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Title: **Decreased hippocampus volume in healthy girls at risk for depression.**

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Abstract:

- **Context.** Researchers have documented that the hippocampus is smaller in depressed than in nondepressed individuals. The temporal or causal association of this reduction in hippocampal volume in depression, however, is not known.
- **Objective.** To test the hypothesis that reduced hippocampal volume precedes and, therefore, may be implicated in the onset of, depression, we used magnetic resonance imaging to examine brain structure volume in individuals at high and low familial risk for depression.
- **Design.** Anatomical images from magnetic resonance imaging were analyzed using both whole-brain voxel-based morphometry and manual tracing of bilateral hippocampus.
- **Setting.** A research university.
- **Participants.** 55 girls between the ages 9 and 15: 23 daughters of mothers with recurrent episodes of depression in the daughter's lifetime (high-risk) and 32 age-matched daughters of mothers with no history of psychopathology (low-risk). None of the girls had any past or current Axis-1 psychopathology.
- **Main Outcome Measures.** Group differences in voxel-based morphometry brain matter density estimates and traced hippocampal volume.
- **Results.** Voxel-based morphometry analyses indicated that individuals at high risk for depression had significantly less gray matter density in clusters in bilateral hippocampus ($p<0.001$) than did low-risk participants. Tracing yielded a volumetric reduction in the left hippocampus in the high-risk participants ($p<0.05$).
- **Conclusions.** Compared with individuals at low familial risk for the development of depression, high-risk individuals have reduced hippocampal volume, indicating that

neuroanatomical anomalies associated with depression may precede the onset of a depressive episode and influence the development and course of this disorder.

Text:

Introduction

Major Depressive Disorder (MDD) is among the most prevalent and burdensome of all psychiatric disorders[1]. With advances in neuroimaging techniques, investigators have been able to examine the function and structure of specific brain regions in this disorder. For several reasons, researchers have focused on the role of the hippocampus in depression. The hippocampus is involved in the regulation of the hypothalamic pituitary adrenal (HPA)-axis, which is responsible for production of stress-related glucocorticoids such as cortisol[2]. In this context, depressed individuals have been found consistently to report high levels of stress[3], which is reflected biologically in elevated rates of hypercortisolemia[4] and disturbed HPA-axis functioning[5]. Moreover, depressed patients have also been found to be characterized by difficulties in hippocampal-dependent learning and memory[6]. These factors, in addition to the high degree of connectivity between the hippocampus and other brain regions critical for emotion and cognition, make this structure a prime candidate for further investigation[7].

Importantly, glucocorticoids produced by the HPA axis are particularly deleterious to hippocampal neurons[8]. Given the association between depression and glucocorticoid production, it is not surprising that investigators have reported reductions in hippocampal volume in individuals diagnosed with MDD[9,10], underscoring the involvement of this structure in the pathophysiology of depression. Indeed, severe stressors such as childhood abuse have been postulated to lead to reduced hippocampal volume in adulthood, and may represent a link between hippocampal volume and psychopathology[11,12,13]. It is important to recognize, however, that the nature of the association between reduced hippocampal volume and depression is not yet clear. For example, although some investigators have failed to find decreased hippocampal

volume in depression[14,15], other researchers have found hippocampal reductions only in individuals with recurrent episodes of MDD[16]. In this context, Sheline and her colleagues[17] found reduced hippocampal volume to be associated with increased lifetime duration of depression in individuals with a history of depression, and a recent meta-analysis indicates that hippocampal volume reductions may be found only in patients with multiple episodes or long duration of illness[18]. Other investigators, however, have documented volumetric anomalies in individuals experiencing their first episode of MDD[19],[20].These inconsistencies have made it difficult to ascertain the causal nature of the association between reduced hippocampal volume and depression. Because reduced hippocampal volume has been found to predict poorer outcome of a depressive episode[21,22,23], it is possible that variation in hippocampal volume precedes and influences the development and course of MDD.

In the present study we examined whether reduced hippocampal volume precedes the onset of MDD by assessing brain morphometry, including hippocampal volume, in individuals who are at elevated risk for MDD but who have not yet experienced a depressive episode. Among the strongest risk factors for depression is a family history of the disorder[24]. Adverse effects of parental depression on the functioning of offspring have been documented in children ranging in age from infancy to adolescence; in fact, having parents who are diagnosed with MDD is associated with a three-fold increase in the risk to the offspring for developing a depressive episode[25]. In this study we used voxel-based morphometry (VBM) as well as manual tracing of bilateral hippocampus to examine brain morphometry in young girls at high or low risk for depression by virtue of the presence or absence of a history of recurrent depression in their mothers. We specifically recruited mothers because of the results of a meta-analysis indicating that maternal depression is more strongly correlated with internalizing problems in children than

is depression in fathers[26]. Indeed, consistent with this conclusion, investigators have found maternal depression to be related to wide-ranging deficits in children's functioning, including academic performance, behavior, cognition, interpersonal relationships, and neuroendocrine regulation[27,28]. We recruited young adolescent daughters as participants because, first, beginning in early adolescence, MDD is twice as prevalent in females as in males[29], and second, girls are likely to experience an earlier onset of depression than are boys, which is associated with poorer course and greater severity of the disorder[30]. We hypothesized that girls at high familial risk for depression would have decreased hippocampal volume compared to their low-risk peers, despite not having experienced current or past psychopathology.

Methods

Participants

Participants were 56 girls between the ages 9 and 15 with no current psychopathology and no history of any Axis I disorder. Thirty-three of these girls had mothers who also had no current or past Axis I disorder (low risk for depression), and 23 had mothers who had a history of recurrent episodes of MDD during their daughters' lifetime (high risk for depression) but no current Axis I disorder or recent substance abuse. Participants were recruited through advertisements posted within the local community. A phone screen established that both mothers and daughters were fluent in English and that daughters were between 9 and 15 years of age. Daughters were excluded if they had experienced severe head trauma, learning disabilities, and/or current or past depression. Both low- and high-risk mothers (as well as all of the daughters in the study) had no current or past substance abuse.

Trained interviewers assessed the diagnostic status of daughters by administering the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and

Lifetime version (K-SADS-PL)[31] separately to the daughters and to their mothers (about the daughters). The K-SADS-PL has been shown to generate reliable and valid child psychiatric diagnoses. A different interviewer administered the Structured Clinical Interview for the DSM-IV (SCID)[32] to the mothers. Both K-SADS-PL and SCID interviewers had previous experience administering structured clinical interviews. To assess inter-rater reliability, an independent rater who was blind to group membership evaluated 30% of the SCID and K-SAD-PL interviews by randomly selecting audiotapes of equal numbers of high-risk and control pairs. In all cases, diagnoses of the presence of two or more depressive episodes in mothers, no history of depressive episodes in mothers, and absence of any current or previous Axis-I disorder in the girls matched the diagnosis made by the original interviewer, κ=1.00, indicating excellent inter-rater reliability. Daughters also completed the 10-item version of the Children's Depression Inventory (CDI-S)[33], a self-report measure of depressive symptomatology for children between the ages of 8 and 17. The CDI-S is derived from the 27-item CDI; the long and short forms have been found to yield comparable results[34]. The CDI-S was administered at the interview as well as before the scan; the mean of these two scores was used in all analyses. Daughters also completed the vocabulary subscale of the Wechsler Intelligence Scale for Children-III (WISC-III[35]) to examine possible group differences in knowledge of word meanings and language development. Finally, to assess pubertal development, daughters were also administered the Tanner stages questionnaire[36].

Daughters in the high-risk group were eligible to participate in the study if: 1) they did not meet criteria for any past or current Axis-I disorder according to both the parent and child K-SADS-PL; and 2) their mothers met the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV)[37] criteria for at least two distinct episodes of MDD since the birth of their daughters,

but did not currently meet criteria for MDD or any other Axis-I disorder. Daughters in the healthy control group were eligible to participate if: 1) they did not meet criteria for any past or current Axis-I disorder based on both the parent and child K-SADS-PL; and 2) their mothers did not meet criteria for any Axis-I disorder during their lifetime. Daughters were excluded if they had experienced traumatic early life events, such as physical or sexual abuse, that may have affected neurological functioning. The Life Events Checklist administered to the daughters revealed only one individual who reported a significant illness or injury; removing this individual from the analysis did not change the results.

Imaging

All subjects were scanned on a 1.5T GE scanner (GE Healthcare Systems, Milwaukee, WI). Anatomical images were obtained using a T1-weighted SPGR sequence with the following parameters: TR = 8.924 msec; TE = 1.792 msec; flip angle = 15°, with an in-plane resolution of 0.859 X 0.859 and a slice thickness of 1.5 mm. Data were analyzed using the default parameters of SPM8 with Matlab 7.5.0 (R2007b). Because Bergoiurgnan et al.[38] have challenged the effectiveness of conventional VBM in detecting volumetric reductions in medial temporal lobe structures in depression, we used a diffeomorphic image registration algorithm (DARTEL)[39] to achieve image registration to a generated template. We followed the general image processing protocol outlined by Bergouignan et al., which includes manually checking images for scanner artifacts and anatomical anomalies that would affect the image analyses and manually aligning images using the reorient tool in SPM8.

Images were initially segmented using the segmentation in SPM8[40]. Using the DARTEL toolbox, we generated templates for image registration that were used to derive Jacobian scaled warped tissue class images for gray and white matter. These resulting 'modulated' and warped

images were then smoothed with an isotropic Gaussian kernel of 8mm full-width at half-maximum and examined with an absolute masking threshold of 0.05. The resulting images had a normalized voxel size of 1.5 X 1.5 X 1.5mm.

Statistical Analysis

Two-sample t-tests were conducted comparing low-risk and high-risk girls. Covariates in the statistical design included participants' age, CDI-S score, and total brain volume on segmented, unmodulated, unsmoothed volumes. As in Bergouignan et al.'s[38] examination of the hippocampus using DARTEL-VBM, whole-brain t-tests were conducted on the smoothed, modulated, and segmented gray and white matter images with a voxel threshold of $p<0.05$, FDR corrected, using additional non-stationary cluster extent correction at that threshold[41,42]. Contrasts were set for low-risk > high-risk and for high-risk > low-risk. Given our specific interest in the hippocampus, a small volume correction was performed with the hippocampal *cornu ammonis* canonical map provided with SPM, with a threshold set to $p<0.001$ uncorrected.

Tracing was performed using Insight Toolkit's SNAP program[43], which visualizes volumes in 3 planes simultaneously while also providing 3-dimensional renderings of traced segmentations of structures. VBM analyses utilize, and indeed require, spatial normalization in order to make comparisons across a variety of sizes and shapes of brains. Because manual tracings were performed in reoriented native space, subsequently measured hippocampal volumes were divided by total brain volume to control for the potentially confounding factor of head size. Segmentations for left and right hippocampus were estimated using the SNAP program’s active contour segmentation, then hand-corrected at each coronal slice by two raters blind to participant risk status and other demographic variables. The resulting segmentations were checked in sagittal and axial planes and using the 3-dimensional render for accuracy. The

hippocampal head-body boundary was delineated by the clear appearance of the uncal recess, while the body-tail boundary was delineated by the opening of the crus of the fornix. Other anatomical features used to guide manual tracing have been described elsewhere[44]. Final volumes were output using SNAP and analyzed with SPSS16. Volumes were divided by the total brain volume and compared across groups, controlling for age and CDI-S.

Results

Demographic and clinical characteristics of the participants and their mothers are presented in Table 1. The two groups of girls did not differ in age, $t(53)=0.28$, Tanner breast, $t(53)=0.18$, Tanner hair, $t(53)=0.2$, the proportion of pre- and post-menarcheal girls, $X^2(1)=0.04$, WISC-III Vocabulary scores, $t(53)=0.44$, or CDI-S scores, $t(53)=1.94$, all *p*s>.05. Importantly, the CDI-S scores of the girls in both groups were well below the cut-off of eight used to indicate possible depression. Consistent with the absence of diagnosed depression in the participants, no participants were currently taking antidepressant medications. The two groups of mothers did not differ in socioeconomic status as measured by household income, $X^2(4)=6.89$, $p>.05$. The recurrent depressed mothers were slightly but significant younger than were the control mothers, $t(54)=2.32$, $p<.05$.

Images of brain structure acquired with magnetic resonance imaging were analyzed using VBM, an unbiased automated procedure that has been used to examine brain-structure volume in depression[45], aging[46], and neurodegenerative disorders[47]. The low- and high-risk daughters did not differ in total segmented gray matter volume, $t(53)=1.11$, total segmented white matter volume, $t(53)=1.62$, or total brain volume, $t(53)=1.35$, all *p*s>.05. Whole-brain voxel-wise analyses of gray and white volumes conducted to compare the two groups of daughters had an individual voxel significance threshold of $p<.05$, FDR corrected with a non-stationary

smoothness correction. Given our specific interest in the hippocampus, we also performed a ROI analysis with a canonical hippocampus mask with a voxel significance threshold of $p<.001$ uncorrected. We entered participants' age, CDI-S score, and total brain volume as covariates in each analysis. In whole brain analyses, there were no significant differences between the low- and high-risk girls in either white matter or non-hippocampus gray matter. Consistent with our predictions, however, ROI analysis with a hippocampal mask found that the high-risk girls had significantly less gray matter density in bilateral posterior hippocampus than did the low-risk girls, with a 31-voxel cluster on the left and a 15-voxel cluster on the right that exceeded the significance threshold (Figure 1). Moreover, adding mothers' age as another covariate did not change the results of the analyses. Thus, using VBM, we found reduced gray matter density in bilateral hippocampus in participants at elevated risk for depression.

Differences in gray matter density obtained from VBM analyses can be due to a number of factors in addition to volumetric differences in a particular area or structure. Spatial normalization to a standard template in VBM may distort neuroanatomical information; moreover, significant differences in gray matter density may also reflect differences in shape or location of a particular structure or area. Finally, the use of a smoothing kernel makes it difficult to localize with precision neuroanatomical group differences. To assess whether the VBM results indexed true volumetric differences, we followed up the VBM analyses with manual tracing. Using the same structural images that we analyzed with VBM, two raters blind to group traced bilateral hippocampi using SNAP, a segmentation and image navigation facility that is part of the Insight Toolkit. Inter-rater reliability for the two raters was .93 for the left hippocampus and .90 for the right hippocampus.

The left and right hippocampus segmentation volumes for the two groups of participants

are also presented in Table 1. Because of the wide range of total brain volumes in this age range, it is critical to control for this potentially confounding variable. Thus, in examining the ratio between hippocampus and total brain volume, high-risk participants had 6.3% smaller left hippocampus volume and 2.2% smaller right hippocampus volume than did low-risk individuals. One-way analyses of variance (ANOVAs) comparing the ratio of unilateral hippocampal volume to total brain volume between the low- and high-risk groups, covarying age and CDI-S score, and mothers' age yielded no significant group difference for the right hippocampus, $F(1,50)=2.69$, $p>.05$, but a significant effect of group for the left hippocampus, $F(1,50)=4.98$, $p<0.05$. Importantly, the data obtained from the manual tracing indicated that healthy girls at high familial risk for depression had a smaller ratio of left hippocampus to total brain volume than did their low-risk counterparts. These findings of reduced left hippocampal volume mirror the results of the VBM analyses, in which the high-risk girls were found to have significantly smaller bilateral hippocampal gray matter density than were the low-risk girls. In sum, therefore, these convergent results from VBM and manual tracing indicate that never-disordered individuals at elevated risk for depression are characterized by reduced hippocampal volume.

Comment

Previous investigations have documented lower hippocampal volume in depressed than in nondepressed persons[9,10]; the present study is the first to report smaller hippocampal volume in healthy girls at high familial risk for depression but who have not yet experienced the disorder. Few studies have examined neuroanatomical anomalies in children at high risk for psychopathology. Recently, Ladouceur and colleagues[48] reported increased hippocampal and parahippocampal volume in individuals at high risk for bipolar disorder. These results both underscore the potential importance of the hippocampal formation in affecting risk for

psychopathology and highlight a possible biological differentiation between risk for bipolar versus unipolar depressive disorders. While the present data do not preclude an association between hippocampal volume reduction and episode duration in currently depressed individuals, they do raise the possibility that the depressed participants characterized in previous studies had reduced hippocampal volumes prior to the onset of their depressive episode.

While we do not know the cause of the reduced hippocampal volume in individuals at risk for depression, it is likely that genetics plays a significant role[49]. Given their family history, the high-risk daughters in this study are likely to have a genetic predisposition for developing depression, which may also contribute to the reduction in hippocampal volume documented here. Several studies have reported associations between specific genes and reductions in hippocampal volume: the long variant of the serotonin transporter promoter region polymorphism in depressed patients[50]; the *met* allele of the brain-derived neurotrophic factor *val66met* polymorphism in depressed patients and controls[51]; and single nucleotide polymorphisms (SNPs) within the disrupted-in-schizophrenia 1 gene in schizophrenics[52]. It is becoming increasingly clear, therefore, that the functional impact of genetic factors, including SNPs, on a complicated endophenotype such as neuroanatomical structure warrants further investigation[53].

Importantly, experiential variables have also been found to influence brain morphometry, especially in the context of depression. For example, a large number of studies have reported that childhood trauma, such as physical or sexual abuse, predicts reduced hippocampal volume in individuals who subsequently develop depression in adulthood[54,55,56], but a recent study by Lenze et al.[57] found no association between childhood adversity and hippocampal volume. It is unlikely that a single gene or environmental stressor is responsible for the decreased hippocampal volume found in girls at risk for depression; it will be important to consider a combination of inherited

characteristics and life experiences in understanding the results of the present study[58].

As we noted earlier, depressed individuals have been characterized by HPA-axis dysfunction and reductions in hippocampal volume[4,5,9,10]. While the precise reasons for this decreased hippocampal volume are not clear from histopathological studies[59], it is well documented that glucocorticoids increase vulnerability of hippocampal neurons to excitotoxic insults[8]. Consistent with its role in the negative feedback regulation of the HPA-axis that controls cortisol production, smaller hippocampal volume has been found to be associated with increased cortisol secretion in response to a stressor[60], increased adrenocorticotropic hormone release and inhibited feedback regulation in response to a stressor[61], as well as with vulnerability to post-traumatic stress disorder[62]. Increased cortisol levels, in turn, could further impair hippocampal regulation and lead to increased cortisol production. Notably, early experiences, such as childhood abuse, can affect epigenetic regulation of the glucocorticoid system in the hippocampus well into adulthood[63]. Indeed, a combination of genetic, epigenetic, and environmental factors may affect hippocampal regulation of the HPA-axis. Thus, high-risk individuals with reduced hippocampal volume may be especially vulnerable to HPA-axis dysregulation and hippocampal damage, especially in the context of the development of MDD.

Given the connection of the hippocampus with other limbic and cortical circuits involved in the regulation of mood and cognition, it is not surprising that reduced hippocampal volume has been associated with executive dysfunction in depressed individuals[64]. The present finding of decreased hippocampal volume in a sample of never-depressed young girls at high risk for the development of this disorder may help to explain why people who have recovered from MDD continue to show deficits in psychological and neurocognitive functioning[65]. In addition, reduced hippocampal volume has been found to predict poorer outcome in depressed individuals[21]; thus,

reduced hippocampal volume may reflect a vulnerability for recurrent depressive episodes. Finally, given evidence that reduction in hippocampal volume in depression may be associated with specific subtypes of depression, such as psychotic depression[66], it will be important to follow these participants to examine the association between reduced hippocampal volume and the probability of developing specific depressive disorders.

Despite the strengths of the present study, there are also a number of limitations. For example, we did not administer measures of neuropsychological functioning or obtain information about school performance in the sample and, therefore, do not know whether reduced hippocampal volume is associated with specific cognitive deficits, such as difficulties in memory[67]. We also do not have data concerning antenatal and early life experiences of these participants aside from major psychopathology, such as PTSD, that might have resulted from these experiences. Obtaining a detailed assessment of early life experiences in future studies may help to elucidate the differential contribution of genetic and experiential factors to hippocampal volume. Finally, while the VBM analysis indicated that there were gray matter density reductions in the high-risk girls in bilateral hippocampus, manual tracing yielded significant volume reductions in high-risk participants only in the left hippocampus. Given that the manual segmentation also yielded reductions, though not statistically significant, in right hippocampal volume in the high-risk girls, VBM may be more sensitive than manual tracing to regional changes. In any case, however, the role of potentially asymmetric volume change in the hippocampi in young girls is an important direction for further study[68].

Identifying the factors that contribute to reduced hippocampal volume in individuals at high risk for MDD will be critical in helping to understand the mechanisms of inheritance of risk for this disorder. In this context, it will be important in future research to integrate brain imaging

techniques with assessments of specific genetic risk factors and neuroendocrinological and psychosocial functioning. Given that the behavioral effects of many antidepressants depend on neurogenesis in the hippocampus[69], as well as the observation that antidepressant treatment prevents stress-related hippocampal volume loss[70] and may reverse hippocampal volume reduction in depression[22], promoting neurogenesis through antidepressants or other interventions in individuals at high risk for depression may prevent or reverse neuronal or glial atrophy and, ultimately, delay or prevent the onset of the disorder.

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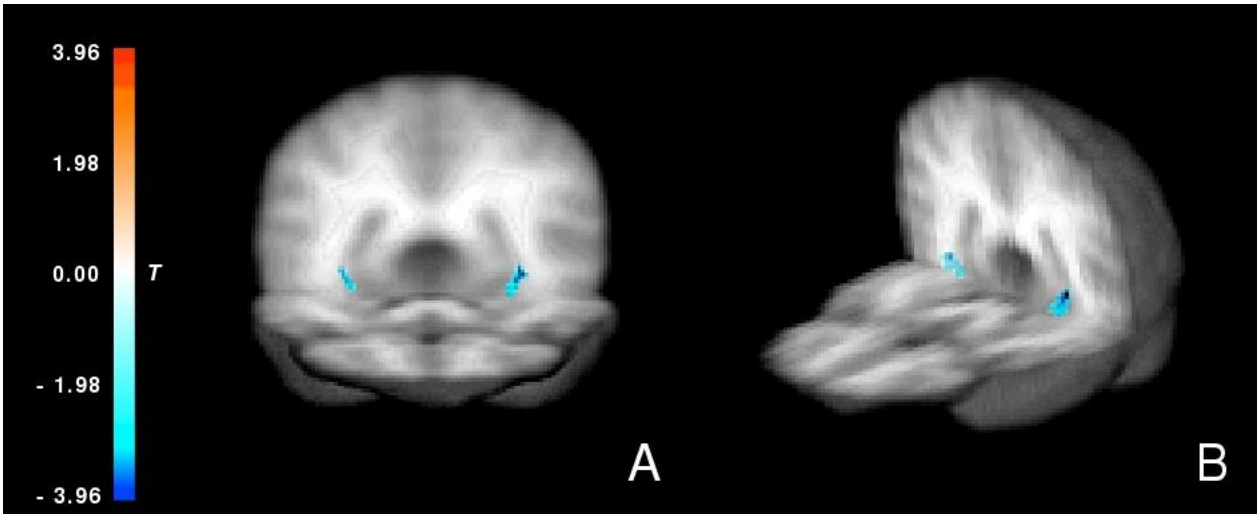
Table 1: Demographic and volumetric variables for all participants, low-risk participants, and high-risk participants (S.D.).

	Sample mean	Low Risk (32 participants)	High Risk (23 participants)	p value diff.
Daughters' age [years]	12.84 (1.56)	12.90 (1.55)	12.76 (1.60)	>0.05
Mothers' age [years]	44.89 (5.44)	45.63 (4.49)	42.39 (5.84)	0.024
Tanner [breast]	3.17 (0.98)	3.19 (0.83)	3.14(1.2)	>0.05
Tanner [hair]	3.15 (1.19)	3.13 (1.28)	3.19 (1.08)	>0.05
Menses [Y/N]	19 / 26	11 / 17	8 / 11	>0.05
CDI-S	1.87 (1.51)	1.55 (1.32)	2.33 (1.66)	>0.05
WISC-III	50.79 (6.94)	50.45 (7.92)	51.29 (5.32)	>0.05
Left hippocampal volume [mm^3]*	3021.28 (338.41)	3066.55 (329.34)	2959.70 (348.49)	0.030
Right hippocampal volume [mm^3]*	2850.31 (300.24)	2848.00 (284.92)	2853.52 (326.89)	>0.05
Total brain volume [mm^3]	1.13×10^6 (0.086×10^6)	1.12×10^6 (0.076×10^6)	1.15×10^6 (0.096×10^6)	>0.05

Note. CDI-S = Child Depression Inventory – Short form; WISC-III = Wechsler Intelligence Scale for Children – III; * = ratio of hippocampal volume to total brain volume, controlling for participants' age, mothers' age, and CDI-S score

Figure 1 Legend: Visualization of voxel-based morphometry analysis showing clusters of gray matter volume difference between high-risk and low-risk girls on a normalized smoothed brain, with positive T values representing clusters of increased matter in high-risk and negative values representing reduced matter in high-risk individuals. (A) coronal and (B) canonical cutouts (Y=-44, Z=-18) show significant gray matter reduction in high-risk individuals compared to low-risk individuals in posterior bilateral hippocampus. Cluster locations, sizes, and significance values for reduced gray matter in high-risk compared to low-risk individuals are shown at bottom of figure.

Figure 1:



Cluster Location	Local maxima x,y,z (mm)	Cluster size (voxels)	Voxel Peak significance (uncorrected *p*)
Left hippocampus	-34, -39, -6	31	<0.001
Right hippocampus	36, -39, -6	15	<0.001

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