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Stress-System Genes and Life Stress Predict Cortisol Levels and Amygdala and Hippocampal Volumes in Children

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Depression has been linked to increased cortisol reactivity and differences in limbic brain volumes, yet the mechanisms underlying these alterations are unclear. One main hypothesis is that stress causes these effects. This is supported by animal studies showing that chronic stress or glucocorticoid administration can lead to alterations in hippocampal and amygdala structures. Relatedly, life stress is cited as one of the major risk factors for depression and candidate gene studies have related variation in stress-system genes to increased prevalence and severity of depression. The present study tested the hypothesis that genetic profile scores combining variance across 10 single nucleotide polymorphisms from four stress-system genes (CRHR1, NR3C2, NR3C1, and FKBP5) and early life stress would predict increases in cortisol levels during laboratory stressors in 120 preschool-age children (3–5 years old), as well as hippocampal and amygdala volumes assessed with MRI in these same children at school age (7–12 years old). We found that stress-system genetic profile scores positively predicted cortisol levels while the number of stressful/traumatic life events experienced by 3–5 years old negatively predicted cortisol levels. The interaction of genetic profile scores and early life stress predicted left hippocampal and left amygdala volumes. Cortisol partially mediated the effects of genetic variation and life stress on limbic brain volumes, particularly on left amygdala volume. These results suggest that stress-related genetic and early environmental factors contribute to variation in stress cortisol reactivity and limbic brain volumes in children, phenotypes associated with depression in adulthood.

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INTRODUCTION

Stress, particularly in early life, is one of the strongest predictors of major depressive disorder (MDD; Green et al, 2010; Kessler and Magee, 2009) making it critical to understand the neurobiological mechanisms underlying this association. Early stress exposure (eg, Carpenter et al, 2007; Ouellet-Morin et al, 2011) and MDD (Heuser et al, 1994) are both associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, the regulatory system for stress/cortisol responsivity. Accumulating evidence from animal models (eg, Conrad et al, 1999; Cui et al, 2008; Vyas et al, 2002; Watanabe et al, 1992) and humans (eg, Brown et al, 2008; Campbell et al, 2004; Sacher et al, 2012; Videbech and Ravnkilde, 2004) has shown that stress exposure, HPA axis dysregulation, excessive corticosteroid levels, and depression relate to structural alterations in the

hippocampus and amygdala, brain regions important in HPA axis regulation (Jacobson and Sapolsky, 1991; Lupien et al, 2009). Thus, differences in these structures are a promising mechanism linking stress to depression and may also arise from individual differences in HPA axis function. While environmental stress can induce HPA axis dysregulation (eg, Carpenter et al, 2007; Lovallo et al, 2012; Ouellet-Morin et al, 2011), polymorphisms within genes coding for HPA axis proteins also relate to individual differences in stress responsivity, depression risk, and related phenotypes (Table 1). These polymorphisms may be important moderators of the effects of environmental stress on depression and brain structure (see Figure 1 for a schematic of these proposed mechanistic interactions).

The present study tested whether early life stressors and genetic variation within four HPA axis-related genes (CRHR1, NR3C2, NR3C1, and FKBP5) predict individual differences in HPA axis function and neural structure in children. Given the increase in depression risk in adolescence, we focused on school-age children to understand pathways contributing to this increasing risk. We adopted a polygenic approach by creating genetic profile scores, which additively combined 10 single nucleotide polymorphisms (SNPs), selected from the literature based on established

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Table 1 Associations Between Selected HPA Axis SNPs, Stress Responsiveness, Brain, and Psychopathology

Gene	SNP	Associations
CRHR1 17q12-q22	rs4792887	Minor allele: increased depression and suicidality among male suicide attempters exposed to low stress (2;31) Major allele: part of dose-dependent haplotype protective against MDD after CM (7)
	rs110402	Minor allele: more seasonal and earlier onset MDD (23); Dex/CRH cortisol in interaction with CM (27); part of haplotype predicting higher depressive symptomology after CM (10;14;24) Major allele: protective effect against depressive symptoms after CM (7;10;24); lower Dex/CRH cortisol response in men (10)
	rs242941	Minor allele: more prevalent among MDD patients than controls (19) Major allele: part of a haplotype associated with greater antidepressant treatment response in high anxiety MDD patients (17;18)
	rs242939	Minor allele: more prevalent among MDD patients than controls (19), increased depression and suicidality among male suicide attempters exposed to low stress (32) Major allele: part of a haplotype associated with greater antidepressant treatment response in high anxiety MDD patients (17;18)
	rs1876828	Major allele: part of a haplotype associated with greater antidepressant treatment response in high anxiety MDD patients (17;18)
NR3C2 4q31.1	rs5522[a]	Minor allele (MR I180V): increased salivary and plasma cortisol to TSST (8); increased prevalence of depressive symptomology among older adults (13), increased amygdala reactivity at low childhood adversity (6)
NRC31 5q31.3	rs41423247	Major allele homozygotes: increased cortisol response to TSST in women but decreased in men (12); increased anticipatory cortisol to TSST (11), increased risk of MDD episode (28)
	rs10482605	Major allele: increased in MDD patients compared to controls, potentially varying by ethnic population (29); part of a haplotype related to prevalence of childhood onset mood disorders (22)
	rs10052957	Minor allele homozygotes: higher evening and total cortisol across the day (25); increased in MDD patients; decreased hippocampal volume (35)
FKBP5 6p21.31	rs1360780	Minor allele: increased FKBP5 protein levels (3); reduced total diurnal cortisol secretion (30); altered cortisol response after DST (4;5;21), TSST (5), and SSP (20); increased incidence of depression (15;16); interacts with CM to predict depression (1;34), PTSD (3), and suicide (26); increased MDD recurrence and more rapid response to antidepressant treatment (5); increased amygdala reactivity in the context of elevated childhood adversity (33), increased threat-bias, increased threat-related hippocampal activity, and morphological changes in the hippocampus (9)

Numbers in parentheses refer to reference list in Supplementary Table S15.
CM = History of Childhood Maltreatment (emotional/physical abuse, trauma, or neglect).
DST = Dexamethasone Suppression Test; Dex/CRH = Dexamethasone/Corticotropin-Releasing Hormone Test.
TSST = Trier Social Stress Test; SSP = Strange Situation Paradigm in Infants.
[a]rs5522 is an exonic, non-synonymous SNP, all other SNPs are intronic.

association with HPA axis dysregulation, risk for depression, and/or related phenotypes (Table 1). We tested the hypotheses that genetic profile scores and early life stress exposure would predict: (1) increased cortisol responses to laboratory stressors in preschool-age children, (2) decreased hippocampal volume, and (3) increased amygdala volume in these same children at school age. Last, because HPA axis dysregulation is a putative mechanism by which stress leads to alterations in neural structure, we tested the hypothesis that (4) stress-related cortisol levels in early childhood would mediate the effects of genetic factors and early life stress on hippocampal and amygdala volumes later in childhood.

MATERIALS AND METHODS

Participants

Data were analyzed from 120 children (58 females; 57.5% White, 30.0% African-American, and 12.5% of other or mixed race) enrolled in the Preschool Depression Study (PDS), a prospective longitudinal study of preschool-age children (N = 306) conducted at the Washington University School of Medicine Early Emotional Development Program (WUSM EEDP) in St Louis.

For the PDS, 3–5-year-old children and their primary caregivers were recruited from daycares, preschools, and primary care sites in the St Louis area (see Luby et al (2009)

for details), using the Preschool Feelings Checklist (Luby et al, 2004) to oversample for children with or at risk for depression. Following an initial session during which psychopathology assessments, behavioral testing, and saliva collection occurred, children underwent annual clinical assessments and completed a neuroimaging session between the ages of 7 and 12 (current subsample mean age = 10.38 years, SD = 1.17 years). Parental written consent and child assent were obtained and the Institutional Review Board at Washington University approved all procedures.

Only children who met all inclusion criteria based on data quality and availability were included. Of the 306 children in the PDS, 168 completed the neuroimaging session. Forty-eight were excluded for missing, unusable, or poor quality structural imaging, genetic, or cortisol data, or for factors influencing cortisol (see Supplementary Materials), leaving a final sample size of N = 120.

Psychopathology and Stress Assessment

Trained WUSM EEDP staff conducted up to seven in-person assessments (current subsample mean = 4.74 sessions, SD = 1.01) with participants and their parents/guardians from study enrollment through the time of scan. Before children were 8, a reliable and age-appropriate semi-structured parent-report diagnostic interview was used to assess psychiatric symptoms, the Preschool-Age Psychiatric
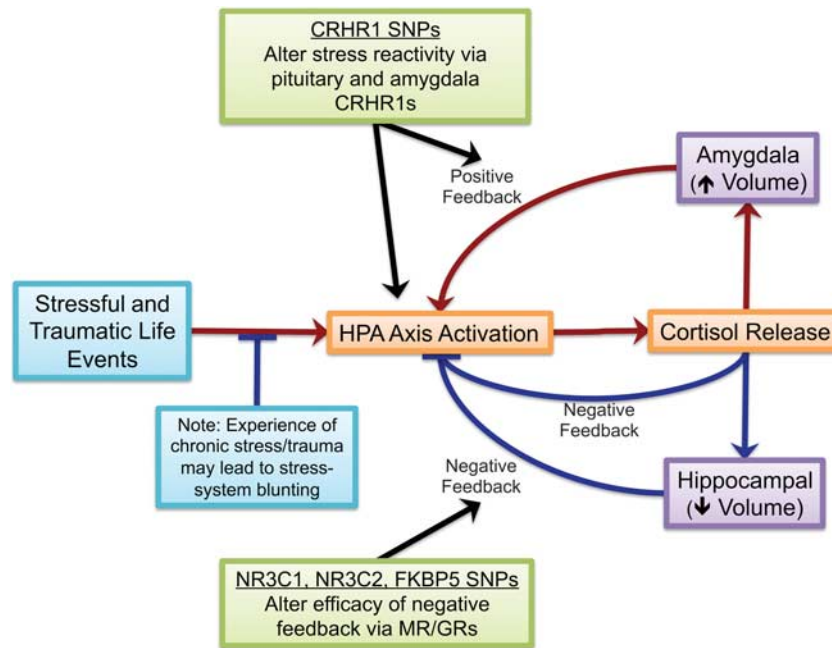
Figure 1 Schematic of potential stress-related mechanisms of change in brain structure. The experience of individual stressful and traumatic life events can activate the hypothalamic–pituitary–adrenal (HPA) axis, which prompts cortisol release having widespread effects on the brain and body. Notably, repeated activation of the HPA axis with life stress may in fact lead to blunting of the stress response to future stressors. Prolonged, elevated cortisol levels in animals are also shown to cause atrophy in the hippocampus and hypertrophy in the amygdala, largely through changes in dendritic remodeling. These structural changes may contribute to deficits in appropriate feedback onto the HPA axis. *CRHR1* is expressed in the amygdala and pituitary, such that genetic variants may alter activation of the HPA axis and thus cortisol release. *NR3C1*, *NR3C2*, and *FKBP5* are highly expressed in the hippocampus, where variants of these genes may alter cortisol negative feedback. Therefore, while stress-system genetic variants may alter the intrinsic reactivity and regulation of the HPA axis, changes in brain volume due to cortisol-mediated mechanism are likely to occur in interaction with the experience of environmental stressors.

Assessment (PAPA; Egger *et al*, 2003). After age 8, the Childhood and Adolescent Psychiatric Assessment (CAPA; Angold and Costello, 2000) was used, which also includes child report. Interviews were audiotaped, reviewed for reliability, and calibrated for accuracy (Luby *et al*, 2009). Four diagnostic groups were created based on the PAPA/ CAPA: Preschool-Onset MDD ($N = 45$; MDD before age 6, note: the 2-week symptom duration criteria was relaxed as previously validated by Gaffrey *et al* (2011)), later MDD ($N = 16$; MDD by the time of scan, but after age 6), other psychiatric conditions ($N = 28$; no diagnosis of MDD ever, but another diagnosis by the time of scan), and healthy controls ($N = 31$; no diagnoses through time of scan). For details, see Supplementary Table S1.

Analyses examined stressors experienced from birth through the baseline assessment when participants were 3–5 years old (current subsample mean age at baseline assessment = 4.45 years, SD = 0.77), at which time cortisol was collected. We summed the instances of stressful and traumatic life events reported by parents during the PAPA. A full list of life events assessed and their frequency is presented in Supplementary Table S2.

Stress Induction, Cortisol Collection, and Analysis

Salivary cortisol was collected three times during the baseline assessment; first upon arrival in the WUSM EEDP laboratory (1 + hours after the child's last meal to control for cortisol changes due to food/drink). As salivary cortisol levels are thought to indicate stress levels ∼ 30 min prior to

collection (Kirschbaum and Hellhammer, 1994), this first cortisol sample should represent time with the parent before the assessment. The second saliva collection occurred ∼ 30 min after separation from the parent, during which time the child performed a variety of behavioral tasks. Following this collection, the child performed more behavioral tasks and several stress-/frustration-inducing episodes from the Laboratory Temperament Assessment Battery (LabTAB; Goldsmith *et al*, 1995; 2004; Supplementary Table S3). A final cortisol sample was taken after these stressful tasks. See Supplementary Materials and Suzuki *et al* (2013) for details.

As we had no specific hypotheses about differences between separation- or stressor-induced changes in cortisol, an area under the curve ground (AUC) value was used to capture variance across all three timepoints (C_1, C_2, and C_3) using the time between collections ($t_{1,2}$ and $t_{2,3}$; in minutes) to yield a single summary variable (AUC = $\frac{(C_1 + C_2) \times t_{1,2}}{2} + \frac{(C_2 + C_3) \times t_{2,3}}{2}$; Pruessner *et al*, 2003). To control for circadian effects, the unstandardized residuals of AUC cortisol were saved from a linear regression with a binary time of day predictor (collection occurred either around 0900 hours or 1300 hours) and used for all subsequent analyses. See Supplementary Materials for additional validation of this approach.

MRI Acquisition and Volume Analysis

Structural images were collected as part of a scan session that also included task-based and functional connectivity

data. Imaging data were collected using a 3T TIM TRIO Siemens scanner. T1-weighted images were acquired in the sagittal plane using an MPRAGE 3D sequence (TR = 2400 ms, TE = 3.16 ms, flip angle = 8°, slab = 176 mm, 176 slices, matrix size = 256 × 256, field of view = 256 mm, voxel size = 1 × 1 × 1 mm).

FreeSurfer v4.5.0 (http://surfer.nmr.mgh.harvard.edu/, Fischl et al, 2002, 2004) was used to segment each participant's anatomical image, allowing estimation of left and right hippocampal and amygdala volumes and whole brain volume (WBV; total gray + cortical white matter volume). FreeSurfer was also used to segment and extract volumes for the hippocampal subfields (for details and validation against hand-tracing, see Van Leemput et al (2009)). This included volume estimates for the left and right presubiculum, CA1, CA2/3, fimbria, subiculum, CA4/dentate gyrus, and hippocampal fissure.

Genetic Analysis

DNA extracted from saliva was genotyped using standard high-throughput methods of the Sequenom Technology Core at Washington University (current subsample call rate = 99.5%; Supplementary Table S4). PLINK v1.07 (Purcell et al, 2007) was used to assure that all SNP genotypes were in Hardy–Weinberg equilibrium (all $ps > 0.05$) and to test whether allelic frequencies differed by ethnicity (Supplementary Table S4).

A recent study documented the utility of combining genetic variants in a biologically informed manner to study polygenic effects on the brain, whereas single polymorphisms alone were not significantly predictive (Nikolova et al, 2011). For the current study, we focused only on genes coding for integral HPA axis proteins and selected SNPs previously associated with increased cortisol, MDD prevalence/severity, and/or related phenotypes (Table 1). Fifteen SNPs were identified from the four genes of interest and were narrowed down to 10 to reduce linkage disequilibrium (LD; all pairwise $r^2 < 0.49$; Supplementary Figure S2) using SNPSpD (Nyholt, 2004). Sum scores across these 10 SNPs were created, where higher genetic profile scores indicate more genotypes previously associated with increased cortisol, MDD prevalence/severity, and/or related phenotypes.

Data Analysis

We used hierarchical linear regressions in IBM SPSS Statistics v20 (Armonk, NY: IBM) to predict cortisol and regional brain volumes. Predictors were added in steps to understand their effects alone and controlling for covariates. Regressions first controlled for ethnicity and sex. Next, centered, continuous variables for genetic profile scores and life events were entered. Interactions between these factors and interactions with sex were then entered. Predictors were assessed with and without controlling for WBV to test the specificity of effects on limbic brain volumes. Relatedly, amygdala volumes were added as covariates in predicting hippocampal volumes and vice versa to assess specificity and the variance shared by these regions predicted by genetic and environmental factors. Finally, we controlled for diagnostic status to test if this accounted for any of the

main/interaction effects. See Supplementary Materials for details.

We used the simple moderation model from the PROCESS tool for SPSS (Hayes, 2013) to parse significant interaction effects. Figures in the main text display simple slopes (split by sex or at mean ± 1 SD values of continuous moderators), at mean levels of the covariates; scatterplots of raw data are presented in the Supplementary Materials. PROCESS was also used to test for mediation effects. This regression-based approach estimates the *indirect effect* of an independent variable on a dependent variable via a mediator, equivalent to the difference between the *total effect* (not controlling for the mediator) and the *direct effect* of the independent variable (controlling for the mediator). To determine significance of the indirect effect, PROCESS uses bootstrapped confidence intervals (CIs; significant when not overlapping zero; Hayes, 2013). See Supplementary Materials for details.

RESULTS

Control Analyses

The distributions of all variables of interest (Supplementary Figure S3) and differences by ethnicity, sex, and diagnostic status (Supplementary Table S5; which were controlled for in the main analyses) are in the Supplementary Materials. There was no correlation between early life stress and genetic profile scores in the whole sample or in the sex or ethnicity subgroups ($ps > 0.5$; Supplementary Figure S4). Age was significantly correlated with the number of life events ($r(118) = 0.29$, $p = 0.001$) but not with genetic profile scores, cortisol, or any brain volumes (*all* $ps > 0.10$). The regression results presented below remained significant when controlling for age (data not shown).

Main Effects of Genetic Profile Scores and Life Events

AUC cortisol levels were positively predicted by genetic profile scores ($\beta = 0.32$, $t = 3.04$, $p = 0.003$) and negatively predicted by life events ($\beta = -0.28$, $t = -3.00$, $p = 0.003$), even when controlling for all covariates. Together these factors accounted for 8% of the variance in cortisol beyond ethnicity and sex (R^2 change $p = 0.01$; Supplementary Table S6). There was a sex difference in the strength of the genetic effect ($\beta = 0.22$, $t = 2.23$, $p = 0.028$). Genetic profile scores were a significant positive predictor of cortisol in females ($\beta = 0.484$, $t = 3.290$, $p = 0.001$), while showing a positive but non-significant relationship in males ($\beta = 0.099$, $t = 0.876$, $p = 0.383$; Figure 2 and Supplementary Figure S5). Genetic profile scores and stressful life events did not significantly predict hippocampal or amygdala volumes (Supplementary Tables S7–S10).

Genetic Profile Scores × Stressful Life Events Interactions

After accounting for main effects, cortisol, and WBV, the interaction between genetic profile scores and life events significantly predicted left hippocampal ($\beta = 0.21$, $t = 2.83$, $p = 0.04$; Supplementary Table S7; Figure 3a and b) and left amygdala volume ($\beta = 0.16$, $t = 2.10$, $p = 0.04$;

Supplementary Table S9; Figure 3c and d). *Post-hoc* simple slope testing revealed that genetic profile scores negatively predicted left hippocampal volume in the context of few stressful life events (mean − 1 SD). Stressful life events positively predicted left hippocampal volume in the context of high genetic profile scores (mean + 1 SD). Note that as *higher* genetic profile scores but *fewer* life events predicted higher cortisol levels, these results may be consistent with a cortisol-related mechanism of hippocampal loss. Stressful life events negatively predicted left amygdala volume in the context of average to low (mean − 1 SD) but not high (mean + 1 SD) genetic profile scores. After controlling for

hippocampal volume, this interaction was no longer significant, suggesting that it was related to shared variance between left amygdala and hippocampal volumes. These interaction effects were not significant for the right hippocampus or amygdala ($ps > 0.10$; Supplementary Tables S8 and S10).

Follow-up Analyses on Hippocampal Subfields

The animal literature shows that stress/corticosteroid administration impact the CA3 subfield and dentate gyrus of the hippocampus (eg, Conrad *et al*, 1999; Gould *et al*, 1998; Pham *et al*, 2003; Watanabe *et al*, 1992). In the current data, while most of the left hippocampal subfields showed a trend towards a genetic profile score × life events interaction, the interaction significantly predicted CA2/3 and CA4/dentate gyrus volumes (both passed Bonferroni correction for seven multiple comparisons per hemisphere), accounting for ∼5% of the variance in each subfield (Supplementary Table S11). On the right, this interaction only predicted presubiculum volume, but did not pass Bonferroni correction (Supplementary Table S12).

Mediation Analyses

As research suggests that chronic stress or glucocorticoid administration may lead to alterations in brain structure (eg, Conrad *et al*, 1999; Cui *et al*, 2008; Vyas *et al*, 2002; Watanabe *et al*, 1992), we tested the hypothesis that individual differences in stress-related cortisol levels in early childhood would mediate the effects of genetic profile
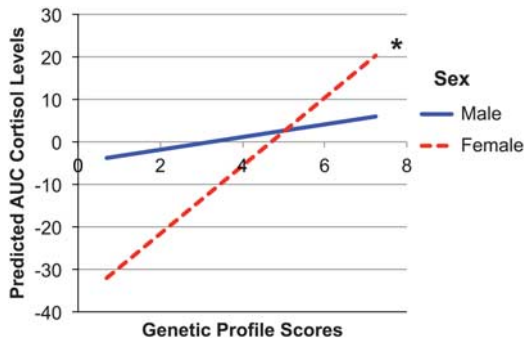

Figure 2 Regression-predicted effects of genetic profile scores on cortisol split by sex. The lines display the simple slopes of genetic profile scores predicting area under the curve (AUC) cortisol levels for males and females at mean values of all covariates (ethnicity, life events, genetic profile scores × life events, regional and WBV, and diagnostic status). Significant simple slopes are marked on the graph *$p < 0.05$.
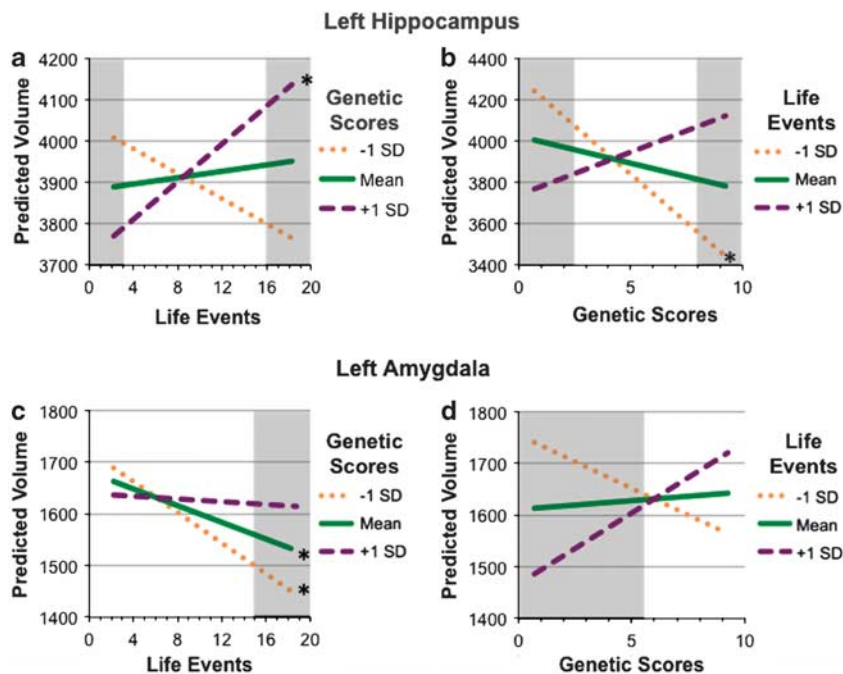

Figure 3 The interaction of genetic profile scores and life events predicting left hippocampal and left amygdala volumes. The lines display the simple slope of (a) life events at mean and mean ± 1 standard deviation values of genetic profile scores and of (b) genetic profile scores at mean and mean ± 1 standard deviation values of life events predicting left hippocampal volume (mm³) events at mean values of covariates (ethnicity, sex, genetic profile scores × sex, life events × sex, genetic profile scores × life events × sex, cortisol, WBV, and diagnostic status). The equivalent simple slopes predicting left amygdala volumes are presented in panels (c) and (d). Gray shaded regions display the Johnson–Neyman results, which indicate the range of moderator values (genetic profile scores or life events) at which there is a significant relationship between the other predictor and brain volume (a: < 3.3 and > 15.7; b: < 2.1 and > 5.3; c: > 14.4; d: < 4.7). Significant simple slopes are marked on the graph * $p < 0.05$.
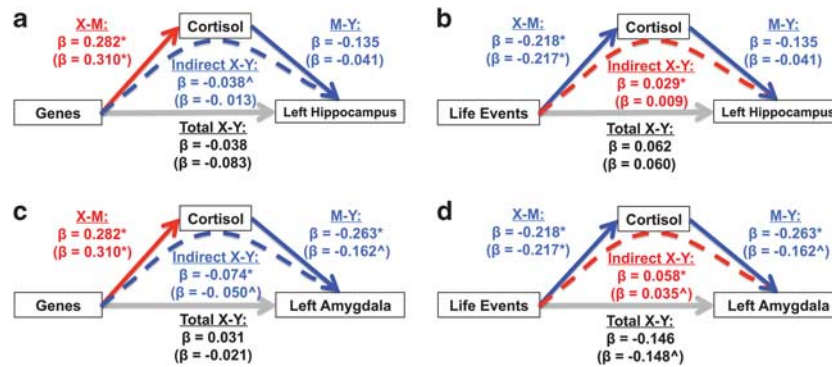
Figure 4 Cortisol mediates the effects of genetic profile scores and life events on left hippocampal volume and left amygdala volumes. The schematic diagrams represented the cortisol-mediated effects of genetic profile scores and life events on left hippocampal volume (a and b, respectively) and on left amygdala volume (c and d, respectively). Solid arrows represent unmediated effects (ie effects on the mediator or the total effect of the independent variables on cortisol or volume) while the dashed arrows represent the indirect effects via cortisol. Red arrows indicate positive relationships, blue arrows indicate negative relationships, and gray arrows indicate non-significant or near-zero total effects. Two standardized regression coefficients (β) are presented for each arrow, the top is the β coefficient from the regression model not controlling for WBV (Supplementary Table S13), while the bottom β in parentheses is the value from the regression model which does control for WBV (Supplementary Table S14). ^$p < 0.10$, * $p < 0.05$.

scores and early life stress on hippocampal and amygdala volumes in later childhood. We first tested this controlling for ethnicity, sex, interactions with sex, and diagnostic status (Supplementary Table S13), and then also controlling for WBV (Supplementary Table S14).

Cortisol-mediated negative indirect effects of genetic profile scores and positive indirect effects of life events on left hippocampal (Figure 4a and b) and left amygdala volumes (Figure 4c and d). Higher genetic profile scores and fewer experiences of stressful life events each predicted smaller left hippocampal and amygdala volumes mediated by higher cortisol levels.

While the genetic profile score × life events interactions described in the previous sections appear specific to the left hippocampus and amygdala (ie controlling for WBV), the mediation results may indicate a more widespread cortisol effect on the brain. Specifically, cortisol was negatively correlated with WBV ($r(118) = -0.216$, $p = 0.018$). After controlling for WBV, the mediation effects on left hippocampal volume were no longer significant, while indirect effects of genetic profile scores and life events on the left amygdala volume via cortisol remained trend-level significant (Supplementary Tables S13–S14).

DISCUSSION

Our results show that stress-system genetic profile scores and early life stress predict cortisol levels and interact to predict left hippocampal and amygdala volumes, putative phenotypes underlying associations between stress and depression.

Stress-system Genes and Life Events Predict Cortisol Reactivity

Genetic profile scores (higher scores indicating more SNPs associated with increased cortisol levels and/or depression) positively predicted cortisol levels during psychosocial stress in preschool-age children, providing validation for our polygenic approach. Sex moderated the effect of genetic profile scores on cortisol levels, such that the positive relationship between genetic profile score and cortisol

was stronger in females than males. While the rates of depression did not differ by sex in this young sample (see Supplementary Materials), the literature shows a clear sex difference beginning in adolescence, with females twice as likely to develop MDD as males (eg, Nolen-Hoeksema and Girgus, 1994). This stronger effect of genetic profile scores on cortisol among females may contribute to the sex differences in MDD prevalence, especially as genetic factors may be more influential in the etiology of MDD in females than in males (Kendler *et al*, 2001).

Congruous with the literature suggesting that life adversity results in blunted HPA axis responses to acute stress (Carpenter *et al*, 2007; Lovallo *et al*, 2012; Ouellet-Morin *et al*, 2011), we found that the number of stressful and traumatic life events experienced by preschool age negatively predicted cortisol levels. High or chronic levels of early life stress may induce stress-system 'burnout' or, relatedly, these children may have perceived the laboratory stressors as less stressful.

Stress-Related Effects on Hippocampal and Amygdala Volumes

The interaction of genetic profile scores and early stressful life events predicted both left hippocampal and left amygdala volumes at school age. While life stress typically does not predict hippocampal volume in childhood (eg, Woon and Hedges, 2008), exploring the genetic risk may be the key to detecting these differences early in development. However, the nature of the interactions between genetic profile scores and life events was somewhat atypical, as a diathesis–stress model would predict an additive interaction (eg, Caspi *et al*, 2003), with smaller volumes associated with both higher genetic risk and more stressful life events. Instead, we found that a greater number of 'risk' SNPs predicted smaller left hippocampal volumes only in the presence of fewer stressful life events. This relationship suggests that the environment may set boundaries on the effects of genetic factors, with higher life stress over-riding genetic influences. Conversely, genetic risk may promote stress-related phenotypes and disorders, even in the

absence of adversity/environmental provocation. Other studies have found conceptually analogous gene × environment interactions (eg, Carballedo et al, 2013; Taylor et al, 2006). Within the left hippocampus, the interaction effect was most predictive of CA2/3 and CA4/dentate gyrus subfield volume. This is convergent with animal studies showing that chronic stress or corticosteroid administration reduce dendritic length and branching in hippocampal CA3 (eg, Conrad et al, 1999; Watanabe et al, 1992) and impair neurogenesis in the dentate gyrus (eg, Gould et al, 1998; Pham et al, 2003).

For the left amygdala, elevated stressful life events predicted decreased volume with lower, but not higher, genetic profile scores. In this case, the level of genetic risk appears to over-riding the environment's effects. Although the literature on amygdala volume with depression and stress is mixed, our findings are consistent with research indicating decreased amygdala volumes in depression (eg, Keller et al., 2008; Sacher et al, 2012) and with cortisol administration (Brown et al, 2008). Controlling for hippocampal volume reduced the effect of the genetic profile score x life event interaction on the left amygdala volume, indicating that this interaction predicted variance shared between the left amygdala and hippocampus. This is particularly interesting given that both structures are thought to be affected by stress-/cortisol-mediated mechanisms, evident in the animal literature showing effects of chronic stress and corticosteroid administration on the hippocampus (Conrad et al, 1999; Pham et al, 2003; Watanabe et al, 1992) and amygdala (Cui et al, 2008; Vyas et al, 2002). Interestingly, the current effects are left-lateralized, consistent with meta-analytic work on amygdala volume in MDD (decreased left but not right volumes (Sacher et al., 2012)) though not with hippocampal meta-analyses that show bilateral effects (Campbell et al, 2004; Cole et al, 2011).

Consistent with a stress-/cortisol-mediated mechanism, our mediation analyses indicated that cortisol levels in early childhood may mediate the influence of genetic profile scores and stressful life events on limbic brain volumes. Interestingly, cortisol levels also negatively predicted WBV. Controlling for WBV, there were no longer unique cortisol-mediated effects on the left hippocampus, suggesting that effects on limbic volume may share mechanisms with broader cortisol effects on the whole brain. However, trend-level cortisol-mediated effects remained for left amygdala volume, suggesting some effects over and above those seen for WBV. The genetic profile scores × life events interactions did show specificity to both the left hippocampus and amygdala, as these effects were not accounted for by broader influences on the whole brain.

Though depression is associated with changes in hippocampal and/or amygdala volume, we found no diagnostic status effects in this sample of children. Hippocampal volume loss may relate to the burden of MDD illness, only becoming visible with a 2 + year history and 1 + episodes (McKinnon et al, 2009) where volume tends to decrease with increasing MDD duration/number of episodes (MacQueen et al, 2003; Videbech and Ravnkilde, 2004). Despite no association with diagnostic status in this young sample, it will be important to test whether stress-related factors account for later depression-related differences in brain volumes. Future work must also determine whether

this genetically influenced stress-/cortisol-mediated pathway affects brain function as well as structure, especially given recent work showing an impact of childhood cortisol levels on adult functional connectivity (Burghy et al, 2012) and evidence of the importance of stressful life events and abnormal stress reactivity in the genesis and maintenance of depression (eg, Kendler et al, 1999; Lopez-Duran et al, 2009).

Limitations and future directions

First, using single continuous summary values increases power by combining variance and reducing the number of tests performed. However, the use of a summed count of life events may not represent the true mechanisms by which risk is accumulated. Likewise, while the assumption of additive effects of SNPs across different genes might be considered a strong one and does not take into account potential epistatic effects, it is somewhat supported in the study of complex traits (Hill et al, 2008). As knowledge regarding the effects of single SNPs and life events on phenotypes of interest accumulates, more sophisticated weighting according to predicted effect sizes may be warranted. Our relatively small sample and insufficient priors regarding the magnitude of expected effects or epistatic relationships prevented us from adopting such methodology at this point. To aid this in the future, we have presented the effects of individual SNPs in the Supplement.

An additional concern is that while the FKBP5 variant is functional, the remaining SNPs are intronic. Therefore, even though we prioritized genes central to HPA axis activation/regulation and only included SNPs with prior evidence for association with cortisol and/or depression, the functional significance of our polygenic score remains to be explored. Yet, it is likely that our SNPs tag functional variants (Supplementary Materials). Notably, different ethnic groups may have different underlying patterns of LD (Supplementary Figure 2). While self-reported ethnicity was included as a covariate in these analyses, our polygenic scores may be further refined as research accumulates on differential markers in Caucasian and non-Caucasian cohorts. Currently, we avoided excluding any subpopulation, as this would have considerably diminished power. Nevertheless, it should be noted that the significant effects of genetic profile scores, life events, and their interaction on cortisol and brain volumes were observed in the Caucasian subsample alone ($N = 69$), either reaching significance or showing a pattern in the same direction as in the full sample (data not shown).

Finally, while genetic profile scores more efficiently approximate the polygenic underpinnings of our outcome measures (but see limitations above), it is possible that our finding reflects a false positive given the relatively modest sample size and potential for low priors (Duncan and Keller, 2011). Novel reports of interactions, such as ours, are particularly vulnerable, though the current study was based on a strong set of a priori hypotheses about biological mechanisms from human and animal studies. Thus, replication is necessary when an analogous cohort of young children with similar data is available. Future studies should build further to explore the role of stress-related genetic/environmental factors in the intergenerational transmission of depression and in understanding the specific and overlapping effects of stress on limbic and WBVs.

CONCLUSIONS

We found that more 'risk' variants in stress-system genes and lower levels of stressful life events from birth to the preschool period predicted higher cortisol levels during lab stressors in preschool-age children. The interaction of these factors predicted left amygdala and left hippocampal volumes in these same children at school age. Cortisol, which negatively correlated with limbic and WBVs, may serve as a mediator of the effects of genes and life stress on limbic brain volumes. The findings elucidate the association between normal variation in the stress-system and limbic brain volumes in children. Although diagnostic status was not strongly associated with differences in limbic volumes, the results may be potential evidence for a stress-mediated mechanism underlying putative depression-related changes in brain structure. This will be important in understanding differences in the normative developmental trajectory of cortisol reactivity and limbic brain structure as well as differences related to stress and psychiatric disorders.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)