Val/Met; male, age 27 years, COMT Val158Met: Val/Met, 5-HTTLPR:  $L_A/L_A$ , BDNF Val66Met: Val/Val) following visual quality control of the segmented structural images.

*Behavioral measures.* All subjects were asked to complete the life events questionnaire (LEQ), which is a short form of the life history calendar, a data collection method for obtaining reliable retrospective data about life events (LEs) and activities (Caspi et al., 1996; Canli et al., 2006). This self-report questionnaire comprises 28 stressful LEs (Fig. 1). Subjects were asked to indicate whether, when, and how often they had experienced a particular event. The sum of all LEs resulted in the total LEQ score. To test for effects of temperament, the German version of the Temperament and Character Inventory (Version 9) was applied (Cloninger et al., 1993).

Genotyping. DNA extraction and genotyping were performed at the Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria. DNA was isolated from EDTA blood samples using the Magna Pure LC DNA Isolation Kit (Roche). A tetra-primer amplification refractory mutation system-PCR (ARMS-PCR) was used for COMT Val158Met (rs4680) genotyping following a previously published protocol (Ruiz-Sanz et al., 2007). All subjects were further genotyped for the SLC6A4 promoter variant (5-HTTLPR), including rs25531 following a previously published protocol (Wendland et al., 2006). Genotyping resulted in 24  $S_A/S_A$ , 56  $S_A/L_A$ , 12  $S_A/L_G$ , 1  $S_G/L_A$ , 12  $L_A/L_G$ , and 48  $L_A/L_A$ carriers. Because the L<sub>G</sub> allele equals the S allele with regard to 5-HTT expression, the L<sub>G</sub> allele was grouped together with the S allele (Hu et al., 2006) and is for simplicity referred to as S allele in the course of the manuscript. BDNF Val66Met (rs6265) genotyping was performed using a TaqMan (Applied Biosystems) 5'-nuclease assay. Sequence detection was accomplished in a 384-well format on an ABI 7900HT RT-PCR thermocycler (Applied Biosystems) applying 10 ng genomic DNA in a

total volume of 10  $\mu$ l consisting of 5  $\mu$ l TaqMan Genotyping Mastermix (Applied Biosystems) and 0.25  $\mu$ l of a 20× TaqMan Genotyping assay (Assay ID C\_11592758\_10, Applied Biosystems) containing sequence-specific primers and probes. PCR was performed under the following conditions: initial denaturation step (10 min, 95°C), followed by 40 cycles of DNA denaturation (15 s, 95°C) and oligonucleotide annealing/ strand elongation (60 s, 60°C). Evaluation of data was realized using SDS 2.3 sequence detection software (Applied Biosystems). BDNF Val66Met genotyping resulted in 5 Met/Met, 52 Val/Met, and 96 Val/Val carriers. There was no indication for deviation from Hardy-Weinberg equilibrium for COMT Val158Met (p = 0.19), BDNF Val66Met (p = 0.64), 5-HTTLPR (p = 0.50), and rs25531 (p = 0.39).

MRI: acquisition. 3 Tesla (3T) TIM Trio scanners equipped with Siemens 12-channel head coils (Siemens Medical Solutions) were used for structural MRI measurements at both study sites using the same scan protocol and quality control procedures. Head movements were restricted using foam pillows. Structural images were acquired using a 3D magnetization-prepared rapid acquisition with a gradient echo sequence (3D MPRAGE, TR/TE = 2300/4.21 ms, flip angle = 9°, inversion time = 900 ms, and voxel size =  $1 \times 1 \times 1.1$  mm). Preprocessing: Anatomical MRI preprocessing was performed on a Linux computer (Red Hat Enterprise Linux 5, x86\_64 architecture) using FreeSurfer version 5.1.0 (http://surfer.nmr.mgh.harvard.edu/), a set of automatic tools for morphological operations that require little or no human interventions. Preprocessing included registration to Talairach space, intensity normalization, removal of nonbrain tissue, and segmentation. Hippocampi were delineated by a subcortical segmentation procedure that assigns to each voxel one of 37 anatomical labels based on voxel intensity, intensity of neighboring voxels, and atlas-based prior probabilities (Fischl et al., 2002). Hippocampal subfields were defined by the use of Bayesian inference within a statistical model of image formation around the hippocampus (Van Leemput et al., 2009). Hippocampal volumes as well as subfields were corrected for total intracranial volume using the residual method (Sanfilipo et al., 2004).

Statistics. To test for interaction effects between genotype and LEs, linear regression models were used with total hippocampal or hippocampal subfield volume as dependent variables and the interactions between COMT Val158Met, BDNF Val66Met, and 5-HTTLPR genotypes and LEs as independent variables. Age, gender, and study site were included as covariates. The following regressors for genotype variables were used: For COMT Val158Met, a linear allele-dose model was assumed by regressing the number of Met alleles (0, 1, 2) given the overwhelming evidence for dosing effects of COMT Val158Met (Smolka et al., 2005; Drabant et al., 2006; Domschke et al., 2012). 5-HTTLPR genotypes were collapsed into risk allele carriers (S allele carriers) and LA homozygotes in analogy to previous imaging genetics studies and coded as 0 (L<sub>A</sub> homozygotes) and 1 (S carriers) (Canli et al., 2006). Similarly, BDNF Val66Met genotypes were grouped into Met carriers (coded as 0) and Val homozygotes (coded as 1) as in previous studies (Kambeitz et al., 2012). A separate regression model, including the same regressors without the interaction terms, was used to assess potential main effects of genotype and LEs. Where applicable, the resulting p values were corrected for multiple comparisons using the Bonferroni-Holm method (Holm, 1979). Likelihood-ratio tests and Akaike information criterion were used for comparison of nested and non-nested models, respectively. To assess the variance explained by the interaction terms for each hippocampal subfield, we used the lmg metric ( $R^2$  partitioned by averaging over orders, provided in R package "relaimpo," function "calc.relimp") to break down  $R^2$  into shares from the individual regressors in analogy to a previous study (Teicher et al., 2012). All statistical analyses were conducted in R (version 2.15.2; http://cran.r-project.org/).

## Results

## Demographics

The sample consisted of 153 subjects (81 females, 72 males) with an average age of  $23.79 \pm 3.03$  years (range: 18-43 years; percentiles: 25th: 22 years; 50th: 23 years; 75th: 25 years). Subjects were generally well educated as reflected by the percentage (98%) re-

Table 1. Distributions, sample sizes, means, and SDs according to COMT Val158Met genotypes<sup>a</sup>

COMT Val158Met V = 153	ValVal N = 30	ValMet $N = 85$	MetMet $N = 38$	n
1 155				٢
Gender (female/male)	20/10	43/42	18/20	0.23
Age	23.77 ± 2.37	23.84 ± 3.4	23.71 ± 2.64	0.98
5-HTTLPR (L <sub>A</sub> L <sub>A</sub> /	9/21	29/56	9/29	0.50
S carrier)				
BDNF Val66Met	10/20	28/57	19/19	0.17
(Met carrier/ValVal)				
LE	$0.22 \pm 0.14$	$0.18 \pm 0.13$	$0.23 \pm 0.13$	0.10
Study site (Vienna/	27/3	72/13	33/5	0.76
Dresden)				
Nonsmoker/smoker	22/8	59/25	24/14	0.63
Novelty seeking	21.9 ± 5.18	21.53 ± 5.13	22 ± 5.19	0.88
Harm avoidance	14.23 ± 5.66	13.08 ± 5.48	12.58 ± 5.68	0.46
Hippocampal volume	8113.79 ± 446.72	8322.59 ± 771.1	8328.82 ± 664.81	0.33
(mm <sup>3</sup> )				

 $^{a}p$ , p value of  $\chi^{2}$  test or ANOVA between genotype groups. LE, Life events/year.

Table 2. Distributions, sample sizes, means, and SDs according to BDNF Val66Met genotypes<sup>a</sup>

BDNF Val66Met N = 153	ValVal N = 96	Met carriers $N = 57$	р
Gender (female/male)	53/43	28/29	0.57
Age	$23.7 \pm 3.36$	$23.95 \pm 2.39$	0.59
5-HTTLPR (L <sub>A</sub> L <sub>A</sub> /S carrier)	29/67	19/38	0.82
COMT Val158Met	19/57/20	19/28/10	0.17
(MetMet/ValMet/ValVal)			
LE	$0.2\pm0.13$	$0.2\pm0.13$	0.86
Study site (Vienna/Dresden)	81/15	51/6	0.52
Nonsmoker/smoker	62/33	43/14	0.26
Novelty seeking	21.68 ± 4.79	21.79 ± 5.68	0.90
Harm avoidance	$13.23 \pm 5.38$	13.11 ± 5.89	0.90
Hippocampal volume (mm <sup>3</sup> )	$8322.51 \pm 735.59$	$8216.98 \pm 617.53$	0.34

 ${}^{a}p$ , p value of  $\chi^{2}$  test or t test between genotype groups. LE, Life events/year.

# Table 3. Distributions, sample sizes, means, and SDs according to 5-HTTLPR genotypes<sup>a</sup>

5 71			
5-HTTLPR	L <sub>A</sub> L <sub>A</sub>	S carrier	
N = 153	N = 48	N = 105	р
Gender (female/male)	23/25	58/47	0.50
Age	$23.21 \pm 2.76$	$24.06 \pm 3.12$	0.09
COMT Val158Met	9/29/10	29/56/20	0.50
(MetMet/ValMet/ValVal)			
BDNF Val66Met (Met carrier/ValVal)	19/29	38/67	0.82
LE	$0.21 \pm 0.13$	$0.2 \pm 0.13$	0.68
Study site (Vienna/Dresden)	40/8	92/13	0.64
Nonsmoker/smoker	35/13	70/34	0.61
Novelty seeking	$22.35 \pm 5.72$	$21.43 \pm 4.83$	0.33
Harm avoidance	13.56 ± 5.7	13.01 ± 5.51	0.58
Hippocampal volume (mm <sup>3</sup> )	$8310.96 \pm 608.05$	$8270.5\pm732$	0.72

 ${}^{a}p$ , p value of  $\chi^{2}$  test or t test between genotype groups. LE, Life events/year.

ceiving 12 or more years of schooling. The distributions of 5-HTTLPR, BDNF Val66Met, and COMT Val158Met did not significantly differ between each other (all p > 0.05; Tables 1, 2, and 3). As shown in Tables 1, 2, and 3, genotype groups did not significantly vary with respect to age, gender, smoking status, or study site. Subjects reported on average 4.77 ± 3.14 LEs (range: 0–15 events; percentiles: 25th: 2 events; 50th: 5 events; 75th: 6 events), which occurred on average 5.75 ± 5.07 years before the MRI scan (range: 0–32 years; percentiles: 25th: 2 years; 50th: 4 years; 75th: 9 years). The type and timeline of all LEs in the whole sample are depicted in Figure 1. The number of LEs was signifi-

Table 4. Gene >	LE interaction	effects on	hippocampal volume <sup>a</sup>
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	Test for main effects				Test for interaction effects					
	b	SE	t	р	b	SE	t	р	p <sub>corr</sub>	
Constant	-0.15	0.20	-0.76	0.451	-0.22	0.19	-1.15	0.250		
Age	0.02	0.08	0.19	0.853	-0.06	0.08	-0.78	0.439		
Gender	0.26	0.17	1.57	0.118	0.37	0.16	2.37	0.019		
Study site	-0.36	0.24	-1.50	0.135	-0.61	0.24	-2.58	0.011		
COMT Val158Met	0.10	0.08	1.19	0.237	0.11	0.08	1.44	0.151		
5-HTTLPR	-0.08	0.18	-0.45	0.652	0.00	0.17	-0.01	0.990		
BDNF Val66Met	0.21	0.17	1.27	0.207	0.22	0.16	1.42	0.158		
LE	0.03	0.08	0.37	0.709	0.70	0.19	3.78	< 0.001		
LE $ imes$ COMT Val158Met					-0.27	0.08	-3.51	< 0.001	0.002	
${\tt LE} imes {\tt SLC6A4}{\tt 5-HTTLPR}$					-0.43	0.17	-2.56	0.012	0.012	
LE $ imes$ BDNF Val66Met					-0.55	0.17	-3.21	0.002	0.003	
	$R^2 = 0.05$	$F_{(7,145)} = 1.15$	<i>p</i> = 0.335		$R^2 = 0.19$	$F_{(10,142)} = 3.40$	p < 0.001			

<sup>a</sup>p<sub>corr</sub>, p value corrected for multiple comparisons using the Bonferroni–Holm correction. LE, Life events/year.

cantly correlated with age ( $\rho = 0.18$ , p = 0.02). To obtain a measure of stressor intensity and to avoid correlated regressors, we corrected the number of LEs by age, resulting in an average of  $0.20 \pm 0.13$  LEs per year. There was no significant difference with regard to LE between COMT Val158Met, BDNF Val66Met, and 5-HTTLPR groups (all p > 0.05; Tables 1, 2, and 3).

#### $G \times E$ effects on hippocampal volume

There were no significant main effects of COMT Val158Met, BDNF Val66Met, 5-HTTLPR, or LE on total hippocampal volume (Table 4; Fig. 2). However, each genotype exhibited a significant and independent interaction effect with LE indicating increased negative impact of LE on hippocampal volume in COMT Met homozygotes, BDNF Val homozygotes, or 5-HTTLPR S allele carriers (Table 4; Fig. 2). There was no evidence for statistical epistasis with regard to these interaction effects (LE  $\times$  Val158Met  $\times$  5-HTTLPR: b = -0.13, SE = 0.17,  $t_{(140)} = -0.76, p = 0.45; LE \times Val158Met \times Val66Met: b = 0.12,$ SE = 0.16,  $t_{(140)}$  = 0.75, p = 0.45; LE × Val66Met × 5-HTTLPR: b = -0.19, SE = 0.36,  $t_{(140)} = -0.53$ , p = 0.60). Given the independence of  $G \times E$  interaction effects in previous analyses, we performed a post hoc analysis of the combined genetic effect on hippocampal volume. Based on above-mentioned results, we constructed a cumulative risk score (CRS) by summing up genetic risk factors (number of COMT Met alleles, 5-HTTLPR S allele, and BDNF Val/Val genotype) for each individual. CRS was highly predictive for hippocampal volume in interaction with LE (b = -0.41, SE = 0.08,  $t_{(146)} = -4.99$ , p < 0.00001; Fig. 3A). Interestingly, this interaction exhibited a gradual effect on hippocampal volume ranging from volume increases to decreases dependent on the load of genetic risk (Fig. 3A). The more parsimonious CRS model explained nearly as much variance as the full model (full model:  $R^2 = 0.19$ , Table 4; additive model:  $R^2 = 0.18$ ,  $F_{(6,146)}$ , p < 0.0001) and reduced the Akaike information criterion by 5.96 indicating improved model performance. Moreover, this additive effect was even detectable when analyzing sites separately (Vienna: n = 132, b = -0.42, SE = 0.10,  $t_{(126)} = -4.10$ , p < 0.0001; Dresden: n = 21, b = -0.39, SE = 0.15,  $t_{(15)} =$ -2.59, p = 0.021). The model fit, however, varied substantially: in the genetically more consistently defined groups, LE explained between 38% and 42% of hippocampal volume variance, notably in opposite directions (CRS = 1: n = 19, b = 0.69, s<sup>2</sup> = 42%; CRS = 4: n = 15, b = -0.68,  $s^2 = 38\%$ ), whereas only 1% and 5% variance was observed in the intermediate groups. We further performed several post hoc analyses to exclude effects of potential confounding variables on these  $G \times E$  interaction effects (de-

tailed statistics available upon request). There were no significant main or interaction effects of smoking status (n = 152, all p >0.6), gender (all p > 0.5), harm avoidance, or novelty seeking (all p > 0.05). Additionally, including all possible covariate  $\times$  environment and covariate × genotype interactions (similar to model 4 in Keller, 2014) revealed no significant differences to the parsimonious models (full model:  $F_{(12,130)}$ , p = 0.79, additive model:  $F_{(6,140)}$ , p = 0.64) and no change in significance or direction of effects. To explore whether these  $G \times E$  interaction effects were driven by recent or early LEs, we conducted separate analyses restricted to specific developmental periods. Participants reported significantly less LEs during childhood (first 15 years of life) than during the last 5 years before the MRI scan  $(1.03 \pm 1.12)$ vs 2.52  $\pm$  2.07,  $t_{(152)} = -8.29$ , p < 0.001). A restriction to the last 5 years showed a similar, but less significant, pattern compared with the full set of LEs (COMT: b = -0.23, SE = 0.09,  $t_{(142)}$  = -2.54, p = 0.01, BDNF: b = -0.39, SE = 0.18,  $t_{(142)} = -2.11$ , p = 0.04, SLC6A4: b = -0.32, SE = 0.17,  $t_{(142)} = -1.89$ , p = -1.890.06, additive model: b = -0.33, SE = 0.09,  $t_{(142)} = -3.76$ , p < -3.760.001). Similarly, a restriction to childhood revealed an almost identical, but also less significant, pattern (COMT: b = -0.17,  $SE = 0.08, t_{(142)} = -2.19, p = 0.03, BDNF: b = -0.26, SE = 0.17,$  $t_{(142)} = -1.58, p = 0.12, \text{SLC6A4: } b = -0.28, \text{SE} = 0.17, t_{(142)} =$ -1.63, p = 0.11, additive model: b = -0.25, SE = 0.07,  $t_{(142)} =$ -3.34, p = 0.001). Nevertheless, both models explained major parts of variance in total hippocampal volume, but considerably less than the full model (last 5 years:  $R^2 = 0.13$ ,  $F_{(10,142)} = 2.18$ , p = 0.023; childhood:  $R^2 = 0.12$ ,  $F_{(10,142)} = 1.92$ , p = 0.047; LE:  $R^2 = 0.19, F_{(10,142)} = 3.40, p < 0.001).$ 

#### $G \times E$ effects on hippocampal subfield volumes

To study observed G × E interaction effects in specific hippocampal substructures, we applied a novel method for hippocampal subfield segmentation (Fig. 3*B*,*C*) allowing for the distinction between presubiculum, subiculum, fimbria, CA1, CA2/3, and CA4/dentate gyrus (Van Leemput et al., 2009). Similar to total hippocampal volume, no main effects of genotype or LE on any of these subfields were present (all p > 0.05). In contrast, G × E interaction analyses revealed spatially distinct effects for single genetic variants that exhibited the same direction as being found for total hippocampal volume (Table 5; Fig. 3*D*). Interestingly, all genetic variants showed no significant effects at the presubiculum or fimbria, whereas most effects were present in subiculum and Ammon's horn (Table 5; Fig. 3*D*). In contrast to single G × E effects, the additive model exhibited effects across almost all subfields. Whereas only the right fimbria fell short of



**Figure 2.** Main effects and gene  $\times$  LE interaction effects of genetic variation in *COMT*, *SLC6A4*, and *BDNF* on hippocampal volume. Left panel side: Main effects of COMT Val158Met (*A*), 5-HTTLPR (*B*), and BDNF Val66Met (*C*) on hippocampal volume for 153 subjects (*n* per genotype group is shown in Tables 1, 2, and 3). Hippocampal volumes are corrected for intracranial volume as well as the remaining variables according to the main effects model described in Table 4. The gray box represents the 95% confidence interval. Right panel side: Interaction effects between LEs/year and COMT Val158Met (*A*), 5-HTTLPR (*B*), and BDNF Val66Met (*C*) on hippocampal volumes for 153 subjects (*n* per genotype group is shown in Tables 1, 2, and 3). Hippocampal volume for 153 subjects (*n* per genotype group is shown in Tables 1, 2, and 3). Hippocampal volumes are corrected for intracranial volume as well as the remaining variables according to the interaction effects model described in Table 4.

significance before multiple comparison correction, nine of the 12 subfields survived Bonferroni-Holm correction, namely, CA1, CA2/3, CA4/dentate gyrus, and subiculum on both hemispheres as well as the left presubiculum. Within these subfields, the inter-

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action effect between LE and CRS explained between 4% and 12% of variance in volume with maximal effects present in right hemispheric regions implicated in neurogenesis and neuroplasticity, such as CA2/3 (12% explained variance) and CA4/dentate gyrus (11% explained variance, Table 5; Fig. 3*D*).

# Discussion

This study provides evidence for the presence of interaction effects between stressful LEs and three functional genetic variants on total hippocampal and hippocampal subfield volumes in healthy individuals in line with previous imaging, animal, and epidemiological evidence (McEwen, 2001; Caspi et al., 2003; Canli et al., 2006; Gianaros et al., 2007). These results highlight the need to model  $G \times E$  interactions at the intermediate phenotype level to sufficiently map the intimate relationship between environmental and genetic variability (Caspi and Moffitt, 2006).

We observed that the impact of stressful LEs on hippocampal volume is significantly modulated by variation in *COMT*, *BDNF*, and *SLC6A4* independent of gender, smoking, and temperament. Notably, these effects were driven by both recent and childhood events, suggesting that environmental effects on hippocampal volume occur throughout life and probably last for years (Teicher et al., 2012; Gray et al., 2013).

For COMT Val158Met, we observed a dose-dependent effect of the Met allele resulting in a gradual change from a positive to a negative correlation between LE and hippocampal volume in line with its previously described pleiotropic effects on brain function and behavior (Mier et al., 2010). Previous studies suggested that Met homozygosity strengthens prefrontal cognitive stability (Mier et al., 2010). However, this benefit comes with a trade-off of disadvantageous emotion-related information processing likely because of increased subcortical tonic and increased cortical phasic dopaminergic signaling (Bilder et al., 2004; Mier et al., 2010). Correspondingly, the Met allele has been associated with stress-related phenotypes, such as exaggerated limbic response to unpleasant stimuli (Smolka et al., 2005, 2007), HPA axis hyperreactivity (Armbruster et al., 2012), and increased pain sensitivity (Zubieta et al., 2003). This balance of costs and benefits suggests that each allele can be

advantageous depending on the environmental context ("warrior vs worrier" model) in line with our data (Goldman et al., 2005). Interestingly, COMT Val158Met effects were most pro-

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**Figure 3.** *A*, Interaction effect between additive genetic risk and LEs/year on hippocampal volume for 153 subjects. Scatter plots between LEs/year and hippocampal volume are shown separately for the number of genetic risk factors (COMT Met alleles, 5-HTTLPR S carrier, BDNF Val/Val carrier). The individual genotype groups are shown above. COMT Val158Met: Val/Val (VV), Val/Met (VM), Met/Met (MM); 5-HTTLPR: L<sub>A</sub> homozygotes (L), S carrier (S); BDNF Val66Met: Met carrier (M), Val homozygotes (V). *B*, Hippocampal subfield segmentation of a randomly drawn subject from the Dresden study site. The subfields are color-matched to Figure 3*D*. *C*, Hippocampal subfield segmentation of a randomly drawn subject from the Vienna study site. The subfields are color-matched to Figure 3*D*. *D*, Percent variance (s<sup>2</sup>) of hippocampal subfield volumes explained by the interaction effects between LEs/year and COMT Val158Met; BDNF Val66Met; 5-HTTLPR, and the additive risk score for 153 subjects. Bonferroni-Holm-corrected significance of the interaction effect is indicated as follows: <sup>+</sup> Corrected *p* < 0.1. \*Corrected *p* < 0.05. \*\*Corrected *p* < 0.01. \*\*\*Corrected *p* < 0.01. COMT, COMT Val158Met; BDNF, BDNF Val66Met; SLC6A4, 5-HTTLPR; LE, LEs/year.

Table 5. Gene ×	LE interaction	effects on h	ippocampal	subfields <sup>a</sup>
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Subfield	COMT Va	COMT Val158Met		5-HTTLP	5-HTTLPR		BDNF Val66Met		Additive model			
	s <sup>2</sup>	р	p <sub>corr</sub>	s <sup>2</sup>	р	<i>p</i> <sub>corr</sub>	s <sup>2</sup>	р	<i>p</i> <sub>corr</sub>	s <sup>2</sup>	р	p <sub>corr</sub>
L presubiculum	0.03	0.020	0.099	0.02	0.094	0.285	0.00	0.696	1.000	0.05	0.007	0.036
R presubiculum	0.01	0.163	0.484	0.03	0.036	0.251	0.00	0.610	1.000	0.03	0.023	0.069
L fimbria	0.01	0.121	0.484	0.02	0.057	0.285	0.00	0.998	1.000	0.02	0.041	0.081
R fimbria	0.00	0.716	0.716	0.03	0.014	0.130	0.00	0.271	1.000	0.02	0.053	0.081
L subiculum	0.04	0.007	0.051	0.05	0.004	0.048	0.00	0.421	1.000	0.08	< 0.001	0.002
R subiculum	0.06	0.001	0.009	0.04	0.013	0.129	0.01	0.046	0.369	0.11	< 0.001	< 0.001
L CA4/DG	0.04	0.005	0.041	0.04	0.012	0.129	0.03	0.007	0.078	0.11	< 0.001	< 0.001
R CA4/DG	0.06	0.001	0.006	0.02	0.087	0.285	0.04	0.004	0.043	0.11	< 0.001	< 0.001
L CA2/3	0.03	0.010	0.061	0.03	0.031	0.246	0.03	0.015	0.138	0.09	< 0.001	0.001
R CA2/3	0.07	< 0.001	0.004	0.02	0.068	0.285	0.03	0.007	0.078	0.12	< 0.001	< 0.001
L CA1	0.01	0.143	0.484	0.03	0.044	0.265	0.01	0.164	0.982	0.04	0.009	0.037
R CA1	0.06	0.001	0.009	0.01	0.134	0.285	0.01	0.080	0.563	0.08	< 0.001	0.001

as 2, Variance explained by the interaction effect between LEs and the specified polymorphism; p conv. p value corrected for multiple comparisons using the Bonferroni–Holm correction; L, left; R, right; CA, cornu ammonis; DG, dentate gyrus.

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nounced in CA1, CA2/3, CA4/dentate gyrus, and subiculum, which is in line with preclinical research suggesting disruptive effects of glucocorticoids and repeated stressors on neurogenesis or neuroplasticity in these hippocampal regions (Sousa et al., 2000; McEwen et al., 2012; MacDougall and Howland, 2013). The specific mechanisms of how dopamine affects hippocampal volume are still obscure but may involve alterations of the dopamine-modulated stress response that translate to changes of HPA axis function (Armbruster et al., 2012; Hernaus et al., 2013). Moreover, given the high hippocampal *COMT* expression, also direct effects on local dopamine signaling and hippocampal plasticity may be possible (Matsumoto et al., 2003; Lisman et al., 2011; Laatikainen et al., 2012).

 $G \times E$  interactions on hippocampal volume were also present for BDNF Val66Met in this study. The Val allele, which drove hippocampal volume loss in our data, has shown to promote social defeat stress susceptibility in mouse models because of increased BDNF signaling within the reward circuitry (Krishnan et al., 2007). In humans, Val/Val individuals have been linked to increased neuroticism (Frustaci et al., 2008), diminished antidepressant response (Niitsu et al., 2013), and heightened stress vulnerability (Yu et al., 2012; Jiang et al., 2013). Interestingly, we found maximal effects of BDNF Val66Met in CA4/dentate gyrus, where BDNF loss has been associated with depressive behavior and attenuated antidepressant efficacy (Adachi et al., 2008; Taliaz et al., 2010). However, this region is also highly susceptible to stressor-induced glucocorticoid signaling (Karst and Joels, 2003). It is therefore possible that an exaggerated stress response in Val/ Val individuals (because of increased BDNF signaling in the mesolimbic dopamine circuit) diminishes the positive effect of increased BDNF signaling in the hippocampus (Krishnan et al., 2007; Alexander et al., 2010).

Furthermore, hippocampal volume loss was mediated by the S allele of 5-HTTLPR in our analyses in line with previous reports and highlighting the specific role of this allele in stress susceptibility (Hariri et al., 2002; Caspi et al., 2003; Canli et al., 2006; Frodl et al., 2010). 5-HTTLPR has been extensively studied and is known to alter serotonergic neurotransmission as well as brain development (Gaspar et al., 2003; Fisher et al., 2012; Migliarini et al., 2013). Accordingly, the observed effects could be driven by both direct and indirect serotonergic effects, including 5-HTTLPR-mediated alterations of HPA axis activity or direct effects of serotonin on neuroplasticity (Martinowich and Lu, 2008; Klempin et al., 2013; Miller et al., 2013). In this study, 5-HTTLPR exerted maximal effects in the subiculum, which is the principal hippocampal relay for HPA axis control and exhibits neuroplastic changes in response to stress (MacDougall and Howland, 2013). Interestingly, the subiculum is also densely packed with 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors, which are involved in the serotonergic antidepressant response (Boeijinga and Boddeke, 1996; Hall et al., 1997; Cai et al., 2013).

There was no evidence for gene–gene interactions in our data, which may be understandable given that these genes are involved in distinct molecular pathways (Hemani et al., 2014). Instead, most of the individual variance was captured by a simple additive model suggesting that the observed  $G \times E$  effects on hippocampal volume accumulate similar to other stress-related endophenotypes (Smolka et al., 2007; Stone et al., 2013) or quantitative traits (Hill et al., 2008; Yang et al., 2010). Notably, this additive effect predominantly affected hippocampal subfields that have been reported to be specifically vulnerable to childhood maltreatment, glucocorticoids,

or stress in general (Sousa et al., 2000; McEwen et al., 2012; Teicher et al., 2012; MacDougall and Howland, 2013).

Interestingly, subjects at low genetic risk exhibited effects that were diametrically opposite to subjects at high genetic risk in our study. Although mathematically obvious, the biological meaning of this finding is less intuitive. Even so, a significant body of evidence exists that implicates factors promoting well-being in hippocampal growth (Kempermann et al., 1997; Pollak et al., 2008; Erickson et al., 2011; Davidson and McEwen, 2012; Arnone et al., 2013). Moreover, resilience, coping behavior, predictable stress, and low-dose glucocorticoids have been reported to stimulate hippocampal neurogenesis and neuroplasticity (Jeanneteau et al., 2008; Lyons et al., 2010; Schloesser et al., 2010; Delgado y Palacios et al., 2011; Parihar et al., 2011; Chen et al., 2012). It is therefore tempting to speculate that more beneficial stress coping behavior in individuals at lower genetic risk may have led to positive associations in our data. However, it needs to be emphasized that this is highly speculative given the correlative nature of our data and unresolved questions with respect to stress-related hippocampal changes (Czéh and Lucassen, 2007; Petrik et al., 2012). Nonetheless, such diametrically opposite effects may explain the lack of a significant main effect of LEs in several large healthy samples, including this study (Dannlowski et al., 2012; Luby et al., 2012).

The present study is not without limitations. The investigated sample is healthy, highly educated, and fairly homogeneous with regard to age, thereby controlling for major confounders, which might have been advantageous for the detection of subtle effects (Uher and McGuffin, 2008). However, this limits, on the other hand, the generalizability of our results, which especially cannot be extrapolated to patients. Subjects participating in this study reported considerably more recent than early LEs, which may reflect both the difficulty of recalling childhood memories as well as the different landscape of childhood stress (Howe, 2013). The reported diminished significance for early LEs could therefore be attributed to difficulties in memory recall rather than to lower  $G \times E$  effects on hippocampal volume per se. It would therefore be interesting to further assess these interactions with regard to early life stressors (Teicher et al., 2012). Furthermore, there are other genetic variants in genes, such as FKBP5, CRHR1, NR3C2, or KIBRA, that have been associated with stress vulnerability or hippocampal structure (Mandelli and Serretti, 2013). However, the study of a higher number of variants would unlikely have led to meaningful results given the expected small effects and the requirement for rigorous Type I error control. We therefore focused on three functional variants that have been related to hippocampal structure and, most importantly, stress reactivity by numerous studies providing strong a priori support for the investigated effects (Moffitt et al., 2005). Another limitation is the lack of functional measures of stress responsiveness in our sample, which would have allowed for assessing the potential mediatory effect of HPA axis reactivity. However, the impact of these genotypes on stress reactivity has repeatedly been demonstrated before and was therefore beyond the scope of this study. Finally, pharmacological or behavioral stress tests offer the possibility of standardized stressors that are free of any recall bias but are not suited to study the long-term consequences of real life stressors as intended in our study (Pilger et al., 2014).

In conclusion, this study provides evidence of interaction effects between three well-studied functional genetic variants and environmental adversity on hippocampal anatomy, thereby resembling previous animal, molecular biology, genetic, and imaging work. These results highlight the importance of  $G \times E$  interactions in imaging studies, which should facilitate a better

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understanding of the complex interplay between genes and the environment in future research.

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