Early Parenting Predicts Hippocampal Subregion Volume Via Stress Reactivity in Childhood

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Abstract

Rodent models indicate that parenting shapes offspring outcomes by programming the hypothalamic-pituitary-adrenal axis response to stress and, ultimately, altering brain structure and function. The present study tested this hypothesis and explored possible timing-dependent associations in a longitudinal sample of children ($N = 63$). At Time 1 ($M = 4.23 \pm .84$ years) and Time 2 ($M = 7.20 \pm .89$ years), children completed parent-child interaction tasks and a laboratory stressor after which salivary cortisol samples were collected. At Time 2, children also completed a structural MRI. Analyses revealed timing- and region-dependent associations between early and concurrent parenting and cortisol reactivity and hippocampal subregion volumes. Moreover, greater negative parenting during early childhood predicted greater cortisol reactivity three years later, which, in turn, led to reduced left hippocampal tail volume. Findings suggest that the hippocampus is sensitive to environmental influences during early childhood, a result that parallels findings from rodent models.

*Keywords*: hippocampus; volume; subregion; parenting; cortisol reactivity; HPA axis; structural MRI.
**Introduction**

Parenting behaviors, both good and bad, matter for child outcomes. A robust body of rodent literature has documented that the early caregiving environment shapes offspring development by epigenetically programming the hypothalamic pituitary adrenal (HPA) axis response to stress, which subsequently, alters the structure and function of the developing brain. Despite a substantial literature linking caregiving behaviors to long-term child outcomes, and a growing literature examining associations between parenting and brain structure, little research has prospectively investigated the effects of the early caregiving environment on neurobiological development in school-age children. The current study aims to fill this gap by examining the concurrent and longitudinal associations between maternal parenting behaviors, child stress reactivity, and hippocampal subregion volume at school age.

Parenting behaviors have been linked to a host of child outcomes spanning cognitive, affective, social, behavioral, and neurobiological domains (Belsky & de Haan, 2011; Borkowski, Ramey, & Brisol-Power, 2002). Entire subfields of developmental psychology exist to explore the effects of early parenting on various aspects of child development (Cassidy & Shaver, 2016). Although many studies are correlational, experimental manipulation and interventions of parenting behaviors have also been found to alter child outcomes (Yap et al., 2016). Despite substantial evidence of the effects of the early caregiving environment on child outcomes, little research has examined the neurobiological processes mediating these associations in humans. In other words, how does the early parenting environment “get under the skin” and influence the brain and behavior during childhood (Fox, Levitt, & Nelson, 2010; McEwen, 2012)?

**Neurobiological Pathways of Parenting**
The past two decades of carefully designed rodent research and converging human evidence point to the role of the early parenting environment in the epigenetic programming of the developing hypothalamic-pituitary-adrenal (HPA) axis and its downstream effects on neural development. The HPA axis, an endogenous stress response system preserved across mammalian species, facilitates adaptive coping to stressors by regulating key biological systems. When an organism is presented with a stressor, a series of neurobiological events unfold, ultimately culminating in the release of the stress hormone cortisol (corticosterone in rodents). Research indicates that the early caregiving environment programs the offspring’s HPA axis response to stress through changes in gene expression. Specifically, offspring from nurturing environments are better able to shut down effectively their stress response and cope with stress, whereas offspring who are exposed to less warmth and nurturance demonstrate a dysregulated HPA axis responses to stress often marked by high and increasing cortisol levels (Barha, Pawluski, & Galea, 2007; Liu et al., 1997). The debated glucocorticoid vulnerability hypothesis proposes that elevated levels of circulating glucocorticoids (i.e., cortisol, corticosterone) may produce dendritic restructuring, or “retraction”, which increases vulnerability to neurotoxic or metabolic changes in regions with high densities of glucocorticoid receptors, such as the hippocampus. While conflicting findings suggest that the primate hippocampus may not have the same negative response to glucocorticoids as the rodent hippocampus, Conrad (2009) proposes that chronic stress history and repeated glucocorticoid exposure do lead to deleterious effects on the human hippocampus. Due to the protracted period of dendritic retraction, lasting up to several years, the hippocampus is particularly susceptible to these metabolic influences (Conrad, 2009; Sapolsky, 1987; Sapolsky, Packan, & Vale, 1988; Sapolsky, Uno, Rebert, & Finch, 1990; Uno et al., 1994). These potential effects are particularly important given the hippocampus’ role in regulating the
HPA axis (Jacobson & Sapolsky, 1991), episodic memory functions (Tulving & Markowitsch, 1998), and its implication in the pathophysiology of depressive disorders (Campbell & Macqueen, 2004). Critically, the neural changes associated with HPA dysregulation mirror the effects of parenting behaviors on hippocampal structure and function (Champagne et al., 2008).

**Human Translational Research**

As reviewed above, the rodent literature has characterized the pathway from early parenting to hippocampal alterations through HPA axis dysregulation. However, to date, examinations of the link between parenting and neural development in humans are only emerging.

**Parenting and cortisol.** Of the existing human research, studies have largely replicated the links between the early parenting environment and HPA axis programming, though with somewhat less consistent and more modest results. For example, while many studies have found associations between early maladaptive parenting practices and evidence of hypercortisolism (increased reactivity, higher basal cortisol) (Bugental, Martorell, & Barraza, 2003; Ellenbogen & Hodgins, 2009; Kuhlman, Olson, & Lopez-Duran, 2014; Taylor et al., 2013), others have found evidence of hypocortisolism (Engert et al., 2010; Marsman et al., 2012; Narita et al., 2012; Zalewski, Lengua, Kiff, & Fisher, 2012). These inconsistencies may be due to methodological differences such as the method for measuring indices of parenting (e.g., observational versus self-report) and various indices of HPA axis function, as well as the age at which these measurements are taken. Moreover, there is evidence that acute versus chronic exposure to stressors may differentially influence the developing HPA system, with chronic stress exposure related to lower cortisol levels (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Kudielka & Wüst, 2015). Despite these methodological differences and some inconsistencies in the literature,
HPA function has been linked to parenting in human offspring (Gunnar & Hostinar, 2015; Koss & Gunnar, 2018).

**Cortisol and hippocampus.** Neuroimaging techniques provide a means to translate findings in rodents to human populations and to explore the effects of cortisol on neural structure and function in humans. The majority of studies to date have examined associations between HPA axis function and hippocampal structure in adults (for a review see Frodl & O’Keane, 2013), with limited research examining these associations during childhood. Higher levels of morning cortisol have been associated with decreased hippocampal volumes in geriatric populations (Knoops, Gerritsen, van der Graaf, Mali, & Geerlings, 2010; Sudheimer et al., 2014). In contrast, in younger adults, greater cortisol release on the dex/CRH test (Narita et al., 2012), greater cortisol awakening response (Dedovic et al., 2010; Pruessner, Pruessner, Hellhammer, Bruce Pike, & Lupien, 2007) and greater cortisol reactivity to a social stressor (Pruessner et al., 2007) have been linked to larger hippocampal volumes. In a sample of 7- to 12-year-old children, morning basal cortisol was not associated with total hippocampal volume, but was associated with regionally-specific inward and outward deformations in lateral and anterior regions of the hippocampus (Wiedenmayer et al., 2006). In a prospective longitudinal sample, higher cortisol levels in children ages 3-6 years mediated associations of both genetic risk and early life stress with decreased hippocampal volumes when children were 7-12 year-olds (Pagliaccio et al., 2014). These patterns of results may suggest an age-related change in the association between cortisol levels and hippocampal volume, with possible negative associations in childhood and old age and positive associations in mid-life adulthood. Thus, it is possible that glucocorticoids exert differential effects on neural substrates at different points in development.
**Parenting and hippocampus.** In a review of the extant literature linking the early parenting environment to neural development in humans, Belsky and de Haan (2011) concluded that the majority of pre-existing human literature has exclusively focused on extreme forms of negative parenting such as maltreatment and neglect. These studies largely replicate the rodent literature – with reports of early physical and sexual abuse linked to reduced hippocampal volume in adults (Andersen et al., 2008; Mehta et al., 2009; Stein, Koverola, Hanna, Torchia, & McClarty, 1997; Teicher, Anderson, & Polcari, 2012). Although very limited, two studies reported links between normative variations in negative parenting and reduced hippocampal volume in youth (Lee et al., 2018; Little et al., 2015). In contrast, more research has attempted to characterize the effects of individual differences in positive parenting on neural development but findings have been mixed. Some studies have found positive associations between maternal support and total hippocampal volume (Luby et al., 2012), left and right hippocampal volume (Rifkin-Graboi et al., 2015), and faster rates of hippocampal volume growth (Luby, Belden, Harms, Tillman, & Barch, 2016). Others, however, have found negative associations between maternal nurturance and left, but not right, hippocampal volume (Rao, Betancourt, Giannetta, Brodsky, Avants, et al., 2010), or no associations between positive parenting and hippocampal volume (Narita et al., 2012; Whittle et al., 2014).

Several factors may contribute to the divergent findings in the parenting literature. For instance, Luby et al. (2012, 2016) oversampled children with pediatric depression, Whittle et al. (2014) measured parenting behaviors relatively late in development (12 years), and Rao et al. (2011) used a sample which included children exposed to drugs prenatally. Another potential explanation for the lack of consistency is due to the difficulty in disentangling the effects of optimal positive parenting from parental overprotection on the hippocampus (Wang, Song, Li,
Zhang, & Liu, 2017). For instance, one study found that parental care in childhood was not associated with hippocampal volume in adulthood; however, when parental overprotection was low, parental care was positively correlated with adult hippocampal volume (Wang et al., 2017).

Together, these methodological differences make it difficult to form firm conclusions from the available evidence. Additionally, the non-linear and distinct developmental trajectories of hippocampal subregions may account for some of the differences in findings (Belsky & de Haan, 2011; Schlichting, Guarino, Schapiro, Turk-Browne, & Preston, 2017). The current study attempts to examine positive parenting, separate from overprotection, as well as negative parenting, to better capture its effects on hippocampal subregion volume in children.

**Gaps in the Human Literature**

Evidence of the associations between parenting, cortisol, and hippocampal volume are less consistent in the human than in the animal literature. Despite rapid advances in the examination of the effects of parenting on brain development, major gaps in our knowledge remain. First, although studies have examined pairwise comparisons between parenting, neuroendocrine functioning, and brain structure in a piecemeal fashion, no study has examined the full model in a young longitudinal population. Therefore, the interconnectedness of all variables within a single human sample is currently unknown. This marks a clear deficit in directly translating the animal literature to human populations.

Second, much of the existing literature has exclusively examined associations with the whole hippocampus volume. However, the hippocampus is a heterogeneous structure with functionally and structurally distinct regions (Blankenship, Redcay, Dougherty, & Riggins, 2017; Demaster, Pathman, Lee, & Ghetti, 2014; Poppenk, Evensmoen, Moscovitch, & Nadel, 2013; Poppenk & Moscovitch, 2011; Riggins, Blankenship, Mulligan, Rice, & Redcay, 2015).
Moreover, hippocampal subregions have different developmental trajectories, with posterior subregions increasing and anterior subregions decreasing in volume through development (Belsky & de Haan, 2011; Schlitching et al., 2017). Failure to examine the hippocampus at a finer resolution may contribute to inconsistencies in the literature and obscure important regionally-specific effects.

Third, much of the existing literature has exclusively investigated the effects of a unidimensional construct of parenting on hippocampal development: one whereby a lack of positive parenting behaviors implies negative parenting. Critically, positive and negative parenting behaviors may not exist on opposite ends of a continuum, and rather, may represent two orthogonal dimensions of parenting behaviors that predict different developmental outcomes (Field, 1998; McLaughlin, Sheridan, & Lambert, 2014; Sheridan & McLaughlin, 2014). Additionally, although positive and negative parenting may predict distinct outcomes on hippocampal structure (Little et al., 2015; Rao, Betancourt, Giannetta, Brodsky, Korczykowski, et al., 2010; Rifkin-Graboi et al., 2015; Whittle et al., 2016), no study has examined the impacts of both positive and negative parenting dimensions on hippocampal volume in children in the same study. Exploration of the potential divergent effects of different parenting dimensions on the developing brain and stress response system is necessary. Finally, the extant literature suggests that early childhood (3-5 years) may be a period of increased hippocampal sensitivity to variations in parenting behaviors (Andersen et al., 2008; Teicher et al., 2012). Additional research is necessary to explore and replicate these possible timing-dependent associations.

**Current Study**

The current study sought to address the current gaps in the literature with an overarching aim of investigating the longitudinal and concurrent associations between parenting and cortisol
reactivity on hippocampal subregion volume. The three aims of the study were to: 1) examine associations between early (3-5 years) and concurrent (5-10 years) parenting and cortisol reactivity on hippocampal subregion volume at 5-10 years, 2) examine whether these associations are timing-dependent, and 3) to test the full mediation model, evident in rodents, whereby parenting shapes hippocampal volume through individual differences in stress reactivity. These aims were tested in a longitudinal cohort that was oversampled for offspring of depressed mothers and a non-depressed comparison group drawn from the same community. Oversampling for mothers with a lifetime history of depressive disorders afforded the ability to capture greater variability in predictor (i.e., parenting, cortisol) and outcome (i.e., hippocampal structure) variables in the present study. At Time 1 and Time 2, parents and their preschool-aged children completed observational assessments of parenting and children were exposed to a laboratory-based, standardized stressor paradigm during which five salivary cortisol levels were collected to assess cortisol reactivity. At Time 2, children also completed a structural MRI. Drawing from rodent models and evidence of developmental sensitivity of the hippocampus, it was hypothesized that greater early maladaptive parenting behaviors (low positive or high negative) would predict increased cortisol reactivity and reduced hippocampal subregion volumes and that the association between parenting and offspring hippocampal volume would be mediated by offspring cortisol reactivity. Due to the relative dearth of literature examining associations between parenting and cortisol reactivity on hippocampal subregion volumes, we are exploring these associations with no a priori hypotheses regarding regional specificity; we hope our findings will inform future hypothesis testing.

Methods
Procedure. All methods were approved by the University’s Institutional Review Board. The present analyses were performed on a subset of children (n = 63) from a longitudinal study (N = 175) of high-risk offspring of depressed mothers and a non-depressed community comparison group (Dougherty, Tolep, Smith, & Rose, 2013a). Informed consent was provided by parents and informed assent was provided by children 7 years or older. At Time 1, children and their parents completed two laboratory behavioral sessions, during which parenting and cortisol reactivity data were collected. At Time 2, children and their parents completed one behavioral visit during which parenting and cortisol reactivity data were collected, followed by a neuroimaging session.

Participants

Recruitment. At Time 1, participants (N = 175) were recruited through flyers distributed to local schools, daycares, and healthcare providers (73.1%) and through a commercial mailing list (26.9%). A subset of children were targeted based on a maternal history of depression via advertisements; 50% (n = 83) of mothers at Time 1 had a lifetime depressive disorder based on the Structured Clinical Interview for DSM-IV Disorders (First, Spitzer, Gibbon, & Williams, 1996). At Time 1, eligible children were between three to five years of age; had an English-speaking biological parent with at least 50% legal custody; had no parent-reported history of significant medical conditions or developmental disabilities; and had biological parents without a history of bipolar or psychotic disorders. Families at Time 1 were invited to participate at the Time 2 assessment approximately 3 years later. At Time 2, 115 families completed questionnaires and 104 completed the Time 2 behavioral session. Families who completed the behavioral visit were also invited to complete the neuroimaging visit; of
these, 64 chose to participate. A total of 63 children completed scanning and contributed data for analysis; one child did not complete any scans due to claustrophobia.

**Demographic and descriptive statistics.** Demographic data are reported on the sample of 63 children (32 female, 50.8%) who completed the parent-child interaction task with their mothers and the MRI assessment. At Time 1, children and their parents completed a parent-child interaction task during the first laboratory visit ($M = 4.23 \pm .84$ years, range = 3.00-5.96 years) and a laboratory stressor task during the second laboratory visit approximately 28 days later ($M = 27.77 \pm 18.21$ days). The Time 2 behavioral assessment occurred approximately 3 years later when children were 5-10 years old ($M = 2.91 \pm .45$, range = 2.09–3.90 years; child age $M = 7.20 \pm .89$, range = 5.57–10.00 years). The neuroimaging session occurred approximately three months after the Time 2 behavioral assessment (child age $M = 7.48 \pm .88$ years).

Participants were racially diverse (30 White, 22 African-American, 11 Multi-Racial/Other; 9 identified as Hispanic/Latino descent, with 1 choosing not to identify); household income ranged from < $20,000 to > $100,000 per year (5 < $20,000, 4 $20,001-$40,000, 13 $40,001-$70,000, 15 $70,001-$100,000; 26 > $100,001); and the majority (74.6%) of children had at least one parent with a 4-year college degree. Thirty-eight mothers (60.3%) had a lifetime history of depressive disorders. See Table 1 for descriptive statistics on all study variables.

We compared the subset of children who completed the neuroimaging assessment at W2 to children who completed the baseline assessment at W1 but not the neuroimaging assessment at W2 and to children who completed the behavioral assessment only at W2 on all variables included in the study. The subsample of children included in the present analyses did not significantly differ from the W1 sample on any variable. In addition, the neuroimaging
samples did not differ from the sample that returned for the Time 2 behavioral visit only on any demographic variable, maternal depression, or cortisol reactivity measures. However, the neuroimaging subsample had lower Time 1 positive parenting $t(96) = -1.98, p = .05$ and higher Time 1 negative parenting, $t(91.96) = 2.34, p = .022$ compared to children who completed the behavioral assessment only at Time 2.

**Observed parenting behavior.** At both Time 1 and Time 2, parents and their children completed parent-child interaction tasks modified from the Teaching Tasks Battery (Egeland et al., 1995). The five tasks at Time 1 included: (1) Book reading: parents were instructed to read a picture book to their children (2) Wheels: assisted their children in naming as many objects with wheels as possible (3) Maze: parents guided their children through completing an Etch A Sketch® maze without touching any of the lines (4) Story: with the aid of their parents, children arranged cards depicting an action sequence in the correct temporal order and (5) Tangoes: children positioned geometric puzzle pieces to match a predetermined shape. At Time 2, the battery included four tasks: (1) Guessing game: parents guide their children in guessing an image on an unseen card (2) Traffic: with their parents’ help, children are required to shift cars up/down and left/right on a board to clear a path (3) Maze: parent and child are required to both collaboratively and competitively to direct a marble into holes on a wooden labyrinth board and (4) Block Buddies: parents and children work together to put together plastic shapes to match designs shown on cards.

We used a detailed coding manual adapted from Egeland and colleagues’ Teaching Tasks Battery (1995), and our coding procedure has been previously described (Dougherty, Klein, Rose, & Laptook, 2011; Dougherty, Tolep, Bufferd, et al., 2013). The principal investigator (LRD) trained two graduate students in the administration and coding of the task using
previously rated videos. Once the graduate students reached at least 80% accuracy with the principal investigator and the previously coded tapes from an independent sample, the graduate students trained undergraduate research assistants until they reached 80% reliability with the graduate students. Weekly coding meetings were held to prevent rater drift. At both Time 1 and Time 2, each task was coded on several domains of parenting behaviors: maternal hostility (expression of anger towards or rejection of the child), maternal intrusiveness (interference with the child’s needs, desires, interests, or actual behaviors), maternal support (expression of positive regard and emotional support to child), maternal positive affect (frequency and intensity of positive affect including facial, vocal, and body language), and maternal negative affect (frequency and intensity of negative affect including facial, vocal, and body language). All domains were coded on a 1-5 scale with the exception of maternal positive and negative affect scale, which were each scored on a 3-point scale. Parenting domains were averaged across tasks and z-scored. Z-scored values of maternal hostility, maternal intrusiveness, and maternal negative affect were averaged to create a composite score of negative parenting. Z-scored values of maternal support and maternal positive affect were averaged to create a composite measure of positive parenting. Resulting composite scores were z-scored according to the larger sample that completed the task with their mother (n = 161 at Time 1, n = 97 at Time 2). The internal consistency (α) of the scales was acceptable (Time 1 negative parenting: α = .75; Time 1 positive parenting: α = .88; Time 2 negative parenting: α = .73; Time 2 positive parenting: α = .85). Based on 38 video recordings at Time 1 and 28 video recordings at Time 2, the intraclass correlation coefficients (ICC) for inter-rater reliability were excellent (Time 1 negative parenting: ICC = .97; Time 1 positive parenting: ICC = .96; Time 2 negative parenting: ICC = .96; Time 2 positive parenting: ICC = .91).
**Cortisol reactivity.** At both Time 1 and Time 2, children completed a laboratory stressor task and provided salivary cortisol samples. At Time 1, the stressor task required children to match colored chips to a corresponding animal as quickly as possible while being evaluated (length of task: $M=8.11$ minutes, $SD=1.96$) (Kryski, Smith, Sheikh, Singh, & Hayden, 2011). The experimenter manipulated the timer so that the child failed to complete the task three times before the child was informed that the timer was broken and received their prize [for complete description of the task, see Dougherty, Tolep, Smith, & Rose, 2013b; Tolep & Dougherty, 2014]. At Time 2, the stressor included a modified version of the Trier Social Stress Task (length of task: $M=10.21$ minutes, $SD=0.52$) (Buske-Kirschbaum et al., 1997) during which children were instructed to tell a 4.5 minute story after 30 seconds of preparation followed by an unsolvable puzzle where children were given 3 minutes to complete a puzzle that contained pieces from two similar but different puzzles [for a complete description of the tasks, see Leppert, Kushner, Smith, Lemay, & Dougherty, 2016]. As cortisol levels vary as a function of time of day, we aimed to assess children’s cortisol reactivity in the afternoon (afternoon assessments: 78.1% at Time 1; 93.8% at Time 2).

At Time 1 and Time 2, salivary cortisol samples were collected at baseline (after 30 minutes of quiet play preceding the stressor), and 20-, 30-, 40-, and 50-minutes post-stressor using a cotton roll dipped in Kool Aid (~.025 mg) and chewed until saturated (~ 1 minute). If used sparingly and consistently, this method does not influence cortisol assays and makes the sampling procedure more pleasant for young children (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005). Samples were frozen at -20° C and assayed in duplicate using a time-resolved fluorescence immunoassay with fluorometric end-point detection (DELFIA) at the Biochemical Laboratory at the University of Trier, Germany. Inter- and intra-assay coefficients of variation
ranged between 7.1-9.0\% and 4.0-6.7\%, respectively. Of the 630 samples collected, 2 were missing and 3 were extreme values (exceeded 44 nmol/L). Extreme values were discarded and the 5 missing data points were interpolated using the average of five multiple imputations (MI; Rubin, 1987). MI is a valid and commonly used method for estimating missing data for cortisol values (e.g., Little, Jorgensen, Lang, & Moore, 2014; Müller, Zietlow, Tronick, & Reck, 2015; Rotenberg & McGrath, 2014; Walker, 2010). MI uses the individual’s data (i.e., the other 4 cortisol samples) and data from the entire sample to estimate the missing data points (de Goeij et al., 2013; Rubin, 1987). Previously used imputation methods, including mean substitution and regression imputation, leave no margin of error around the predicted missing value, artificially shrinking the standard error, and yielding biased estimates. In contrast, MI involves computing a series of plausible estimates of what the missing values may have been, creating variability in the predicted estimates (Little et al., 2014). Given that there is debate as to whether extreme cortisol values should be discarded or winsorized (Adam & Kumari, 2009), we chose to discard the extreme samples and treat the values as missing. However, results were similar when the three extreme values were winsorized. Following procedures outlined by Pruessner and colleagues, we calculated area under the curve of the five cortisol samples with respect to increase (AUCi). These values were log transformed and z-scored (T1: $M=.017, SD=0.42$; T2: $M=.079, SD=0.83$) (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

**MRI assessment.** During the neuroimaging assessment, children participated in a mock scanner session to become acclimated to the scanner environment and to provide motion feedback. Children were scanned in a Siemens 3.0-T scanner (MAGNETOM Trio Tim System, Siemens Medical Solutions, Erlangen, Germany) using a 12-channel coil. In addition to a number of functional scans (not included in this report), children completed a 4 minute and 18
second high-resolution T1 magnetization-prepared rapid gradient-echo (MPRAGE) structural 
scan sequence consisting of 176 contiguous sagittal slices (1.0 x 1.0 x 1.0 mm voxel dimensions; 
1900 ms TR; 2.52ms TE; 900ms inversion time; 9° flip angle; pixel matrix= 256 x 256). Images 
were visually inspected during the scan session to ensure high quality data. If motion artifacts 
were identified, the structural scan was repeated, pending the child’s willingness and ability. In 
total, 15 children had their structural scan repeated: 4 scans (n=1); 3 scans (n= 2); 2 scans 
(n=12). Structural images were processed in the automatic segmentation software Freesurfer 
(version 5.1.0; surfer.nmr.mgh.harvard.edu). Resulting hippocampal segmentations were visually 
inspected for accuracy. Manual edits were performed when necessary for gross over- or under-
inclusions (n = 7).

Freesurfer-generated hippocampal segmentations were further manually divided into 
hippocampal subregions (head, body, tail) based on standard anatomical landmarks. To eliminate 
distortions introduced by reorientation, volumes were aligned to the anterior commissure-
posterior commissure (Poppenk & Moscovitch, 2011). The posterior boundary of the 
hippocampal head was identified as the last coronal slice in which the uncal apex was visible 
(Riggins et al., 2015; Weiss, Dewitt, Goff, Ditman, & Heckers, 2005). The anterior boundary of 
the hippocampal tail was identified as the slice at which the fornix separates from the 
hippocampus and becomes clearly visible (Riggins et al., 2015; Watson et al., 1992). The 
hippocampal body is determined as the area between these regions. These divisions were 
identified twice for each participant by independent raters with a high degree of consistency 
between raters (intraclass correlation coefficients for the posterior boundary of the right and left 
head and the anterior boundary of the right and left tail were .92, .94, .90, and .88, respectively). 
Left and right hippocampal head, body, and tail volumes were adjusted for intracranial volume
(ICV) calculated in Freesurfer, as recommended by Raz et al. (2005), to ensure analyses were not confounded by variations in total brain size. Resulting volumes are included in all analyses as the dependent measures of interest.

**Data analysis plan.** There were three primary aims of the present analyses: 1) to assess associations between early (Time 1) and concurrent (Time 2) parenting and cortisol reactivity on Time 2 hippocampal subregion volume in separate models, 2) determine whether these associations were timing-dependent, and 3) evaluate whether cortisol reactivity mediated associations between parenting and hippocampal subregion volume.

All statistical analyses were conducted in SPSS (Version 23.0). We first inspected data for univariate outliers (>3SD) on each study variable and no univariate outliers were present in the dataset. Bivariate correlations were run to assess main effects between our predictors of interest (i.e., T1 and T2 negative parenting and positive parenting and T1 and T2 cortisol reactivity) and left and right hippocampal subregion volumes. Significant bivariate associations were further examined using multiple linear regression models that included child age and sex as covariates in all models. Both age and sex have been linked to hippocampal volume in prior samples (Noble et al., 2012; Perlaki et al., 2014) and the current sample. In the current sample, child age at the time of the neuroimaging assessment was significantly associated with right hippocampal body volume ($r = .27, p = .034$), with older age predicting larger volume. Child sex was significantly associated with both left ($r = -.30, p = .017$) and right ($r = -.27, p = .030$) hippocampal head volumes, with males having larger left (male: $M = 2058.50, SE = 318.17$, range = 1431.12 - 2842.79 mm$³; female: $M = 1884.96, SD = 240.39$; range = 1455.03 - 2527.68 mm$³) and right (male: $M = 2197.82, SD = 276.13$, range = 1649.32 - 2771.44 mm$³; female: $M = 2047.39, SD = 27$, range = 1574.82 - 2649.33 mm$³) head volumes than females. For models
examining the influence of cortisol reactivity, time of cortisol assessment was included as an additional covariate given that cortisol follows a diurnal rhythm and is thus sensitive to the timing of the assessment (Dickerson & Kemeny, 2004; Gunnar, Talge, & Herrera, 2009).

Controlling for covariates, we tested the main effects of Time 1 and Time 2 negative parenting, positive parenting, and cortisol reactivity on hippocampal subregions in separate linear regression models. Significant main effects were tested for timing-dependent associations by entering the significant predictor at one time point along with the corresponding predictor at the other time point, into a multiple regression model predicting the hippocampal subregion of interest. Multicollinearity analyses were conducted, and all variance inflation factor (VIF) values ranged from 1-2.2 (which is in the acceptable range of .1-10; Field, 2009). We also tested for multivariate outliers using Mahalanobis distance, which represents the distance of a datapoint from the intersection of the mean of the variables being assessed (Mahalanobis, 1936), and winsorized the identified outliers (see Supplementary Material Figures 1 and 2, which denote all multivariate outliers). Winsorized results are reported in the manuscript. Results using non-winsorized data were similar and are reported in Supplemental Material.

Finally, we used non-parametric bootstrapping procedures to evaluate the indirect effect of early parenting at Time 1 on hippocampal subregion volume at Time 2 via cortisol reactivity at Time 2. Unlike hypothesis testing based on parametric statistics, bootstrapping procedures do not assume normality (Preacher & Hayes, 2008). An indirect effect and corresponding confidence interval is calculated by examining the product of the path from the independent variable (positive or negative parenting at Time 1, tested in separate models) to the mediator (cortisol reactivity at Time 2) and from the mediator to the dependent variable (hippocampal subregion volume). We were therefore only interested in cortisol reactivity at Time 2 as the
mediator to ensure that early parenting (the independent variable) temporally preceded cortisol reactivity at Time 2 (the mediator). In addition, we conducted mediation analyses when there was a significant association between the mediator (cortisol reactivity at Time 2) and the dependent variable (hippocampal subregion volumes). A confidence interval that does not contain zero would indicate a statistically significant indirect effect of early parenting on hippocampal subregion volume through cortisol reactivity at Time 2. We employed the PROCESS Macro Version 3.0 in SPSS Version 24 to conduct all mediation analyses with 5,000 bootstrapped samples as recommended by (Hayes, 2009; Preacher & Hayes, 2008; Shrout & Bolger, 2002).

Results

All bivariate correlations are reported in Table 2.

Parenting and Hippocampal Volume

After controlling for age and gender, Time 1 positive parenting predicted larger right hippocampal head volumes ($b=92.84$, $SE=33.53$, $pr=.35$, $p=.008$) and left hippocampal head volumes ($b=79.91$, $SE=36.19$, $pr=.28$, $p=.031$; Figure 1a, b). Time 2 positive parenting was concurrently associated with smaller right body volume at the trend-level ($b=-42.28$, $SE=22.20$, $pr = -.25$, $p = .062$). Time 2 negative parenting was significantly associated with larger right body volume ($b=68.76$, $SE=30.18$, $pr = .29$, $p = .027$; Figure 1c) and no associations were observed for Time 1 negative parenting.

Timing-dependent analyses. To assess timing dependent effects, we followed up the significant effects by including both Time 1 and Time 2 positive parenting in models predicting left and right hippocampal head volumes and right body volumes, controlling for covariates (i.e., age and sex). In these models, Time 1 positive parenting continued to significantly predict larger
right hippocampal head volume \((b=85.80, \text{SE}=36.78, pr=.30, p=.023)\) but the association between Time 1 positive parenting and left hippocampal head volume reduced to a trend-level association \((b=73.85, \text{SE}=39.47, pr=.25, p=.067)\) after accounting for Time 2 positive parenting and covariates. Lastly, Time 2 negative parenting was not associated with larger right body volume \((b=68.54, \text{SE}=42.97, pr=.21, p=.117)\) after accounting for Time 1 negative parenting and covariates.

**Cortisol Reactivity and Hippocampal Volume**

Bivariate associations between cortisol reactivity at Time 1 and Time 2 and hippocampal subregion volumes at Time 2 are presented in Table 2. After controlling for age, child sex, and time of the cortisol assessment, greater Time 1 cortisol reactivity predicted smaller right \((b=-346.17, \text{SE}=84.55, pr=-.49, p<.001)\) and left body volumes \((b=-313.87, \text{SE}=119.95, pr=-.34, p=.011; \text{Figure 2a, b})\). Greater Time 2 cortisol reactivity predicted smaller left hippocampal tail volume \((b=-60.01, \text{SE}=20.67, pr=-.36, p=.005; \text{Figure 2c})\).

*Timing-dependent analyses.* To examine the timing-dependent effects of cortisol reactivity at Time 1 and Time 2 on hippocampal subregion volume, we included both cortisol reactivity at Time 1 and Time 2, along with covariates, in all models predicting hippocampal subregion volume. All associations persisted: Time 1 cortisol reactivity remained significant when controlling for Time 2 cortisol reactivity for both right \((b=-347.97, \text{SE}=84.74, pr=-.50, p<.001)\) and left hippocampal body volumes \((b=-343.45, \text{SE}=121.53, pr=-.37, p=.007)\); Time 2 cortisol reactivity remained a significant predictor of left tail volume controlling for Time 1 cortisol reactivity \((b=-56.72, \text{SE}=20.52, pr=-.36, p=.008)\).

**Mediation Analyses**
We examined whether Time 1 parenting predicted hippocampal subregion volume at Time 2 via cortisol reactivity at Time 2. We only focused on subregions that were significantly associated with Time 2 cortisol reactivity, which consisted of the left hippocampal tail volume. Our independent variables included Time 1 positive parenting or Time 1 negative parenting, in separate models. Our mediator was Time 2 cortisol reactivity, and our dependent variable was left hippocampal tail volume. Thus, we conducted 2 mediation analyses of interest. Analyses revealed a significant indirect effect (path $ab$, see Figure 3) of Time 1 negative parenting on Time 2 left hippocampal tail volume acting through Time 2 cortisol reactivity ($ab \cdot 5,000$ bootstrapped samples) = -11.14, $SE = 8.67$, bias corrected 95% CI [-33.42, -.09]). Specifically, greater Time 1 negative parenting predicted increased Time 2 cortisol reactivity, which, in turn, predicted reduced Time 2 left hippocampal tail volume.¹

**Discussion**

The present investigation examined the longitudinal associations between early (3-5 years, Time 1) and concurrent (5-10 years, Time 2) observed maternal parenting behaviors and cortisol reactivity on children’s hippocampal subregion volume at 5-10 years of age. Results revealed timing- and region-dependent associations between parenting and the cortisol response to stress on hippocampal subregion volume. Greater positive parenting during early childhood predicted larger bilateral hippocampal head volumes three years later, and greater negative parenting at Time 2 was concurrently associated with larger right hippocampal body volumes. Cortisol reactivity during early childhood predicted smaller bilateral body volumes three years later, whereas cortisol reactivity at Time 2 was concurrently associated with smaller left

---

¹ Given that the sample was oversampled for parents with a history of depression, we examined whether maternal depression history was associated with children’s hippocampal subregion volumes. No significant associations were observed; nevertheless, we explored maternal depression history as an additional covariate in analyses and all results were similar.
hippocampal tail volume. Critically, a significant indirect effect was observed, with greater negative parenting during early childhood predicting greater cortisol reactivity three years later, which, in turn, predicted reduced left hippocampal tail volume.

These findings lend important insights into existing contradictions in the literature regarding associations between parenting behaviors and hippocampal volumes. Investigations of the association between parenting and hippocampal volume have provided mixed results, with some studies finding positive, negative, or no associations (Luby et al., 2012, 2016; Narita et al., 2012; Rao, Betancourt, Giannetta, Brodsky, Avants, et al., 2010; Whittle et al., 2014). However, in most previous studies, whole hippocampal volume was used as the dependent variable. The present results indicate that the effects of parenting on hippocampal volume may be regionally-specific in children and obscured by use of whole hippocampal volumes.

The present results add to a growing body of literature, which suggests that the hippocampus may be particularly sensitive to environmental influences during the preschool period (Andersen et al., 2008; Luby et al., 2012, 2016; Rao, Betancourt, Giannetta, Brodsky, Avants, et al., 2010; Tottenham & Sheridan, 2009) versus later childhood (Luby et al., 2016) or adolescence (Whittle et al., 2014). However, our study also found evidence that the hippocampus may remain sensitive to parenting behaviors and cortisol reactivity into middle childhood. This suggests that hippocampal sensitivity to the parenting environment may last longer than previously expected, but is consistent with what would be predicted by the glucocorticoid vulnerability hypothesis and replicates findings in rodents, non-human primates, and human children (Pagliaccio et al., 2015; Wiedenmayer et al., 2006). The exact timing and specificity of this developmental sensitivity should be explored by future research in larger samples that collect neuroimaging data at multiple time points.
The existence of timing- and region-dependent associations suggest that hippocampal subregions have different developmental sensitivities to the parenting environment and HPA axis functioning. In the current dataset, the hippocampal head appears preferentially sensitive to the early parenting environment, the hippocampal body is sensitive to the later parenting environment and early cortisol reactivity, and the hippocampal tail is sensitive to later cortisol reactivity. Neural regions undergoing developmental change are more susceptible to the effects of environmental perturbations – for better or for worse – coincidentally or by design (Pechtel & Pizzagalli, 2011). Differences in the trajectories of hippocampal subregion development are likely interacting with early or late parenting and cortisol reactivity to drive the region-, timing-, and input-specific effects observed in the present report. The findings that greater positive parenting during early childhood predicts increased hippocampal head volumes three years later and greater cortisol reactivity at Time 1 and Time 2 was associated with reduced hippocampal volumes are consistent with what would be hypothesized from the glucocorticoid vulnerability hypothesis. The unexpected finding that larger right hippocampal body volume was associated with higher levels of Time 2 negative parenting may indicate that the hippocampal body, or its subfields, respond differently to this input than other regions (Malykhin, Lebel, Coupland, Wilman, & Carter, 2010). These regionally-specific differences in the direction of effects could reflect different underlying intra- or extra-cellular processes such as changes in the number, size, or complexity of neurons, synapses, and/or glia. Although these possibilities cannot be differentiated using the current data, it is a question that could be answered by future research using technologies better suited for measuring cellular changes.

Of perhaps greatest interest is the finding that cortisol reactivity mediated the association between early negative parenting and hippocampal volume in a young human population.
Consistent with the hypothesis derived from the rodent literature, greater early negative parenting predicted increased cortisol reactivity three years later, which, in turn, predicted smaller left hippocampal tail volumes. This provides the first evidence that a similar mechanistic pathway may exist in human children. Observation of a significant indirect effect supports the utility of rodent model systems for the investigation of risk transmission and the assumption that similar cellular mechanisms may be driving these cross-species effects. Additional research is needed in larger samples to both confirm this mediation model in humans and better understand whether, and why, there might be distinct regional vulnerabilities to negative parenting in the left hippocampal tail. This finding supports growing evidence suggesting the differential susceptibility of subregions of the hippocampus (Lee et al., 2018; Rao, Betancourt, Giannetta, Brodsky, Avants, et al., 2010; Rifkin-Graboi et al., 2015).

Although associations were found between both parenting and cortisol and hippocampal subregion volumes, the functional implications of these associations are unknown. Each hippocampal subregion has been linked to specific cognitive, behavioral, or affective abilities and, depending on the specific period of development, larger or smaller subregion volumes may be associated with better or worse outcomes. Given that the hippocampus undergoes known developmental change, the present results must be considered in light of typical developmental trajectories and the mechanisms which shape them (Demaster et al., 2014; Giedd et al., 1996; Hu, Pruessner, Coupé, & Collins, 2013; Uematsu et al., 2012; Wierenga et al., 2014; Yang, Goh, Chen, & Qiu, 2013). The interpretability of whether an increase or decrease in volume is beneficial will be dependent on the region and the period of development in which it is measured.
The present study has many strengths that expand upon and make novel contributions to the existing literature. This is the first study in a young human population to examine the hypothesis, derived from evidence in rodents that the early caregiving environment shapes hippocampal structure by programming the HPA axis response to stress. Second, use of a longitudinal sample afforded the capability to probe timing-dependent effects of early and concurrent parenting and cortisol reactivity on hippocampal structure. To our knowledge, this is the first study to examine the timing-dependent effects of cortisol reactivity on hippocampal volume in a young population. Third, investigation of subregions provided the advantage of determining regionally-specific differences in hippocampal volume that may be obscured by use of a whole hippocampal segmentation. Finally, unlike many previous studies examining the effects of parenting on hippocampal development in children, the present study used observational measures of both positive and negative parenting behaviors. Testing both positive and negative indices of parenting enabled the evaluation of potential development differences in their effects on hippocampal structure and function.

Despite providing necessary insights into how the early parenting environment and a child’s neurobiological response to stress may shape neural architectures, the conclusions which can be drawn from the present results are restricted by some methodological limitations. First, given that no previous study has examined time-dependent associations between parenting behaviors, cortisol reactivity, and regional-specific hippocampus volume, we used an exploratory approach and did not correct for multiple comparisons; our findings should inform future hypothesis testing in this burgeoning area of research. Second, despite 104 children and families participating in the Time 2 behavioral assessments, MR contraindications and participant (parent and/or child) interest in participating in the MRI assessment significantly reduced the number of
mother-child dyads included in the present analyses ($n=63$). Although our sample size is comparable to other studies examining these associations, low power in the present analyses may have impaired our ability to detect effects (i.e., increasing the type II error rate), especially as it applies to statistical tests of mediation. Third, we had a high rate of attrition from Time 1 to Time 2, as a number of families moved away from the target area. Even though we observed few differences between the MRI subsample and the original sample, future longitudinal studies are needed with larger sample sizes and increased efforts targeting participant retention over time.

Fourth, despite investigating timing dependent associations, we did not examine timing dependent effects of both Time 1 and Time 2 positive and negative parenting in the same model given our limited power; this is an important additional question, as it will be useful to assess the unique influences of positive and negative parenting behaviors on child brain structure across development. Fifth, we used two different stressor paradigms to be developmentally appropriate and avoid dampened stress responses due to habituation to a repeated stressor. However, our use of different stressor tasks could have contributed to differences in findings between cortisol reactivity across time points. Finally, although longitudinal measures of parenting and cortisol reactivity were collected, the present investigation only acquired neuroimaging data at Time 2, limiting the determination of temporal relations between variables. It is unknown whether or not baseline differences were present earlier in life, at what point in development individual differences emerged, and how these associations and the underlying neural substrates may change throughout development.

In light of these limitations, the present study serves as a springboard for future work on this topic and provides some suggestions on how to move forward. Future studies should strive to include multiple data points of longitudinal neuroimaging data in order to draw conclusions
regarding developmental change or long-term outcomes of childhood experiences on brain development. Together, larger samples with more frequent data acquisition will provide greater sensitivity to detect possible sensitive periods and the onset of individual differences in outcome measures as well as increased statistical power to test more sophisticated and nuanced models (e.g., mediated moderation). Larger samples would also allow for testing of more complex models that incorporate possible moderating influences of maternal lifetime depression status (Dougherty, Tolep, Smith, et al., 2013b), gender (Pechtel & Pizzagalli, 2011; Teicher et al., 2003), genetics (Frodl et al., 2010; Gotlib, Joormann, Minor, & Hallmayer, 2008; Hayden et al., 2010; Thomason, Yoo, Glover, & Gotlib, 2009; Wiggins et al., 2012), or interactions between parenting and stress reactivity (Buodo, Moscardino, Scrimin, Altoè, & Palomba, 2013; Kopala-Sibley et al., 2015; Sheikh et al., 2014).

In addition, the field would greatly benefit from designing and incorporating higher resolution imaging technologies to capture hippocampal changes at the subfield level. This would enable volumetric measurements of more functionally- and cytoarchitecturally- distinct units and facilitate more accurate cross-species comparisons. The use of intervention designs would permit examination of causal relations between the timing and quality of parenting experiences and brain structure and function. This research should be expanded to explore the effects of parenting and cortisol reactivity on hippocampal function. Observed associations with hippocampal structure may be mirrored or complemented by functional changes within the hippocampus and/or the larger brain networks with which it is integrated. Finally, and most critically, additional research is necessary to explore the immediate and long-term cognitive and behavioral implications of observed individual differences in hippocampal subregion volume, including potential risk for later psychopathology.
In sum, the present study advances our knowledge of how and when the early environment may alter developing neural architectures and provides insight into the neural changes that may underlie the etiology of later impairments. This is the first study to find evidence that the early parenting environment shapes brain development through programming of the HPA axis response to stress, a pathway that has been known to exist in rodents. Observed timing- and region-dependent changes of early parenting and stress reactivity on hippocampal structure are important as they may reflect increased risk for or resilience to later cognitive, emotional, and behavioral difficulties. Identification of the early factors that shape neurobiological development can inform the development of more effective clinical interventions. Specifically, interventions designed to address the presence and quality of these factors during childhood may have great potential for improving developmental outcomes.
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Figure 1. Associations between Time 1 and Time 2 parenting and hippocampal subregion volumes: a) Association between Time 1 positive parenting and Time 2 right hippocampal head volume; b) Association between Time 1 positive parenting and Time 2 left hippocampal head volume; c) Association between Time 2 negative parenting and Time 2 right hippocampal body volume.

Figure 2. Significant bivariate associations between Time 1 and Time 2 cortisol reactivity and hippocampal subregion volumes: a) Association between Time 1 cortisol reactivity and Time 2 right hippocampal body volume; b) Association between Time 1 cortisol reactivity and Time 2 left hippocampal body volume; c) Association between Time 2 cortisol reactivity and Time 2 left hippocampal tail volume.

Figure 3. Standardized regression coefficients for the association between Time 1 negative parenting and Time 2 left hippocampal tail volume, as mediated by Time 2 cortisol reactivity.
Table 1

**Descriptive statistics**

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**Dependent Measures**

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<sup>a</sup> Volume, measured in mm<sup>3</sup>.
Table 2

*Bivariate correlations among major study variables.*

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<td><strong>Cortisol Reactivity</strong></td>
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<tr>
<td>13. Time 1 cortisol</td>
<td>-.21</td>
<td>.11</td>
<td>-.45***</td>
<td>-.16</td>
<td>-.19</td>
<td>.12</td>
<td>-.36**</td>
<td>.16</td>
<td>.13</td>
<td>.13</td>
<td>.06</td>
<td>.08</td>
<td></td>
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<tr>
<td>14. Time 2 cortisol</td>
<td>-.17</td>
<td>-.07</td>
<td>-.03</td>
<td>-.19</td>
<td>-.17</td>
<td>-.17</td>
<td>.13</td>
<td>-.35**</td>
<td>-.03</td>
<td>-.01</td>
<td>.28*</td>
<td>.12</td>
<td>.12</td>
</tr>
</tbody>
</table>

*Note.* *+* p < .10, *p* < .05, **p* < .01, ***p < .001.
Time 1 Parenting

a) Right Head

b) Left Head

c) Time 2 Parenting

Right Body
Time 1 Cortisol Reactivity

a) Right Body

b) Left Body

Time 2 Cortisol Reactivity

c) Left Tail
$\alpha = 16.18 \ (14.84)$
95% CI [-13.55, 45.91]

$\beta = -54.60 \ (20.25)^*$
95% CI [-95.16, -14.03]

$c = 16.18 \ (14.84)$
95% CI [-13.55, 45.91]

$c' = -11.14 \ (8.98)^*$
95% CI [-33.95, -0.07]
Supplemental Material

Results with non-winsorized data:

Parenting and Hippocampal Volume

After controlling for age and gender, Time 1 positive parenting predicted larger right ($b=92.84$, $SE=33.53$, $pr=.35$, $p=.008$) and left hippocampal head volumes ($b=79.91$, $SE=36.19$, $pr=.28$, $p=.031$); Time 2 positive parenting was concurrently associated with smaller right body volume ($b=-45.32$, $SE=18.52$, $pr=-.31$, $p=.018$); Time 2 negative parenting was concurrently associated with larger right body volume ($b=64.22$, $SE=23.49$, $pr=.35$, $p=.008$); and no associations were observed for Time 1 negative parenting.

Timing-dependent analyses. To assess timing dependent effects, we followed up these significant effects by including both Time 1 and Time 2 positive parenting in models predicting left and right hippocampal head volumes and right body volumes, controlling for covariates (i.e., age and sex). In these models, Time 1 positive parenting continued to significantly predict larger right ($b=85.91$, $SE=36.07$, $pr=.31$, $p=.021$) and marginally significantly predict left hippocampal head volumes ($b=75.37$, $SE=38.75$, $pr=.26$, $p=.057$) after accounting for Time 2 positive parenting and covariates; Time 2 positive parenting continued to be associated with smaller right body volume ($b=-47.81$, $SE=19.77$, $pr=-.31$, $p=.019$), even after accounting for Time 1 positive parenting and covariates. Lastly, Time 2 negative parenting continued to be associated with larger right body volume ($b=81.36$, $SE=34.16$, $pr=.31$, $p=.021$), even after accounting for Time 1 negative parenting and covariates.
Supplemental Figure 1

Time 1 Parenting

a) Left Head

b) Right Head

Time 2 Parenting

c) Right Body

d) Right Body

Supplemental Figure 2. Significant bivariate associations between Time 1 and Time 2 parenting and hippocampal subregion volumes: a) Association between Time 1 positive parenting and Time 2 left hippocampal head volume; b) Association between Time 1 positive parenting and Time 2 right hippocampal head volume; c) Association between Time 2 negative parenting and Time 2 right hippocampal body volume; d) Association between Time 2 positive parenting and Time 2 right hippocampal body volume. Unfilled circles denote identified multivariate outliers.
Cortisol Reactivity and Hippocampal Volume

After controlling for age, gender, and time of the cortisol assessment, higher Time 1 cortisol reactivity predicted smaller left ($b=-187.28, SE=66.02, pr=-.36, p=.006$) and right body volumes ($b=-206.20, SE=45.89, pr=-.52, p<.001$; Supplemental Material Error! Reference source not found.a,b). Higher Time 2 cortisol reactivity was associated with smaller left hippocampal tail volume ($b=-54.51, SE=18.74, pr=-.36, p=.005$; Supplemental Material Error! Reference source not found.c).

Timing-dependent analyses. To examine the timing-dependent effects of cortisol reactivity at Time 1 and Time 2 on hippocampal subregion volume, we included both cortisol reactivity at Time 1 and Time 2, along with covariates, in all models predicting hippocampal subregion volume. All associations persisted: Time 1 cortisol reactivity remained significant when controlling for Time 2 cortisol reactivity for both left ($b=-203.63, SE=66.24, pr=-.40, p=.003$) and right ($b=-208.21, SE=45.50, pr=-.54, p<.001$) hippocampal body volumes; Time 2 cortisol reactivity remained a significant predictor of left tail volume controlling for Time 1 cortisol reactivity ($b=-51.59, SE=18.49, pr=-.36, p=.007$).
Supplemental Figure 2. Significant bivariate associations between Time 1 and Time 2 cortisol reactivity and hippocampal subregion volumes: a) Association between Time 1 cortisol reactivity and Time 2 right hippocampal body volume; b) Association between Time 1 cortisol reactivity and Time 2 left hippocampal body volume; c) Association between Time 2 cortisol reactivity and Time 2 left hippocampal tail volume. Unfilled circles denote identified multivariate outliers.