Effects of early life adversity on hippocampal structures and associated HPA axis functions

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ABSTRACT

Early life adversity (ELA) is one of the major risk factors for serious mental and physical health risks later in life. ELA has been associated with dysfunctional neurodevelopment, especially in brain structures such as the hippocampus, and with dysfunction of the stress system, including the hypothalamic-pituitary-adrenal (HPA) axis. Children who have experienced ELA are also more likely to suffer from mental health disorders such as depression later in life. The exact interplay of aberrant neurodevelopment and HPA axis dysfunction as risks for psychopathology is not yet clear. We investigated volume differences in the bilateral hippocampus and in stress-sensitive hippocampal subfields, behavior problems and diurnal cortisol activity in 24 children who experienced documented ELA (including out-of home placement) in a circumscribed duration of adversity only in their first three years of life in comparison to data of 25 control children raised by their biological parents. Hippocampal volumes and stress-sensititve hippocampal subfields (Cornu ammonis (CA) 1, CA3 and the granule cell layer of the dentate gyrus (GCL-DG)) were significantly smaller in children who experienced ELA taking psychiatric diagnoses and dimensional psychopathological symptoms into account. ELA moderated the relationship between left hippocampal volume and cortisol: In the control group, hippocampal volumes were not related to diurnal cortisol, while in ELA children, a positive linear relationship between left hippocampal volume and diurnal cortisol was present. Our findings show that ELA is associated with altered development of the hippocampus, and an altered relationship between hippocampal volume and HPA axis activity in youth in care, even after living in stable and caring foster family environments for years. Altered hippocampal development after ELA could thus be associated with a risk phenotype for the development of psychiatric disorders later in life.

Key words: Early life adversity, childhood maltreatment, hippocampus, HPA axis, children.

1 INTRODUCTION

Physical or emotional maltreatment, neglect, or separation from the primary caregiver are forms of early life adversity (ELA), which can cause extreme stress in infants [1], when the stress system, the hypothalamic-pituitary-adrenal (HPA) axis, is still immature. Excessive stress hormone levels harm neurocognitive and neurobiological development in infancy [2]. Persistent HPA axis overactivation in infants is associated with aberrant HPA axis maturation in later childhood [3]. Most studies in ELA have observed downregulation of HPA activity with lower diurnal cortisol levels after chronic ELA exposure, although findings differ across age groups and regarding pubertal development [4-6]. Dysfunctional regulation of the stress hormone system also increases the vulnerability for psychiatric disorders like depression, or posttraumatic stress disorder (PTSD) [7, 8] later in life. The plasticity of the neural system is greatest early in life characterized by processes such as neurogenesis, axonal and dendritic growth, and synaptic pruning [9, 10]. The hippocampus is particularly susceptible to altered levels of stress hormones in infancy [11, 12]. It is also involved in the regulation of HPA axis activity [13]. Through its high glucocorticoid and mineralocorticoid receptor expression, the hippocampus seems involved in cortisol-mediated inhibition of the HPA axis, basally and in acute stress, through glutamatergic excitation of GABAergic inhibitory interneurons on the hypothalamic paraventricular nucleus [14]. Neural damage might have an impact on the hippocampal influence on the HPA axis [15]. Thus, ELA might cause HPA axis dysfunction and consecutive disruptions in the maturation of the hippocampus at a time, when both interfere with the other's development [16].

ELA is associated with smaller hippocampal volumes in adolescence and adulthood [17-21], for a review see [22]. Reduced hippocampal volume has also been associated with psychiatric disorders such as depression in adolescence [23]. Disentangling the effects of ELA and psychopathology on hippocampal development is difficult, because many studies investigating hippocampal differences in childhood maltreatment include children with psychopathology [24]. In addition, ELA is oftentimes assessed using subjective retrospective report (e.g., questionnaires), which can lead to a reporting bias [25].

A few recent studies have identified specific subregions within the hippocampus, which are particularly vulnerable to ELA in adults [26-29] and adolescents [30]. Studies in animals of adversity in infancy have shown dendritic shrinkage and a reduction of branching in pyramidal cells in the Cornu ammonis (CA) 1 and CA3, a reduction of granule cells in the dentate gyrus (DG), and a loss of spines in CA1 in childhood [31-34]. However, the question whether ELA has an impact on the stress-sensitive hippocampal subfields in children has not yet been investigated yet.

To address the aforementioned issues, we investigated whether children, who experienced objective and extreme ELA within the first three years of life in the form of parental separation (and who were free of any current or past diagnosis of an affective or trauma-related mental disorder) differ from typically developing children without a history of separation from the primary caregiver with respect to hippocampal volumes. In addition, differences regarding the volumes of the stress-responsive hippocampal CA1, CA3 subfields and the granule cell layer of the DG (GCL-DG) were investigated. A secondary aim was to explore whether the relationship between hippocampal volumes and diurnal cortisol secretion differs in children with ELA.

2 MATERIALS AND METHODS

Participants

Our sample consisted of twenty-five children, who experienced different forms of maltreatment before separation from their biological parents during their first three years of life and permanent placement in German adoptive or foster care families (ELA group). Twenty-six children who were never separated from their biological parents and had never been in contact with Child Protective Services carefully matched on demographic variables served as a control group (also see [35, 36]). The children's histories before placement were assessed by conducting semi-structured qualitative interviews [37] with the foster/adoptive parents by trained staff members to explore the pre-placement history, the main reason for separation and types of maltreatment and by inspecting all of their available medical records. The data were screened to extract the main reason for separation. Exclusion criteria for participation were (i) pervasive developmental disorders (such as Autism spectrum disorders, genetic disorders), (ii) neurological disorders or previous head trauma, (iii) current pharmacological treatment except for methylphenidate (discontinued 24 hours before assessment), (iv) IQ lower than 85, and (v) contraindications for MRI measurement (e.g., metal implants, claustrophobia or epileptic seizures). Study procedures were in accordance with the declaration of Helsinki and approved by the ethical committee of the Medical Faculty of RWTH Aachen University, Aachen, Germany. All children and their legal guardians gave written informed consent and received financial compensation for participation and travel expenses.

Psychological assessment

Intellectual abilities were assessed with the German four-subtest version of the Wechsler Abbreviated Scale of Intelligence [38]. The participants were screened extensively for present and lifetime mental disorders, according to DSM-IV criteria, via semi-structured diagnostic

interviews [39] separately conducted with both the child and the caregivers by trained staff members. According to the DSM-IV criteria five children fulfilled the criteria for attention-deficit/hyperactivity disorder (ADHD) or dyslexia/dyscalculia in the control group. Of the ELA participants, nine fulfilled the criteria for ADHD, dyslexia/dyscalculia, enuresis or conduct disorder (see table 1). None of the participants fulfilled diagnostic criteria for a current or past affective disorder, PTSD or a pervasive developmental disorder, substance abuse or psychosis.

To additionally assess dimensional measures of psychiatric symptoms and behavior problems, caregivers filled out the Child Behavior Checklist (CBCL) [40], a caregiver-report that assesses children's problem behaviors in youth between 4 and 18 years. The internalizing symptom score was calculated by combining the scores on the subscales "social withdrawal", "physical complaints", and "symptoms of anxiety and depression". The subscales "delinquent behavior" and "aggressive behavior" were combined to calculate the externalizing symptom score. The T-values of both scores were used in the analyses.

All children rated the relationship quality with their caregivers on a German self-report questionnaire (EBF-KJ, [41]). The EBF-KJ is a clinically oriented questionnaire assessing the parental representation of children. The questionnaire consists of 36 items representing three resource-scales ("cohesion", "identification", "autonomy"), five risk-scales ("conflicts", "rejection/neglect", "punishment", "emotional burden", "fears/overprotection") and one additional scale "aid". The resulting global score is a measure of the parent-child-relationship. The T-values of the global score were used in the analyses.

MRI acquisition and volumetric assessment

Magnetic resonance imaging was conducted on a Siemens 3T MAGNETOM Trio MRI scanner (Siemens, Erlangen, Germany) using a T1-weighted, three dimensional, magnetization

prepared rapid gradient echo (MPRAGE) sequence covering the whole brain (TE=2.96 ms, TR=2250 ms, flip angle = 7°, 176 slices, matrix 265 x 265 mm², field of view=265 ms, slice thickness= 1 mm, voxel size=1 mm³). The structural images were acquired in a session with an overlapping sample of children that also collected functional task-based and diffusion-tensor-imaging (DTI) data, which have been reported previously (see [35, 36]). Cortical reconstruction and volumetric segmentation were performed using the freely available FreeSurfer image analysis pipeline (version 5.3, http://surfer.nmr.mgh.harvard.edu/) [42]. FreeSurfer analysis tools have been validated in pediatric populations [23]. The segmentations were visually inspected for accuracy by a trained researcher. One control child was excluded due to a previously unknown structural brain abnormality, and one ELA child had to be excluded because of excessive head motion. To overcome problems of estimating hippocampal volume in children [43] and inconsistencies of hippocampal subfield delineation with previous automated procedures [44], the novel improved automated routine from FreeSurfer was used to calculate hippocampal volumes and subfield volumes in accordance with previous studies [45, 46]. It is built on a computational atlas built from *ex vivo* and *in vivo* MRI data and has been proven reliable in estimating hippocampal volume and its subfields according to histological and anatomical boundaries [46]. In addition, it allows for a more precise comparison with animal studies, because all subfields (e.g., all CA-subfields and the granule cell layer of the dentate gyrus) are labelled separately. All volumes were visually inspected for errors. The bilateral hippocampal volumes and the hippocampal subfield volumes of interest, the CA1, CA3 and the GCL-DG were used in the analyses. In line with previous studies, individual total intracranial volumes estimated from FreeSurfer's automated analysis pipeline were used to correct for differences in head size [45].

Cortisol collection and analysis

Diurnal cortisol levels were measured via collection of saliva samples to assess associations between adaptations of neuroendocrine parameters and psychopathology after ELA [47]. Saliva samples were collected at home by the caregivers three times a day (each approximately 30 minutes after awakening, before lunch and before bedtime) on two consecutive days using Salivette sampling devices (Sarstedt, Nürmbrecht, Germany). The participants were asked to restrain from eating, drinking caffeinated beverages, brushing teeth or exercising 60 minutes before sampling. Caregivers filled out a detailed sampling protocol for compliance monitoring. Samples were assayed in the central laboratory of the University Hospital Aachen by electrochemiluminescence-immunoassay (ECLIA, Cobas e601, detection limit 0.5 – 1750 nmol/l). Note that the current sample overlaps with the sample described in [47], see [47] for more detailed procedures of the salivary cortisol assessment. To model HPA axis activity, the area under the curve (AUC) with respect to ground was calculated according to the trapezoid formula [48] on the averaged cortisol values (across both days). In seven children, complete day profiles could not be retrieved due to missing sampling times, missing measurements, measurement error, or sampling in discordance with the protocol. In total, cortisol data of 18 control children and 17 ELA children were analyzed. The descriptives of the children with cortisol data in comparison to the children without cortisol data are displayed in Supplementary Table 1.

Statistical analyses

All statistical analyses were performed using SPSS Statistics v23 (IBM corp., Armonk, New York, USA). All data were visually inspected and tests of normality and homogeneity of variance were performed to assure that the necessary assumptions were met. Extreme outliers were winsorized to the mean ± 3 SD [6]. Group differences in demographic variables (age, gender, ethnicity (Caucausian vs. non-Caucasian), IQ, maternal education as an approximation of

socioeconomic status (SES)), differences in internalizing and externalizing symptoms, perceived relationship quality, psychiatric diagnoses, and diurnal cortisol values were calculated using independent samples t-tests or Chi-Square tests. According to previous studies [49], the demographic variables were examined as potential covariates for the hippocampal volumes using independent samples t-tests and Pearson correlations. Results are reported after removing all non-significant interaction terms and predictors. Gender and total intracranial volume were kept in the analyses due to their known influence on the primary outcome variables.

Volumetric hippocampal analyses

Repeated-measures Analysis of Covariance (rmANCOVA) was used to calculate group differences with group and gender as between-subject factors and hemisphere as within-subject factor using total intracranial volume as a covariate. RmANCOVAs were performed for whole hippocampus, CA1, CA3 and GCL-DG separately. Because psychiatric disorders have been associated with smaller hippocampal volume, the analyses were conducted with psychiatric diagnosis as an additional between-subject factor and internalizing and externalizing symptoms as additional covariates in the rmANCOVAs.

Associations between volume and cortisol

To investigate a possible effect of group on the relationship between diurnal cortisol levels and hippocampal volumes (left and right), linear regressions were conducted: The respective volume, group, psychiatric diagnosis and the interaction terms were included as independent variables and diurnal cortisol as the dependent variable correcting for total intracranial volume, internalizing symptoms, externalizing symptoms, and gender. Hippocampal volumes and internalizing and externalizing symptoms were centred prior to analysis to avoid

multicollinearity. In case of a significant result for the whole hippocampal volume, linear regressions, following the above mentioned procedures, were also performed for the hippocampal subfield volumes of interest of the corresponding hemisphere.

The ELA group was exploratively split according to the median of the left hippocampal volumes into ELA subgroups with larger and smaller left hippocampal volumes. Their mean cortisol values across the day for the three time points (morning, noon, evening) were plotted against the mean cortisol values of the control group to identify differences in diurnal cortisol rhythm.

3 RESULTS

Demographic variables

The ELA children were all separated from their biological families before the fourth year of life (mean age at separation: 11.82 months, SD 12.01, range: .25 – 39 months) and permanently placed in German adoption or foster care families (mean duration of placement: 9.24 years, SD 2.25, range: 54 – 162 months). The control and the ELA group did not differ significantly with respect to age (t(47)=-.53, *p*=.60), gender (X²=.18, *p*=.67), IQ (t(47)=1.02, *p*=.31), SES (t(46)=.00, *p*=1.0) or relationship quality with their parents (t(44)=1.1, *p*=.29) (see table 1). The ELA group contained a significantly higher percentage of children with a non-Caucasian ethnicity (X²=5.13, *p*=.02). ELA children suffered from significantly higher internalizing symptoms (t(46)=-3.4, *p*=.002; borderline clinical range 60-63 [40], T mean = 60.1) and significantly higher externalizing symptoms (t(46)= -4.64, *p*<.001; clinical range > 63 [40], T mean = 63.8) than control children. Internalizing and externalizing symptoms were not significantly related to age at assessment, gender, ethnicity, SES or IQ, nor were they related to age at separation or short-term institutionalization in the ELA children only. A slightly higher

percentage of ELA children had at least one psychiatric diagnosis (37,5% vs. 20%), but the difference did not reach statistical significance (X^2=1.84, *p*=.18). Diurnal cortisol values did not differ between the groups (t(33)=.25, *p*=.80). The sample demographics are presented in table 1.

Simple correlations

In line with previous studies [49, 50], all hippocampal volumes were significantly larger in males than in females (range: t=3.46 – 2.14, all *p*<.05), except for the right CA3 (t=1.82, *p*=.07). Gender was included as a covariate in all subsequent analyses. The other demographic variables (age, ethnicity, IQ, SES, relationship quality with parents) were not significantly associated with hippocampal volume.

Volumetric hippocampal analysis

According to the rmANCOVA, there was a significant effect of group on whole hippocampal volumes (F(1,45)=8.10, *p*=.007) and, as expected, a marginally significant effect of gender (F(1,45)=4.01, *p*=.051). In addition, a significant interaction effect of hemisphere by group was evident (F(1,45)=4.25, p=.045). Neither psychiatric diagnosis nor internalizing neither externalizing symptoms explained variance significantly, so they were removed from the analysis. The separate posthoc univariate ANCOVAs revealed a significant effect of group on the left hippocampal volume (F(1,45)=4.86, *p*=.033) and right hippocampal volume (F(1,45)=10.49, *p*=.002), which were both significantly smaller in the ELA children as compared to the control children, displayed in figure 1. Regarding right hippocampal volume, there was an additional significant effect of gender (F(1,45)=4.52, *p*=.039), which was not present for left hippocampal volume (F(1,45)=2.92, *p*=.095). The significant group effect regarding right hippocampal volume remained after again including the psychopathological variables into the

univariate model (F(1,37)=8.40, p=.006), although the psychopathological variables did not explain variance significantly. The effect of gender was not significant anymore. Regarding left hippocampal volume, inclusion of the psychopathological variables showed no significant effect of group (F(1,37)=3.40, p=.073), and again, neither psychiatric diagnosis, nor internalizing or externalizing symptoms did explain a significant amount of variance.

Regarding subfield CA1, there was a significant effect of group (F(1,45)=9.33, p=.004) and a significant effect of gender (F(1,45)=6.02, p=.018). Regarding subfield CA3, there was a significant effect of group (F(1,45)=7.76, p=.008), but, in line with the simple correlations, no main effect of gender. In GCL-DG, also a significant effect of group was present (F(1,45)=7.99, p=.007) and a significant effect of gender (F(1,45)=4.74, p=.035). In all three subfields, psychiatric diagnosis, internalizing and externalizing symptoms did not explain variance significantly, so they were removed from the analyses. Also, there was no significant interaction effect of hemisphere by group. Because there were no differences in laterality, the estimated average subfield volumes across hemispheres are displayed in figure 2.

Associations between volume and cortisol

The regression model containing ELA, left hippocampal volume and their interaction explained a significant amount of variance of diurnal cortisol (F(5,29)=2.96, p=.028, corr. R^2=.224). Again, psychiatric diagnosis, internalizing and externalizing symptoms were removed in the analyses, because they did not explain variance significantly. The regression model is displayed in supplementary table 2a. ELA significantly moderated the relationship between the left hippocampal volume and diurnal cortisol. The separate regression models showed that for control children, there was no relationship between left hippocampal volume and cortisol, while for ELA children smaller hippocampal volume was significantly associated with lower diurnal cortisol (F(3,13)=4.12, p=.029). Regarding right hippocampal volume, psychiatric

diagnosis, internalizing and externalizing symptoms again did not explain variance significantly, so they were removed. The model containing ELA, right hippocampal volume and their interaction closely failed to predict a significant amount of variance ($F_{(5,29)}=2.24$, $p=.077$), even though the interaction term (group by right hippocampal volume) was significant. The whole regression model is summarized in supplementary table 2b.

Regarding the left hippocampal subfields, both the models containing ELA, left CA1 volume and their interaction, and ELA, left GCL-DG volume and their interaction also closely failed to predict variance significantly (left CA1: $F_{(5,29)}=2.45$, $p=.058$; left GCL-DG: $F_{(5,29)}=2.51$, $p=.053$), even though the interaction terms (group by left CA1/left GCL-DG volume) was significant. The model containing ELA, left CA3 volume and their interaction was not significant, although again, the interaction term was significant.

Exploratively splitting the ELA group according to left hippocampal volume differences into two groups (smaller vs. larger volume) and comparing the mean cortisol levels across the day showed consistently lower cortisol values of the ELA children with smaller left hippocampal volume. The subgroup of ELA children with larger left hippocampal volume showed typical waking cortisol levels, but elevated values at bedtime in comparison with the control group. The diurnal rhythm curves are plotted in figure 3 for descriptive purposes only.

4 DISCUSSION

Our results reveal persistent changes in brain development, HPA axis involvement, and behavior problems in a group of children who experienced extreme ELA at a circumscribed time period in infancy. Children with ELA had smaller bilateral hippocampal volumes, smaller

stress-sensitive hippocampal subfields, and a significant linear relationship between left hippocampal volume and diurnal cortisol. They displayed significantly higher caregiver-reported internalizing symptoms in the borderline clinical range and just above the clinical cut-off externalizing behavior problems. To the best of our knowledge, our study is the first to show smaller stress-sensitive hippocampal subfields and a different relationship between hippocampal volume and diurnal cortisol after documented ELA during the first three years of life in a childhood sample.

Bilateral hippocampal volumes and stress-sensitive hippocampal subfields were significantly smaller in the ELA children than in the control children, suggesting persistent neurodevelopmental alterations even years after ELA exposure. The difference in right hippocampal volume was more pronounced indicated by the significant hemisphere by group interaction effect. This finding partially agrees with and extends upon the findings of previous studies. Hodel and colleagues (2015) investigated extremely neglected children adopted internationally from institutional care. Previously institutionalized children had significantly smaller left hippocampal volumes than children who were raised in their biological families and the right hippocampal volume differed significantly between early and late adopted children [19]. Hanson and colleagues (2015) showed that left hippocampal volume and both amygdala volumes were smaller in children as a function of accumulating life stress in childhood (maltreatment, neglect, poverty, previous institutionalization) [21]. Children who experience adverse care environments are an at-risk population for psychiatric disorders later in life [51]. In both studies, psychiatric disorders were not taken into account, which could explain the difference in laterality in comparison to our findings. Stronger left-sided effects on hippocampal volume seem to be observed more frequently if participants have suffered from psychiatric disorders [e.g., 52]. In our study, internalizing and externalizing symptoms were

significantly higher in the ELA group, although none of our participants had experienced a self- or parent-reported depressive episode or affective disorder. Despite our small sample size, our findings suggest the right hippocampal volume differed more between children with documented objective ELA during infancy and control children, and psychopathology did not explain variance in differences in hippocampal volume. Our finding is in line with a recent metaanalysis suggesting that right hippocampal volume is significantly reduced in youths with childhood maltreatment [53]. Gray matter deficits in the hippocampus after ELA may sensitize children for future stress [20] and might present a vulnerability factor for affective disorders later in life. As the mean age of our sample was below the typical age of onset for depressive disorders, follow-up investigations of the present sample would further evaluate this hypothesis.

Previous studies investigating the effects of maltreatment experience or stress in adults have identified volume reductions in hippocampal subfields CA1, CA2-CA3, CA4-DG, and the subiculum (e.g., [26, 27, 29]) and effects on the development of the left CA4-DG and a larger presubiculum in adolescence [30]. Using a novel automated labelling procedure allowed us to investigate the stress-sensitive hippocampal subfields identified by animal studies in ELA more accurately. In our sample, ELA was associated with volume reductions in CA3 and GCL-DG. In addition, similar to studies in juvenile animals [34], CA1 was also affected. Interestingly, Whittle and colleagues (2017) found a marked but not statistically significant volume reduction in right CA1 in females, who developed a mental illness in late adolescence [30], which was not related to self-reported maltreatment experience.

Most studies to date conducted in adults have assessed maltreatment experience by retrospective self-reports with the potential of reporting bias [25]. Higher perceived stress is also related to significant hippocampal volume and CA2/3 and CA4/DG subfield reductions in

older adults [49]. It is not clear, whether the differences in hippocampal volumes result from the accumulation of stressful experiences or are part of the individual vulnerability, but could imply that individuals who are more stress-sensitive might over-report negative experiences. A strength of our study and our sample is that ELA was not obtained by subjective reports, but according to an objective and validated extreme ELA experience at a circumscribed age in early infancy.

The volumes of the left hippocampus were significantly linearly associated with diurnal cortisol in the ELA participants suggesting an association, which was absent in the control group. The regression models containing right hippocampal volume, left CA1, and left GCL-DG volumes closely failed to detect a significant difference between the ELA and the control group in predicting diurnal cortisol. However, this finding has to be considered as preliminary given the small power in the current sample to detect significant associations. Previous data suggested that projections from the CA1 might be actively involved in the regulation of the HPA axis [54]. Early dendritic loss and local neuronal damage in the hippocampus due to an overexpression of stress hormones in acute phases of extreme adversity might add to a disturbed HPA axis regulation [3]. Abnormal functioning of the HPA axis and decreases in hippocampal volume have been identified as vulnerability markers in at-risk individuals for depression [8]. Our findings of smaller left hippocampal volume associated with lower diurnal cortisol in the ELA group might represent a precursor of the vulnerability to affective disorders in this population later in life. Further longitudinal studies with larger samples are required to clarify this complex issue.

The mean diurnal cortisol measures were not significantly different between the ELA and the control group, although the diurnal cortisol curves suggest different subgroups within the ELA group. The ELA children with lower diurnal cortisol and corresponding smaller hippocampal

volumes might have been affected more severely. The ELA children with larger hippocampal volumes had typical waking cortisol but elevated cortisol values throughout the day. Elevated evening cortisol values have been observed in temporarily stunted post-institutionalized children who caught up in physical development after family placement in comparison with family-reared or chronically stunted post-institutionalized children [55], and also in healthy children who experienced recent increases in parent-child conflict [56]. We did not assess recent parent-child conflict and the subgroup sample is small. Thus, these exploratory findings cannot be interpreted with confidence. Replication in larger samples will be necessary prior to generalizing any conclusions.

A possible developmental mechanism of inhibited hippocampal growth might come about through a change in gene expression. Wei and colleagues (2015) discovered that ELA decreased rRNA levels in the hippocampus, increased DNA methylation and blunted hippocampal growth in mice [34]. In addition to ELA, a higher genetic risk profile might also contribute to differences in hippocampal volume. Rao and colleagues (2010) reported a decrease in left hippocampal volume in adolescents after ELA and in children with a higher genetic risk for depression [57]. Based on our data, we cannot rule out a higher genetic risk profile for affective disorders in the ELA group. However, recent research suggests that, when faced with strong environmental stressors, the genetic risk for abnormal neurobiological development associated with increased risk for affective disorders might be overridden by the environmental stressor [58]. Being separated from the primary caretaker in infancy is clearly one of the strongest stressors in early life, and therefore, the genetic risk in these children might contribute less to the observed changes.

A few limitations have to be kept in mind when considering the results of this study. First, even though the ELA children did not fulfill the criteria of an affective disorder, the number of psychopathological symptoms differed significantly from the symptoms in the control group. The observed effect of the smaller hippocampal volume might not be due to ELA alone but also to subliminal depressive symptoms. However, psychiatric diagnosis and behavior problems did not explain variance in hippocampal volumes or the relationship between cortisol and hippocampal volume significantly. Additionally, recent studies in adults investigating depressed patients have shown that, if maltreatment experiences are controlled for, the differences in hippocampal volume were strongly reduced, suggesting that ELA has an independent additive effect on hippocampal volume [e.g., 59].

We did not include measures of pubertal development in our data. However previous studies in youths with ELA demonstrated reductions of hippocampal volumes independent of pubertal stage [16, 18]. During the transition from childhood to early adulthood, limbic structures such as the hippocampus and its subfields continue to increase in volume [60, 61]. We would suspect that, if the ELA children were accelerated in pubertal development with a concomitant growth in hippocampal volume, there would be a less pronounced difference between groups regarding hippocampal volume. However, it cannot be completely ruled out that the differences between hippocampal volumes of our groups and the associations between diurnal cortisol and hippocampal volume may have been affected by pubertal status.

There were no data on prenatal status, dietary deficiencies such as iron deficiency or stressful life events during pregnancy, which have also been associated with hippocampal development [62, 63]. This is a common limitation in studies of children who were removed from the care of their birth parents, because medical histories of the birth family or pregnancy cannot always be retrieved. Further longitudinal studies taking into account pre- and perinatal risk factors

for hippocampal development in children with ELA are needed to investigate early influences in different developmental periods. Also, pre-placement histories and the forms of maltreatment in addition to the separation experience were only assessed through the foster/adoptive parents, which hampered receiving information on timing, severity, and chronicity of maltreatment experiences. Because we considered the separation experience as ELA, which differentiated between the groups, control children were not specifically screened for maltreatment experience, which presents a further limitation.

Taken together, our findings add two important aspects to previous work in the field. First, we demonstrated that alterations in stress-sensitive hippocampal subfields can be already observed in children exposed to ELA in infancy, which add further to the idea that ELA impairs maturation in the developing hippocampus. Second, our data suggest a possible association between hippocampal volume and diurnal cortisol activity in children exposed to ELA as a possible neurobiological mechanism. Because of the small sample size, our conclusions have to be drawn with caution. Further studies specifically investigating the exact interplay between hippocampal volumes and HPA axis functions after ELA might investigate the mechanisms of adaption after ELA further.

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Table 1. Final sample participants' demographic information.

	ELA group (n=25)	Control group (n=24)	t/X²	p-value
Age (years)	10.6±1.8	10.4±1.7	-.53	p=.60
IQ[1]	101.2±10.4	104.0±9.3	1.0	p=.31
Gender			.18	p=.67
Female	50%	44%		
Male	50%	56%		
SES	1.3±.5	1.3±.4	.00	p=1.0
Ethnicity			5.1	p=.02
Caucasian	46%	16%		
Non-Caucasian	54%	84%		
Relationship quality[2]	68.6±8.4	71.1±7.7	1.1	p=.29
Age at separation (months)	11.8±12.0	-		
Time spent in permanent placement (months)	110.9±27.0	-		
Institutionalization		-		
No institutionalization	83%			
Short-term institutionalization	17%			
Main reason for separation		-		
Emotional/physical neglect	67%			
Abandoned after birth	21%			
Physical abuse	8%			
Witnessing domestic violence	4%			
Psychiatric diagnoses[3]	37.5%	20%	1.8	p=.18
Externalizing symptoms	63.8±9.6	51.2±9.1	-4.6	p<.001
Internalizing symptoms	60.8±10.1	52.0±7.8	-3.4	p=.002
Diurnal cortisol AUC (nmol/L*h)	4587.1±2845.1	4414.7±1503.8	.25	p=.80

[1]assessed with the German 4-subtest version of the Wechsler Abbreviated Scale of Intelligence [37, Wechsler].
[2]assessed with the EBF-KJ [39, Titze].
[3]assessed through standardized clinical interviews according to DSM-IV criteria [38, Unnewehr].
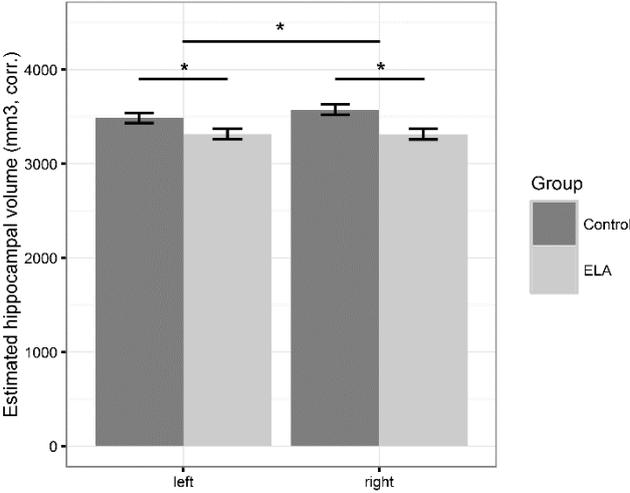Abbreviations: IQ, intelligence quotient; SES, socioeconomic status.

Figure 1.

Figure 1. Differences in estimated hippocampal volume (corrected for gender and total intracranial volume) between the groups. *p<.05. Error bars indicate one standard error. Left, left hemisphere; right, right hemisphere; ELA, early life adversity; corr. , corrected.
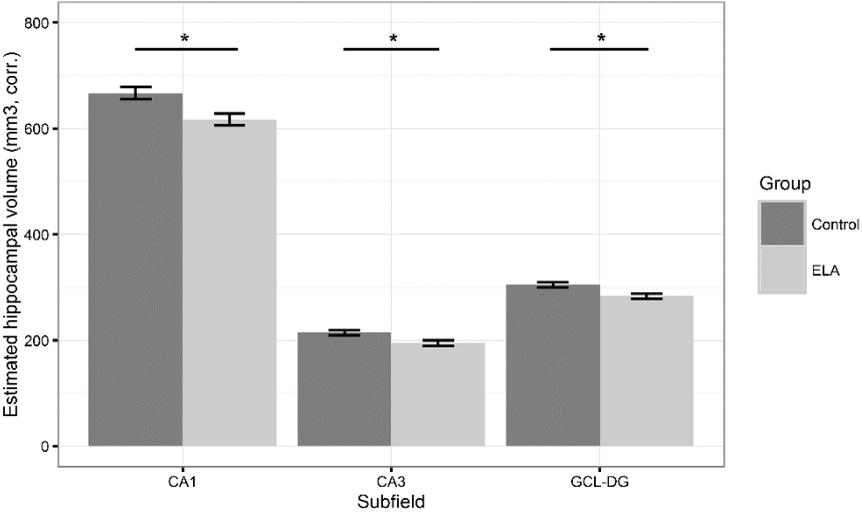
Figure 2.

Figure 2. Differences in estimated hippocampal subfields' volume between the groups (corrected for gender and total intracranial volume). *p<.05. Error bars indicate one standard error. CA1, Cornu ammonis 1; CA3, Cornu ammonis 3; DG, Granule Cell Layer of the Dentate Gyrus; ELA, early life adversity.
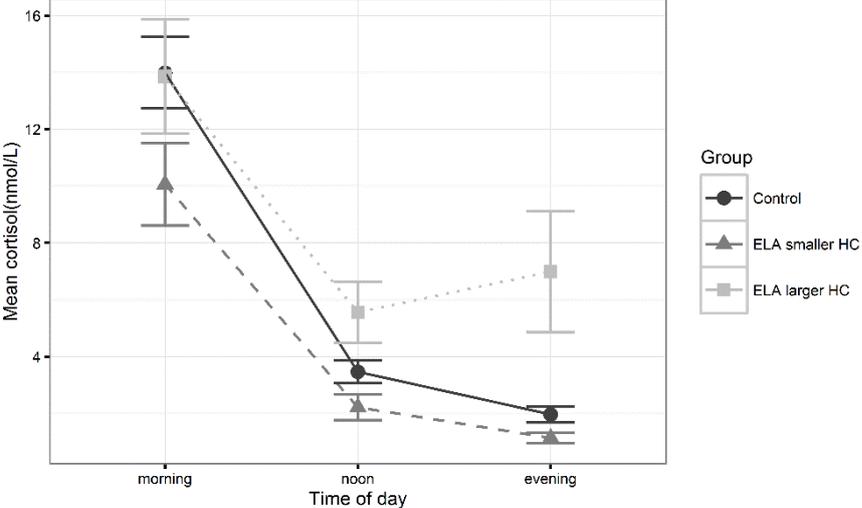
Figure 3.

Figure 3. Mean diurnal cortisol across the day for ELA children with smaller and larger left hippocampal volume separately (median split) and for control children. HC, hippocampal volume; ELA – early life adversity.

Supplementary Table 1. Demographic variables of participants with and without cortisol data.

	Cortisol group (n=35)	No cortisol group (n=14)	t/X²	p-value
Age (years)	10.4±1.8	10.7±1.6	.57	*p*=.57
IQ[1]	101.0±9.4	106.2±9.9	1.9	*p*=.07
Gender			.82	P=.37
Female	50%	43%		
Male	50%	57%		
SES	1.2±.5	1.4±.5	1.7	*p*=.10
Ethnicity			2.5	*p*=.12
Caucasian	63%	86%		
Non-Caucasian	37%	14%		
Relationship quality[2]	69.5±8.3	70.6±7.9	.42	*p*=.68
Age at separation (months)	8.3±8.0	20.4±16.4	1.9	*p*=.10
Time spent in permanent placement (months)	112.8±25.9	106.2±31.0	.92	*p*=.70
Psychiatric diagnoses[3]	29%	29%	.00	*p*=1.0
Externalizing symptoms	57.0±10.8	58.7±12.7	.45	*p*=.65
Interalizing symptoms	56.9±10.2	55.2±9.7	-.50	*p*=.62

[1]assessed with the German 4-subtest version of the Wechsler Abbreviated Scale of Intelligence [37].
[2]assessed with the EBF-KJ [39].
[3]assessed through standardized clinical interviews according to DSM-IV criteria [38].
Abbreviations: IQ, intelligence quotient; SES, socioeconomic status.

Supplementary Table 2A. Coefficients of the model predicting diurnal cortisol (left HC)

	B	SE B	T	p
Constant	5809.89	4296.41	1.35	.19
Group	1287.50	741.51	1.74	.09
Gender	1083.98	750.35	1.45	.16
ICV	-.002	.003	-.54	.60
Left HC	1.19	1.25	.96	.35
Left HC X Group	5.01	1.76	2.85	.008

Model: F(5,29)=2.96, p=.028, corr. R²=.224
Abbreviations: ICV, intracranial volume; HC, hippocampal volume.

Supplementary Table 2B. Coefficients of the model predicting diurnal cortisol (right HC)

	B	SE B	T	p
Constant	3903.60	4299.54	0.91	.37
Group	1277.51	816.47	1.57	.13
Gender	1140.19	800.57	1.42	.17
ICV	-.000	.003	-.080	.94
Right HC	0.5	1.21	.415	.68
Right HC X Group	5.13	1.83	2.8	.009

Model: F(5, 29)= 2.24, p=.077, corr. R²=.154
Abbreviations: ICV, intracranial volume; HC, hippocampal volume.