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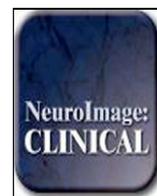
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Links between white matter microstructure and cortisol reactivity to stress in early childhood: Evidence for moderation by parenting

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ABSTRACT

Activity of the hypothalamic–pituitary–adrenal axis (measured via cortisol reactivity) may be a biological marker of risk for depression and anxiety, possibly even early in development. However, the structural neural correlates of early cortisol reactivity are not well known, although these would potentially inform broader models of mechanisms of risk, especially if the early environment further shapes these relationships. Therefore, we examined links between white matter architecture and young girls' cortisol reactivity and whether early caregiving moderated these links. We recruited 45 6-year-old girls based on whether they had previously shown high or low cortisol reactivity to a stress task at age 3. White matter integrity was assessed by calculating fractional anisotropy (FA) of diffusion-weighted magnetic resonance imaging scans. Parenting styles were measured via a standardized parent–child interaction task. Significant associations were found between FA in white matter regions adjacent to the left thalamus, the right anterior cingulate cortex, and the right superior frontal gyrus (all $ps < .001$). Further, positive early caregiving moderated the effect of high cortisol reactivity on white matter FA (all $ps \leq .05$), with high stress reactive girls who received greater parent positive affect showing white matter structure more similar to that of low stress reactive girls. Results show associations between white matter integrity of various limbic regions of the brain and early cortisol reactivity to stress and provide preliminary support for the notion that parenting may moderate associations.

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1. Introduction

A growing literature suggests that hypothalamic–pituitary–adrenal (HPA) axis dysfunction may be an important feature of internalizing disorders (i.e., depressive and anxiety disorders). For example, distinct HPA axis responses measured via salivary cortisol to stress have been reported in adults and adolescents with depression and anxiety (Barden, 2004; Rao et al., 2008; Risbrough and Stein, 2006; Shea et al., 2005). Research also suggests that antidepressant treatment reduces HPA axis abnormalities and that such reductions may be necessary for stable remission of depression (DeBellis et al., 1993; Ising et al., 2005; Kling et al., 1994; Nemeroff et al., 1991). However, HPA axis function may be more than a state marker of disorder. Research from our group and others has shown that considerable individual differences in cortisol reactivity to stress exist from an early age (Bosch et al., 2012; Gunnar, 1989, 1992; Jansen et al., 2010; Kryski et al., 2011); further, this individual variability in HPA axis reactivity may be a

mechanism by which the associations between environmental stress and disorder are mediated (Holsboer, 2000), although longitudinal work is needed to provide evidence for such models. Unfortunately, little is known about the structural brain correlates of early stress reactivity. This is an important gap in the literature, as brain circuitry and neural organization are posited to influence stress reactions, arousal, emotional regulation, brain development, and cognitive development and may thus contribute to risk for mood and emotion problems (Hart and Rubia, 2012; McCrory et al., 2010; Twardosz and Lutzker, 2010). Thus, more comprehensive models of the biology of early emerging internalizing disorder risk require a better understanding of relations between brain structure and makers of stress reactivity, such as cortisol reactivity to stress.

Most research on the neurological mechanisms related to individual differences in HPA axis reactivity comes from clinical and preclinical studies (see detailed reviews of this literature by Hart and Rubia, 2012; Jankord and Herman, 2008; Pruessner et al., 2010). These studies show that components of the brain's limbic system act as primary regulators of the HPA axis response to stress. For example, bilateral lesions of the medial prefrontal cortex (mPFC) enhance adrenocorticotrophic

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hormone (ACTH) and corticosterone responses (Brake et al., 2000). In adult rhesus monkeys exposed to stress, lower tissue densities were observed in the limbic system, particularly the thalamic nuclei, the anterior cingulate cortex compared to controls (Willette et al., 2012), suggesting an important role of these regions in HPA axis regulation.

However, associations between cortisol reactivity to stress and brain structure likely emerge through complex pathways that also entail environmental influences. Supporting this notion is the large literature implicating early care in children's cortisol reactivity and a small literature examining early caregiving and children's brain structure. For example, significant differences in cortisol reactivity have been widely documented in young children exposed to neglect and abuse (Hunter et al., 2011). Even more normative aspects of early care may shape cortisol reactivity; for example, positive parenting was found to moderate the effect of negative life events on children's cortisol reactivity (Barry and Kochanska, 2010; Engert et al., 2010; Hagan et al., 2011; Pendry and Adam, 2007). Regarding the literature on the neural correlates of early care, Eluvathingal et al. (2006) found decreased white matter tract density in the left uncinate fasciculus in post-institutionalized children who experienced early deprivation. Additionally, decreased white matter density (a strong correlate of lower FA) was observed in adolescents and young adults who were exposed to parental verbal abuse and physical neglect during childhood (Choi et al., 2009; Huang et al., 2012). Specifically, these studies reported white matter abnormalities in areas implicated in stress evaluation and regulation, such as the anterior temporal lobe, the cingulum bundle and the fornix. However, extant research has focused on extreme forms of early stress, such as sexual or emotional abuse and physical neglect. While these are important aspects of early stress, additional research is needed on links between more common forms of contextual risk factors, such as more normative aspects of parenting, and children's neural structure. Given the literature implicating parent positive and negative caregiving behaviors in an array of child outcomes (Lueken and Lemery, 2004; Tarullo and Gunnar, 2006), such behaviors may be relevant to associations between stress reactivity and neural structure as well.

Taken together, these lines of research suggest that white matter connectivity may be linked to both children's cortisol reactivity to stress as well as early caregiving. We therefore conducted a preliminary investigation of links between cortisol reactivity to stress and structural connectivity, indexed using diffusion tensor imaging (DTI), which yields fractional anisotropy (FA) indices of white matter. FA is a measure of directionally dependent restriction of water diffusion due to axonal myelin insulation and is widely used to evaluate white matter integrity in the brain. Myelination increases nerve conduction velocities and facilitates synchronous firing of neurons by reducing travel distance effects in distributed networks (Fields, 2008). Therefore, high anisotropy is generally interpreted as white matter with more efficient movement of water molecules by diffusion parallel to the course of the axon fiber bundles. Lower FA in white matter suggests underlying disruption of myelin insulation or axonal integrity (Fields, 2008; Song et al., 2002).

In a community sample of 6-year-old girls, we examined what is, to our knowledge, the first investigation of the neural correlates of early neuroendocrine stress reactivity. Because we were interested in identifying associations between constructs implicated in depression vulnerability (i.e., cortisol stress reactivity, early care; Azar et al., 2007; Dougherty et al., 2011, 2013), we examined girls prior to the age of risk for depression (Kendler et al., 1993, 1997; Wittchen et al., 1994) to increase the likelihood that we were examining risk markers, rather than the sequelae of current or past depression, and used a community sample to increase the representativeness of any findings to typically developing girls and families. Based on evidence for white matter disruptions in limbic regions of the brain and stress-related mood disorders (Osoba et al., 2013; Yap et al., 2008), we hypothesized that early cortisol reactivity would be associated with white matter differences in the limbic regions of the brain. Additionally, based on research linking early care and cortisol function, we investigated whether parenting moderated associations between cortisol reactivity and the brain's white matter microstructure.

2. Methods

2.1. Participants

We recruited 45 6-year-old (mean = 6.13 years, $SD = 0.73$) girls for the current imaging study from a larger community sample of 409 (208 girls) children participating in an ongoing longitudinal study. Children were aged three at the time the larger study was initiated and were screened for significant medical or psychological problems via a procedure administered by trained study personnel; children with such problems were ineligible to participate. Because this was a pilot study of a small sample, only girls were recruited to eliminate the need to use sex as a covariate in a small data set, given the well-established impact of sex differences in young children's brain development (Gong et al., 2011; Paus et al., 2008), although we discuss the significant limitations of this decision in the Discussion section. Girls were either high or low in cortisol reactivity to stress based on data collected at the baseline assessment, operationalized as described in the following section.

Demographics for the current study subsample are provided in Table 1. Girls were administered the Peabody Picture Vocabulary Test (PPVT; Dunn and Dunn, 1997) at baseline to screen for gross cognitive impairment and English proficiency; girls were of average cognitive ability ($M = 112.3$, $SD = 13.9$). We found no differences in family demographics such as family income, parent education and ethnicity and child cognitive ability between the sample recruited for imaging analyses and the original study sample (all ps > 0.28), which was representative of the region from which participants were recruited. The study protocols were reviewed and approved by the University of Western Ontario Health Sciences Research Ethics Board.

2.2. Assessment of cortisol reactivity to stress

The stress task and cortisol collection procedures used have been described in great detail previously (Kryski et al., 2011; Kryski et al., 2013). In brief, at the baseline assessment, cortisol data were collected from children during a visit to the family's home.[1] All visits began between 12:00 pm and 3:30 pm to minimize the effects of diurnal variation on cortisol samples (de Weerth et al., 2003; Donzella et al., 2008). Caregivers were asked to prevent children from eating or drinking for a half hour prior to the visit to remove the influence of food/drink on cortisol assays (Magnano et al., 1989; Schwartz et al., 1998). None of the children were taking corticosteroids. After 30 min of quiet play with the experimenter, a baseline salivary cortisol sample was collected, followed by the stress task described below.

The stress task was a downward adaptation of one developed and validated by Lewis and Ramsey (2002) for use with older children and was designed to emphasize social evaluation under motivated and uncontrollable circumstances, which has been shown to elicit large cortisol responses in both adults (Dickerson and Kemeny, 2004; Magnano et al., 1989) and preschool-aged children (Gunnar et al., 2009). Briefly, each child attempted to complete a matching task by matching colored game pieces to animal icons based on an answer key. A large toy replica of a traffic light was placed adjacent to the board, and the child was instructed that the traffic light would show how much time they had to complete the task and win a prize, with "green" indicating that they had time to work and "red" indicating that they were out of time. The experimenter surreptitiously controlled the traffic light via remote control so that no child could complete the task on time during any of the three trials conducted. The mean duration of the task for children who completed all three trials in Study 1 was 15.01 min ($SD = 1.5$), including

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[1] In total, 392 (95.8%) children provided all six cortisol samples, while the remaining fourteen (3.4%) children did not provide at least one sample.

Table 1
Study sample demographics.

Child Age [yrs (SD)]	6.13 (0.73)
Child Race, [% (N)]	
Caucasian	83.7 (41)
Asian	2.0 (1)
Other	10.2 (5)
Maternal Age [mean years (SD)]	33.96 (5.6)
Paternal Age [mean years (SD)]	35.87 (7.8)
Parent marital status [married, % (N)]	81.6 (40)
Education, % (N)	
High School	8.9 (4)
Some 4-year college	80.0 (36)
Graduate and post-graduate degree	11.1 (5)
Family income [% (N)]	
< $40,000	12.2 (6)
$40,001- $70,000	22.4 (11)
$70,001-$100,000	28.6 (14)
> $100,000	30.6 (15)

the instruction period. Supporting the validity of this task as a means of eliciting stress, our group has previously shown that it successfully elicits an increase in cortisol and that child negative affect increases during the course of participating in this task (Kryski et al., 2011). We have also previously reported that heightened reactivity to this task is associated with elevated child symptoms of anxiety, especially for girls (Kryski et al., 2013), as well as parent history of anxiety disorder (Kryski, in preparation), supporting the notion that heightened cortisol reactivity during this task is meaningfully related to internalizing disorder vulnerability. Following the stress task, the child and experimenter resumed quiet play while the remaining cortisol samples were collected every 10, 20, 30, 40, and 50 min.

To obtain cortisol, children chewed on a 2-inch absorbent cotton dental roll until it was wet with saliva, which was expunged into a microtube and frozen at −20 °C. The saliva samples were assayed in duplicate using salivary cortisol enzyme immunoassay kit (Salimetrics, PA, USA). Studies consistently report high correlations in saliva to serum cortisol concentrations (Daniel et al., 2006; Dorn et al., 2009). Optical density was read on a standard plate reader at 450 nm and corrected at 650 nm (Molecular Devices, Sunnyvale, CA, USA). All samples from the same child were assayed in the same batch with no duplicates varying more than 5%. The average intra- and interassay coefficients were 3.5 and 5.1%, respectively. Standard curve and concentration of unknown samples were generated according to manufacturer's instructions using a 4-parameter sigmoid minus curve fit. Cortisol data were skewed and were therefore $\log_{10}$ transformed prior to all analyses, a standard procedure with human cortisol data (Schwartz et al., 1998).

We created high and low reactivity groups for the purposes of recruitment for the present study. Specifically, we created four quartile scores for children based on their mean cortisol levels across time and cortisol change scores (i.e., the difference from baseline to each child's peak cortisol level following the task). Children from the lowest (1st) quartile on both selection variables were recruited first for the low reactivity group, followed by children in the 1st quartile on one variable (mean cortisol or peak change) and the 2nd quartile on the other. For the high reactivity group, children were first recruited who were in the highest (4th) quartile on both selection variables (mean cortisol and peak change), followed by children who were in the 4th quartile on one variable and the 3rd quartile on the other. Based on these recruitment criteria, as well as other exclusion criteria (e.g., having dental work or metal implants somewhere on the body), we had a total of 22 girls in the low reactivity group and 24 girls in the high reactivity group who participated.

2.3. Parenting assessment

At age three, parenting behavior was assessed using a task designed to elicit child misbehavior and, thus, negative parenting. This task, called the Prohibition Task, entailed a parent–child interaction task that lasted approximately 10 min. Parent–child dyads were presented with two bins of toys, one filled with exciting toys (electronic guitar, under-the-sea gear set, and magnet board), the other containing toys with batteries or key parts missing and age-inappropriate toys. The parent was told to engage the child in play with the unappealing toys and to prohibit the child from playing with the appealing toys. After 3 min the parents were instructed to allow their child to play with the appealing toys for 6 min and were then instructed to have their child put away the toys. Parents were instructed to refrain from helping their child put toys away.

The parent–child interaction task was video recorded and subsequently coded by trained graduate and undergraduate raters using a coding manual based on the Teaching Tasks coding manual (Weinfield et al., 2002) and the Qualitative Ratings for Parent–Child Interactions scale (Payley et al., 2001). Raters coded a minimum of 10 consecutive tapes with an intraclass correlation (ICC) of .80 with a master coder before coding independently. Once this standard was established, intermittent reliability checks were performed on 15% of all recordings (mean ICC = .86). Coders periodically met and reviewed recordings together to prevent observer drift. For the present study, we elected to focus on parent negative and positive affectivity, as these and related parenting styles have been previously implicated in children's stress reactivity, depression risk, and other important developmental outcomes (Dougherty et al., 2011, 2013; Kryski et al., 2013).

2.4. MRI scanning

The MRI assessment occurred approximately two and a half years (mean = 31.68 months, SD = 3.24) after the baseline data were collected. Scans were performed on a 3 Tesla Siemens TIM Trio scanner equipped with a 32-channel head coil. Diffusion-weighted scans were collected at 30 directions (B_1 = 700 s/mm²) following a non-diffusion weighted b_0 scan in the transverse plane (iPAT parallel EPI sequence, GRAPPA acceleration factor = 2; TR = 9100 ms; TE = 91 ms; voxel size = 2 mm³; 62 slices; in-plane FOV = 192 mm²). A T1-weighted anatomical MRI scan was also obtained within-session using a T1-weighted MPRAGE sequence (iPAT GRAPPA acceleration factor = 2; TR = 2300 ms; TE = 3.01 ms; voxel size = 1 mm³; 192 slices; in-plane FOV = 256 mm²).

2.5. Data pre-processing

First, the raw DTI data were corrected for head motion and eddy currents by registering the diffusion-weighted images with the null image through the affine transformations using FMRIB's Diffusion Toolbox v2.0 (FDT, part of FSL) (Smith et al., 2004). To compare movement between the groups, for each subject we quantified movement as the mean of the absolute value of the rotations along each of the Cartesian axes. These values were obtained from the movement correction algorithm. We used t-tests to compare the high and low cortisol reactivity groups on each of the movement variables (pitch, yaw and roll) and found no significant group differences in head movements during image acquisition (all ps > 0.24). Subsequently, DTI data sets were skull stripped using Brain Extraction Tool v2.1 (BET, part of FSL) (Smith, 2002) to remove non-tissue components, and the diffusion tensor was calculated with the DTIFIT program for whole brain volumes to yield FA.

2.6. Fiber tract visualization

Fiber tractography was generated via Diffusion Toolkit version 0.6 with interpolated streamline algorithm and visualized using TrackVis version 0.6 (http://www.trackvis.org/, Harvard Medical School, Boston, MA, USA) (Wang et al., 2007). The threshold for fiber tracking termination was set at a voxel FA value lower than 0.20. The ROIs were drawn

manually with the reference to the b_0 image. The streamline and voxel counts and the length of tracked fibers in ROIs were calculated. The density of tracked fibers was calculated as the ratio of the track count over the voxel count in the ROIs. The length of the tracked fiber to and from the designated ROI was generated using a 2 mm seed region for each participant and used for subsequent statistical analyses.

2.7. White matter microstructure analysis

Individual anatomical scans were aligned to a standard neuroanatomical template (the TT_N27 'Colin' brain, transformed to the stereotaxic space of Talairach & Tournoux) using an automatic 12-parameter affine transform (least-squares cost function), and resampled to 1 mm³. This transformation was then applied to each individual's FA image. In order to reduce the effects of anatomic misregistration due to spatial normalization and to reduce noise and signal variations, spatial smoothing was applied to all the normalized image data with a Gaussian kernel width of 5 mm. Following these steps, data were fed into voxel-wise cross-subject statistical analyses which compared high cortisol reactivity vs. low cortisol reactivity groups. Volume-of-interest (VOI) masks were first extracted based on the clusters showing significant inter-group FA differences. These VOI masks were then back-projected to the individual FA images of each subject, and the mean FA subjectwise values within the VOIs were calculated.

Demographic variables such as family socioeconomic status and child age were not associated with children's cortisol stress responses or FA values (all $ps > 0.18$); therefore, we did not include these variables as covariates in our analyses. T-tests were used to examine associations between study variables and significant inter-group FA differences. Following Forman et al. (1995), we restricted our analysis to VOIs that met a voxel-wise cut-off p-value of .001. As a further safeguard against false positive results, we only retained clusters that were greater than 50 mm³. Pearson correlations were used to characterize associations between FA changes within the VOI and behavioral measures. For correlating behavioral measures with FA values, p values less than 0.05 were considered statistically significant. From the results of VOI group comparisons, the brain regions showing significant inter-group differences were located and labeled anatomically in Talairach space (Lancaster et al., 2000).

3. Results

We first examined links between demographic variables and girls high and low in cortisol reactivity. Demographic variables such as family income, ethnicity, or child cognitive function were not significantly different between the cortisol response groups (all $ps > 0.05$).[2]

3.1. Cortisol reactivity is associated with regional differences in fractional anisotropy

Regional differences in FA as a function of cortisol reactivity are presented in Table 2. We found significant associations between white matter tract integrity and cortisol reactivity to stress in the prefrontal and basal regions of the brain. Specifically, girls high in cortisol reactivity had significantly lower FA values in tracts adjacent to the left thalamus, the right anterior cingulate cortex (rACC) and the right superior frontal gyrus (sFG) (all $ps < 0.001$) (Fig. 1). Regions where FA was significantly different between the two stress reactivity groups are presented in Fig. 2.

3.2. Parenting as a moderator of links between cortisol reactivity and white matter microstructure

We then examined whether the main effects of cortisol reactivity found on FA values in the left thalamus, rACC and sFG were moderated by early caregiving, testing moderation using standard procedures in multiple regression (Aiken and West, 1991). No evidence for moderation of cortisol reactivity–FA associations by parent negative affect in the left thalamus, rACC and sFG was found (all $ps > .05$). However, we found that the association between cortisol reactivity and FA values in the sFG and rACC was moderated by positive parent affectivity during caregiving, with a significant interaction term showing that the associations between cortisol reactivity and FA values were conditional based on parent positive affectivity. Specifically, parent positive affect moderated the effect of child cortisol reactivity on the white matter microstructure adjacent to the sFG ($\beta = 0.04$; $se = 0.02$; $p = 0.02$) and the rACC ($\beta = 0.05$; $se = 0.03$; $p = 0.05$). Plots of these interactions (see Fig. 2) showed that positive parenting moderated associations between cortisol reactivity and white matter microstructure, such that children with parents showing high positive affect during parent–child interactions had better FA in the regions adjacent to the sFG and rACC. We next used Hayes and Matthes' (2009) guidelines for testing regions of significance according to the Johnson–Neyman technique (Johnson and Fay, 1950). In both cases of moderation, the difference between children high and low in cortisol stress reactivity in FA values was only significant at low and moderate levels of parent positive affectivity; when parent positive affectivity was high, the two groups of children did not significantly differ in FA values.[3]

3.3. Group differences in white matter microstructure by spatial tractography

Based on previous literature in which altered interregional connectivity of the cortex has been found in patients with stress-related psychopathologies such as MDD (Korgaonkar et al., 2012), we examined whether similar differences existed in our sample in connectivity, measured via the number of tracts and mean tract length in regions that were associated with group differences in FA. We found a significantly higher mean number of tracts (mean difference: 96.77 (22.14), $t = 4.37$, $df = 41$, $p < 0.001$) and higher length of tracts (mean difference: 14.75 (3.28), $t = 4.50$, $df = 41$, $p < 0.001$) projecting from the left thalamus in the high cortisol reactive group compared to children with low cortisol reactivity (Fig. 3). The mean number and length of tracts were not significantly different in the rACC and sFG (all $ps > 0.05$). We also examined whether parenting behavior moderated associations between child cortisol reactivity and the length or number of tracts to and from the left thalamus. However, no evidence for moderation was found (all $ps > 0.31$).

4. Discussion

To our knowledge, this is the first study to investigate the neural correlates of early neuroendocrine stress reactivity. We found associations between white matter microstructure disruptions and cortisol reactivity in three important emotion regulation regions of the limbic system. Specifically, the white matter adjacent to the left thalamus, sFG and rACC was significantly disrupted in girls with high cortisol reactivity. Our findings are consistent with the extant literature implicating these regions in the regulation of HPA axis function. For example, recent work by Hermans et al. (2011) showed large-scale neural network reconfiguration related to differences in stress reactivity in adults. Specifically, the interconnectivity within a neural network including limbic

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[2] Child symptom measures were collected using the Child Behavior Checklist completed by the child's primary caregiver (Achenbach, 1991). In this smaller sample, cortisol reactivity was not associated with child depressive or anxious symptoms, attention deficit hyperactivity disorder or oppositional defiant disorder (all $ps > 0.05$), although see Kryski et al. (2013) for findings linking certain cortisol reactivity parameters to children's symptoms in a much larger, overlapping sample of children.

[3] The Johnson–Neyman value of positive parenting was 2.95 in the model predicting FA values adjacent to the superior frontal gyrus and was 2.77 for the model predicting FA values adjacent to the rACC. Positive parenting ranged from 1 to 3.

Table 2
Voxel-wise differences in fractional anisotropy (FA) in the high reactivity (N = 24) and low-cortisol reactivity groups (N = 20). The peak coordinates, cluster size, mean FA value (±SD) and post-hoc p-value is provided.

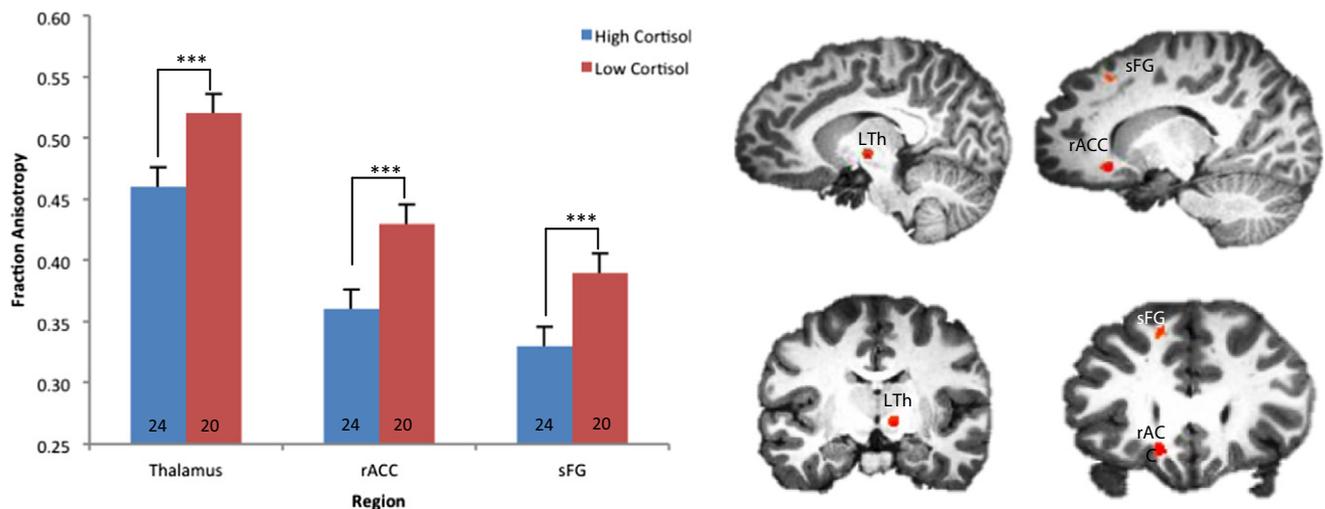| Brain region (coordinates)	Peak Coordinates (x, y, z)	Cluster size (voxels)	Mean FA values		p <
			Low reactivity group (SD)	High reactivity group (SD)	
Left Thalamus	+9, +10, -2	259	0.52 (.04)	0.46 (.05)	0.001
Right Anterior Cingulate	-14, -26, -8	141	0.43 (.06)	0.37 (.06)	0.001
Right Superior Frontal Gyrus	-14, -26, +44	80	0.39 (.03)	0.34 (.04)	0.001

regions such as the anterior cingulate, amygdala, and thalamic nuclei increased as a function of stress response magnitudes. Furthermore, rats with lesions in the thalamus exhibit unresponsive hypophyseal and adrenocortical reactivity to maternal separation stress (Suarez et al., 1998, 2002), suggesting the regulatory role of the thalamus in activation of the HPA axis to stress stimuli. Functional magnetic resonance imaging (fMRI) studies of human adults show a gender-specific effect on limbic activation, the thalamus in particular. In these studies, a significant increase in thalamic nuclei blood-oxygen-level dependent (BOLD) activity was observed upon hydrocortisone administration; this was evident in women only (Merz et al., 2010; Stark et al., 2006). Sex-based regional differences in microstructural integrity have been recently documented in the thalamus in a community sample of healthy adults as well, where women had significantly lower thalamic FA than males (Menzler et al., 2011). As our study included only girls, we cannot say whether there were sex differences in the pattern of findings we obtained, although our data are consistent with the possibility that FA differences in thalamus characterize girls' stress reactivity during early childhood. Further work is needed to determine whether a similar pattern of findings is also evident in boys.

In addition to FA differences in the region adjacent to the thalamus, we examined group differences in neuronal fiber projection from the thalamus. Our tractography data showed that the thalamic region in girls with high cortisol reactivity had significantly higher neuronal projections to and from the dorsolateral prefrontal cortex. Although speculative, it is likely that regions with reduced FA may compensate for lack of neuronal signaling via an increase in number of neuronal projections. However, as the literature has not documented links between cortisol response and long distance axonal tracts, it is difficult to interpret these findings as either adaptive or maladaptive, and longitudinal research is required to explain these links. Recent studies have documented tract specific deficits in the prefrontal cortex of patients with major

depressive disorder (for a review of these studies, see Barch, 2009; Osoba et al., 2013). Moreover, differences in axonal tract radiations from the thalamic nuclei have been documented in females diagnosed with bipolar disorder (Wade et al., 2002). Our findings extend this literature by showing that microstructure disruptions and neuronal tracts of the thalamic nuclei are linked to cortisol reactivity to stress in young girls, suggesting that the connectivity of this region is important in HPA axis regulation early in development.

We also found group differences in white matter FA in a region adjacent to the rACC. A number of fMRI studies have shown that rACC activation is linked to cortisol increases following a psychosocial stress task in adults (Stark et al., 2006; Thomason et al., 2011). Similarly, functional neuroimaging in patients with major depression indicates increased activation of the rACC in response to negative stimuli compared to healthy controls. A meta-analysis of fMRI studies suggests that the abnormal circuitry involving the rACC and negative stimuli processing is more pronounced in females with major depression (Hamilton et al., 2012). The rACC is a key component in the brain's fear/anxiety circuitry due to its functional coupling with the amygdala (Quirk et al., 2003), which implicates it in regulating the activation of the HPA axis in response to stress (Heim et al., 2000; Shin and Liberzon, 2010). Our data add to this literature by showing that decreases in white matter FA surrounding the rACC could be a mechanism by which functional coupling of the rACC and amygdala may be affected. Although speculative, it is possible that the individual differences in connectivity of the limbic system structures influence the perception and evaluation of stimuli as stressful, and, in turn, influence cortisol responses to stress.

Our analyses also showed a link between white matter microstructure adjacent to the sFG and children's cortisol reactivity. Although relatively few studies have implicated this region in stress reactivity, a report by Kremens et al. (2010) showed a significant association between thickness of the sFG and diurnal cortisol levels. In addition, the

Fig. 1. Mean FA (±SEM) values as a function of cortisol reactivity in young girls. Brain regions with group differences in FA are labeled in coronal and axial images. Talariach coordinates and voxel sizes are presented in Table 2. High reactive = 1; low reactive = 0; LTh, left thalamus; rACC, right anterior cingulate; sFG, right superior frontal gyrus (N = 44, ***p < 0.001).

A.

B.
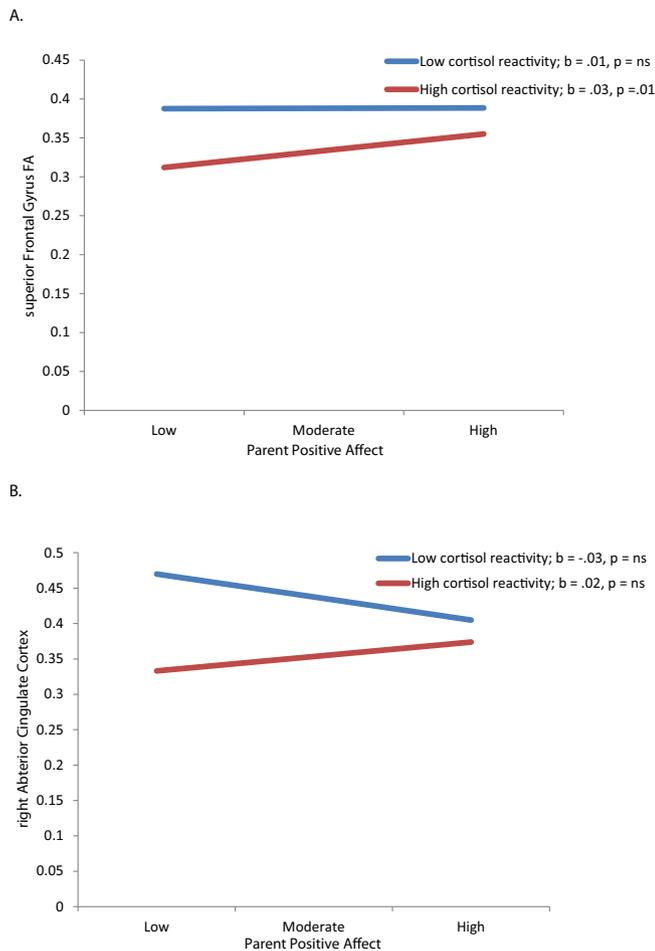
Fig. 2. The main effect of cortisol reactivity on white matter integrity is moderated by parent positive affectivity. Children with high cortisol reactivity to stress showed better white matter FA of the superior frontal gyrus (A) and the right anterior cingulate cortex (B) as a function of positive parenting during the parent–child interaction task.

sFG has also been linked to stress-related psychopathologies such as major depression in adolescents (Cullen et al., 2009) and post-traumatic stress disorder in adults (Lanius et al., 2004, 2005). Similarly, cortisol administration has been shown to increase BOLD activity in the sFG, but only in women (Stark et al., 2006). Additionally, maternal separation activated the sFG and higher cortisol reactivity in rhesus monkeys (Rilling et al., 2001), suggesting the role of this region in stress response. Our findings suggest that structural disruptions in this region are also associated with cortisol reactivity to stress during childhood.

We also found evidence for moderation of main effects of cortisol on children's FA by parenting. Specifically, positive parenting moderated the effect of high cortisol reactivity on child FA values in the rACC and sFG. Children with high cortisol reactivity to stress, but whose parent showed positive affect during interactions, showed white matter integrity that was more similar to children with low cortisol stress reactivity. Our findings complement studies reporting associations between parenting styles and stress-regulating brain regions, including such associations in the context of disorders such as depression. For example, supportive maternal style was positively associated with orbitofrontal gray matter volume and caudate activation (Schneider et al., 2012). Similarly, interactions between maternal aggressive behavior and limbic structures such as the hippocampus, amygdala and anterior cingulate cortex predicted adolescent depressive symptoms (Whittle et al., 2011; Yap et al., 2008). While the current data cannot conclusively speak to causal processes, our findings extend this work by suggesting that the developing brains of some children may be differentially influenced by positive caregiving by virtue of the extent to which children

exhibit heightened stress reactivity. However, longitudinal data are needed to understand the pattern of relationships between stress reactivity, caregiving, and neural structure.

To our knowledge, this study is the first to examine the neural correlates of early neuroendocrine stress reactivity. We used a state-of-the-art protocol to carefully assess girls' cortisol stress reactivity (Kryski et al., 2011), reactivity to which has been linked to internalizing disorder vulnerability (Kryski et al., 2013; Kryski, in preparation). However, our study also had several important limitations. In particular, we recruited girls only for the present study, which allowed us to eliminate the influence of sex differences on neural development and avoid adding covariates to analyses of what was a small sample of participants, thus increasing statistical power. However, the exclusion of boys means that the current findings cannot speak to associations between boys' cortisol stress reactivity and neural structure nor determine whether boys differ from girls in such associations. Future work on this important question is therefore needed. We did not correct for multiple tests of interactions between parenting and cortisol reactivity to stress lest we overlook an important new finding in this understudied area of investigation; however, it is important to note that some of our interactions may not have reached full significance had we made such a correction. Thus, these findings should be interpreted with caution pending future replication. In addition, our stress task indexed reactivity to a brief, standardized laboratory stressor. While previous work supports the relevance of reactivity indexed using this task for children's internalizing disorder risk (Kryski et al., 2013; Kryski, in preparation), we are unable to say whether other indices of the cortisol response, especially those relevant to chronic stress reactivity (e.g., basal cortisol or hair cortisol; Knoops et al., 2010; Wolf et al., 2002), are related to children's brain structure.

Additionally, our study design does not speak clearly to causal relationships between cortisol stress reactivity, caregiving, and neural development. Even though the cortisol measures we collected were gathered at age 3 and the imaging was conducted two and a half years later, we do not claim that cortisol reactivity caused the structural brain differences we found between high- and low-reactive girls. In particular, it is certainly possible that earlier brain development and structure shaped individual differences in children's cortisol stress reactivity. Decisions regarding the timing of the data collection in the present study were based on feasibility and expense. More specifically, we were concerned about the feasibility of collecting imaging data concurrent to the cortisol task, when children would have been quite young, and because cortisol data are quite expensive to analyze, we did not reassess stress reactivity concurrent to the imaging assessment. Future work using cross-lagged analyses of cortisol reactivity and brain structure assessed across time is needed to understand causal relationships between these constructs. As such work will be quite expensive, it is our hope that the current data provide preliminary support for the feasibility and potential value of this type of research. Finally, although we are interested in linking the findings we obtained to depression risk, we cannot say whether the patterns of neural structure associated with cortisol reactivity to stress are pathognomonic. In the absence of longitudinal work linking the current findings to girls' negative outcomes, we conclude only that there are differences in brain organization between high- and low-reactive girls, but whether these are meaningful regarding depression and other outcomes is unclear.

In conclusion, our study suggests that there are white matter microstructural differences related to cortisol reactivity to stress in young girls. These white matter alterations were found in brain regions widely implicated in emotion regulation and mood disorders in adults. We also found a moderating influence of positive parenting of links between cortisol reactivity and early brain development. While further research is needed to replicate our findings and explore whether such associations are also evident in boys, the possibility that early care mitigates high-risk neural trajectories toward negative outcomes has implications for early intervention and prevention.
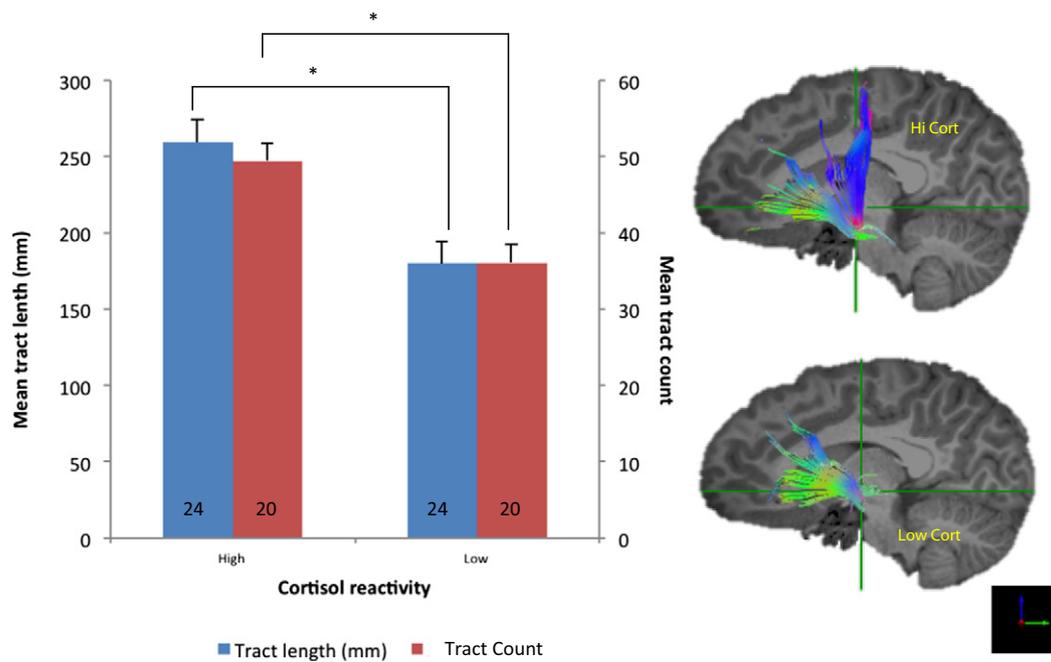
Fig. 3. Mean number of tracts (±SEM) and mean fiber length (±SEM) as a function of cortisol reactivity in region adjacent to the left thalamus ($N = 44$). The contrast of the original image is altered to illustrate the direction of neuronal tracts. For visualization, neural tract data is presented for girls with the highest and the lowest number of fibers. Tract colors represent the direction of neuronal fibers based on diffusion tensor anisotropy data. Green = anterior–posterior; blue = superior–inferior, red = dorsal–ventral. Note: hi Cort = high cortisol group; low Cort = low cortisol group; *$p \leq 0.05$.

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References

Achenbach, T.M., 1991. Manual for the Child Behavior Checklist/4–18 and 1991 ProfileUniversity of Vermont, Department of Psychiatry, Burlington.

Aiken, L.S., West, S.G., 1991. Multiple Regression: Testing and Interpreting Interactions. Sage, Newbury Park, CA.

Azar, R., Paquette, D., Zoccolillo, M., Baltzer, F., Tremblay, R.E., 2007. The association of major depression, conduct disorder, and maternal overcontrol with a failure to show a cortisol buffered response in 4-month-old infants of teenage mothers. Biological Psychiatry 62 (6), 573–579. http://dx.doi.org/10.1016/j.biopsych.2006.11.00917336943.

Barch, D.M., 2009. Neuropsychological abnormalities in schizophrenia and major mood disorders: similarities and differences. Current Psychiatry Reports 11, 313–319. http://dx.doi.org/10.1007/s11920-009-0045-619635240.

Barden, N., 2004. Implication of the hypothalamic–pituitary–adrenal axis in the physiopathology of depression. Journal of Psychiatry & Neuroscience: JPN 29, 185–19315173895.

Barry, R.A., Kochanska, G., 2010. A longitudinal investigation of the affective environment in families with young children: from infancy to early school age. Emotion (Washington, D.C.) 10, 237–249. http://dx.doi.org/10.1037/a001848520364900.

Bosch, N.M., Riese, H., Reijneveld, S.A., Bakker, M.P., Verhulst, F.C., et al., 2012. Timing matters: long term effects of adversities from prenatal period up to adolescence on adolescents' cortisol stress response. The TRAILS study. Psychoneuroendocrinology 37, 1439–1447. http://dx.doi.org/10.1016/j.psyneuen.2012.01.01322365483.

Brake, W.G., Flores, G., Francis, D., Meaney, M.J., Srivastava, L.K., Gratton, A., 2000. Enhanced nucleus accumbens dopamine and plasma corticosterone stress responses in adult rats with neonatal excitotoxic lesions to the medial prefrontal cortex. Neuroscience 96 (4), 687–69510727787.

Choi, J., Jeong, B., Rohan, M.L., Polcari, A.M., Teicher, M.H., 2009. Preliminary evidence for white matter tract abnormalities in young adults exposed to parental verbal abuse. Biological Psychiatry 65 (3), 227–234. http://dx.doi.org/10.1016/j.biopsych.2008.06.02218692174.

Cullen, K.R., Gee, D.G., Klimes-Dougan, B., Gabbay, V., Hulvershorn, L., et al., 2009. A preliminary study of functional connectivity in comorbid adolescent depression. Neuroscience Letters 460 (3), 227–231. http://dx.doi.org/10.1016/j.neulet.2009.05.02219446602.

Daniel, M., Moore, D.S., Decker, S., Belton, L., DeVellis, B., et al., 2006. Associations among education, cortisol rhythm, and BMI in blue-collar women. Obesity (Silver Spring, Md.) 14, 327–335. http://dx.doi.org/10.1038/oby.2006.4216571860.

De Bellis, M.D., Gold, P.W., Geracioti, T.D., Listwak, S.J., Kling, M., 1993. Association of fluoxetine treatment with reductions in CSF early adversity, HPA axis, and depression concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. American Journal of Psych. 150, 656–657.

de Weerth, C., Zijl, R.H., Buitelaar, J.K., 2003. Development of cortisol circadian rhythm in infancy. Early Human Development 73, 39–52. http://dx.doi.org/10.1016/S0378-3782(03)00074-412932892.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychological Bulletin 130, 355–391. http://dx.doi.org/10.1037/0033-2909.130.3.35515122924.

Dorn, L.D., Kolko, D.J., Susman, E.J., Huang, B., Stein, H., et al., 2009. Salivary gonadal and adrenal hormone differences in boys and girls with and without disruptive behavior disorders: contextual variants. Biological Psychology 81, 31–39. http://dx.doi.org/10.1016/j.biopsycho.2009.01.00419428966.

Donzella, B., Talge, N.M., Smith, T.L., Gunnar, M.R., 2008. To spear or not to spear comparison of saliva collection methods. Developmental Psychobiology 50, 714–717. http://dx.doi.org/10.1002/dev.2034018767029.

Dougherty, L.R., Klein, D.N., Rose, S., Laptook, R.S., 2011. Hypothalamic–pituitary–adrenal axis reactivity in the preschool-age offspring of depressed parents: moderation by early parenting. Psychological Science 22, 650–658. http://dx.doi.org/10.1177/095679761140408421460339.

Dougherty, L.R., Tolep, M.R., Smith, V.C., Rose, S., 2013. Early exposure to parental depression and parenting: associations with young offspring's stress physiology and oppositional behavior. Journal of Abnormal Child Psychology 41 (8), 1299–1310. http://dx.doi.org/10.1007/s10802-013-9763-723722864.

Dunn, L.M., Dunn, L.M., 1997. Peabody Picture Vocabulary Testthird edition. Guidance Service, Circle Pines, MN, American.

Eluvathingal, T.J., Chugani, H.T., Behen, M.E., Juhász, C., Muzik, O., et al., 2006. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. Pediatrics 117 (6), 2093–2100. http://dx.doi.org/10.1542/peds.2005-172716740852.

Engert, V., Efanov, S.I., Dedovic, K., Duchesne, A., Dagher, A., Pruessner, J.C., 2010. Perceived early-life maternal care and the cortisol response to repeated psychosocial stress. Journal of Psychiatry & Neuroscience: JPN 35 (6), 370–377. http://dx.doi.org/10.1503/jpn.10002220964960.

Fields, R.D., 2008. White matter in learning, cognition and psychiatric disorders. Trends in Neurosciences 31 (7), 361–370. http://dx.doi.org/10.1016/j.tins.2008.04.00118538868.

Forman, S.D., Cohen, J.D., Fitzgerald, M., Eddy, W.F., Mintun, M.A., Noll, D.C., 1995. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 33 (5), 636–6477596267.

Gong, G., He, Y., Evans, A.C., 2011. Brain connectivity: gender makes a difference. Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry 17 (5), 575–591. http://dx.doi.org/10.1177/107385841038649221527724.

Gunnar, M.R., 1989. Studies of the human infant's adrenocortical response to potentially stressful events. New Directions for Child Development 45, 3–182687726.

Gunnar, M.R., 1992. Reactivity of the hypothalamic–pituitary–adrenocortical system to stressors in normal infants and children. Pediatrics 90 (3 Pt 2), 491–4971513614.

Gunnar, M.R., Talge, N.M., Herrera, A., 2009. Stressor paradigms in developmental studies: what does and does not work to produce mean increases in salivary cortisol. Psychoneuroendocrinology 34 (7), 953–967. http://dx.doi.org/10.1016/j.psyneuen.2009.02.01019321267.

Hagan, M.J., Roubinov, D.S., Gress-Smith, J., Luecken, L.J., Sandler, I.N., Wolchik, S., 2011. Positive parenting during childhood moderates the impact of recent negative events on cortisol activity in parentally bereaved youth. Psychopharmacology 214, 231–238. http://dx.doi.org/10.1007/s00213-010-1889-520521029.

Hamilton, J.P., Etkin, A., Furman, D.J., Lemus, M.G., Johnson, R.F., Gotlib, I.H., 2012. Functional neuroimaging of major depressive disorder: a meta-analysis and new integration of baseline activation and neural response data. American Journal of Psychiatry 169 (7), 693–703. http://dx.doi.org/10.1176/appi.ajp.2012.1107110522535198.

Hart, H., Rubia, K., 2012. Neuroimaging of child abuse: a critical review. Frontiers in Human Neuroscience 19, 52. http://dx.doi.org/10.3389/fnhum.2012.0005222457645.

Hayes, A.F., Matthes, J., 2009. Computational procedures for probing interactions in OLS and logistic regression: SPSS and SAS implementations. Behavior Research Methods 41, 924–936. http://dx.doi.org/10.3758/BRM.41.3.92419587209.

Heim, C., Newport, D.J., Heit, S., Graham, Y.P., Wilcox, M., et al., 2000. Pituitary–adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA: the Journal of the American Medical Association 284, 592–597. http://dx.doi.org/10.1001/jama.284.5.59210918705.

Hermans, E.J., van Marle, H.J., Ossewaarde, L., Henckens, M.J., Qin, S., et al., 2011. Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. Science (New York, N.Y.) 334 (6059), 1151–1153. http://dx.doi.org/10.1126/science.120960322116887.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology 23 (5), 477–501. http://dx.doi.org/10.1016/S0893-133X(00)00159-711027914.

Huang, H., Gundapuneedi, T., Rao, U., 2012. White matter disruptions in adolescents exposed to childhood maltreatment and vulnerability to psychopathology. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology 37 (12), 2693–2701. http://dx.doi.org/10.1038/npp.2012.13322850736.

Hunter, A.L., Minnis, H., Wilson, P., 2011. Altered stress responses in children exposed to early adversity: a systematic review of salivary cortisol studies. Stress (Amsterdam, Netherlands) 14 (6), 614–626. http://dx.doi.org/10.3109/10253890.2011.57784821675865.

Ising, M., Künzel, H.E., Binder, E.B., Nickel, T., Modell, S., Holsboer, F., 2005. The combined dexamethasone/CRH test as a potential surrogate marker in depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry 29 (6), 1085–1093. http://dx.doi.org/10.1016/j.pnpbp.2005.03.01415950349.

Jankord, R., Herman, J.P., 2008. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. Annals of the New York Academy of Sciences 1148, 64–73. http://dx.doi.org/10.1196/annals.1410.01219120092.

Jansen, J., Beijers, R., Riksen-Walraven, M., de Weerth, C., 2010. Cortisol reactivity in young infants. Psychoneuroendocrinology 35 (3), 329–338. http://dx.doi.org/10.1016/j.psyneuen.2009.07.00819651476.

Johnson, P.O., Fay, L.C., 1950. The Johnson–Neyman technique, its theory and application. Psychometrika 15, 349–36714797902.

Kendler, K.S., Neale, M.C., Kessler, R.C., Heath, A.C., Eaves, L.J., 1993. A longitudinal twin study of personality and major depression in women. Archives of General Psychiatry 50 (11), 853–862. http://dx.doi.org/10.1001/archpsyc.1993.018202300230028215811.

Kendler, K.S., Davis, C.G., Kessler, R.C., 1997. The familial aggregation of common psychiatric and substance use disorders in the National Comorbidity Survey: a family history study. British Journal of Psychiatry: the Journal of Mental Science 170, 541–548. http://dx.doi.org/10.1192/bjp.170.6.5419330021.

Kling, M.A., Geracioti, T.D., Licinio, J., Michelson, D., Oldfield, E.H., Gold, P.W., 1994. Effects of electroconvulsive therapy on the CRH–ACTH–cortisol system in melancholic depression: preliminary findings. Psychopharmacology Bulletin 30 (3), 489–4947878187.

Knoops, A.J., Gerritsen, L., van der Graaf, Y., Mali, W.P., Geerlings, M.I., 2010. Basal hypothalamic pituitary adrenal axis activity and hippocampal volumes: the SMART-Medea study. Biological Psychiatry 67 (12), 1191–1198. http://dx.doi.org/10.1016/j.biopsych.2010.01.02520299006.

Korgaonkar, M.S., Cooper, N.J., Williams, L.M., Grieve, S.M., 2012. Mapping inter-regional connectivity of the entire cortex to characterize major depressive disorder: a whole-brain diffusion tensor imaging tractography study. Neuroreport 23 (9), 566–571. http://dx.doi.org/10.1097/WNR.0b013e328354626422562047.

Kremen, W.S., O'Brien, R.C., Panizzon, M.S., Prom-Wormley, E., Eaves, L.J., et al., 2010. Salivary cortisol and prefrontal cortical thickness in middle-aged men: a twin study.

NeuroImage 53 (3), 1093–1102. http://dx.doi.org/10.1016/j.neuroimage.2010.02.02620156572.

Kryski, K.R., Smith, H.J., Sheikh, H.I., Singh, S.M., Hayden, E.P., 2013. HPA axis reactivity in early childhood: associations with symptoms and moderation by sex. Psychoneuroendocrinology 38 (10), 2327–2336. http://dx.doi.org/10.1016/j.psyneuen.2013.05.00223764193.

Kryski, K.R., Smith, H.J., Sheikh, H.I., Singh, S.M., Hayden, E.P., 2011. Assessing stress reactivity indexed via salivary cortisol in preschool-aged children. Psychoneuroendocrinology 36, 1127–1136. http://dx.doi.org/10.1016/j.psyneuen.2011.02.00321388745.

Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., et al., 2000. Automated Talairach atlas labels for functional brain mapping. Human Brain Mapping 10 (3), 120–131. http://dx.doi.org/10.1002/1097-0193(200007)10:3<120::AID-HBM30>3.0.CO;2-810912591.

Lanius, R.A., Williamson, P.C., Densmore, M., Boksman, K., Neufeld, R.W., 2004. The nature of traumatic memories: a 4-T FMRI functional connectivity analysis. American Journal of Psychiatry 161 (1), 36–4414702248.

Lanius, R.A., Williamson, P.C., Bluhm, R.L., Densmore, M., Boksman, K., et al., 2005. Functional connectivity of dissociative responses in posttraumatic stress disorder: a functional magnetic resonance imaging investigation. Biological Psychiatry 57 (8), 873–884. http://dx.doi.org/10.1016/j.biopsych.2005.01.01115820708.

Lewis, M., Ramsay, D., 2002. Cortisol response to embarrassment and shame. Child Development 73, 1034–1045. http://dx.doi.org/10.1111/1467-8624.0045512146731.

Luecken, L.J., Lemery, K.S., 2004. Early caregiving and physiological stress responses. Clinical Psychology Review 24 (2), 171–191. http://dx.doi.org/10.1016/j.cpr.2004.01.00315081515.

Magnano, C.L., Diamond, E.J., Gardner, J.M., 1989. Use of salivary cortisol measurements in young infants: a note of caution. Child Development 60, 1099–1101. http://dx.doi.org/10.2307/11307842805888.

McCrory, E., De Brito, S.A., Viding, E., 2010. Research review: the neurobiology and genetics of maltreatment and adversity. Journal of Child Psychology and Psychiatry, and Allied Disciplines 51 (10), 1079–1095. http://dx.doi.org/10.1111/j.1469-7610.2010.02271.x20546078.

Menzler, K., Belke, M., Wehrmann, E., Krakow, K., Lengler, U., et al., 2011. Men and women are different: diffusion tensor imaging reveals sexual dimorphism in the microstructure of the thalamus, corpus callosum and cingulum. Neuroimage 54 (4), 2557–2562. http://dx.doi.org/10.1016/j.neuroimage.2010.11.02921087671.

Merz, C.J., Tabbert, K., Schweckendiek, J., Klucken, T., Vaitl, D., et al., 2010. Investigating the impact of sex and cortisol on implicit fear conditioning with fMRI. Psychoneuroendocrinology 35 (1), 33–46. http://dx.doi.org/10.1016/j.psyneuen.2009.07.00919683399.

Nemeroff, C.B., Bissette, G., Akil, H., Fink, M., 1991. Neuropeptide concentrations in the cerebrospinal fluid of depressed patients treated with electroconvulsive therapy. Corticotrophin-releasing factor, beta-endorphin and somatostatin. British Journal of Psychiatry: the Journal of Mental Science 158, 59–63. http://dx.doi.org/10.1192/bjp.158.1.591673078.

Osoba, A., Hänggi, J., Li, M., Horn, D.I., Metzger, C., et al., 2013. Disease severity is correlated to tract specific changes of fractional anisotropy in MD and CM thalamus — a DTI study in major depressive disorder. Journal of Affective Disorders 149 (1–3), 116–128. http://dx.doi.org/10.1016/j.jad.2012.12.02623489404.

Paus, T., Keshavan, M., Giedd, J.N., 2008. Why do many psychiatric disorders emerge during adolescence? Nature Reviews. Neuroscience 9 (12), 947–957. http://dx.doi.org/10.1038/nrn251319002191.

Payley, B., Cox, M.J., Kanoy, K.W., 2001. The young family interaction coding system. In: Kerig, P.K., Lindahl, K.M. (Eds.), Family Observational Coding Systems. LEA, Mahwah, pp. 273–288.

Pendry, P., Adam, E.K., 2007. Associations between parents' marital functioning, maternal parenting quality, maternal emotion and child cortisol levels. International Journal of Behavioral Development 31, 218–231. http://dx.doi.org/10.1177/0165025407074634.

Pruessner, J.C., Dedovic, K., Pruessner, M., Lord, C., Buss, C., et al., 2010. Stress regulation in the central nervous system: evidence from structural and functional neuroimaging studies in human populations — 2008 Curt Richter award winner. Psychoneuroendocrinology 35 (1), 179–191. http://dx.doi.org/10.1016/j.psyneuen.2009.02.01619362426.

Quirk, G.J., Likhtik, E., Pelletier, J.G., Paré, D., 2003. Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. Journal of Neuroscience: the Official Journal of the Society for Neuroscience 23 (25), 8800–880714507980.

Rao, U., Hammen, C., Ortiz, L.R., Chen, L.A., Poland, R.E., 2008. Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. Biological Psychiatry 64, 521–526. http://dx.doi.org/10.1016/j.biopsych.2008.05.01218597740.

Rilling, J.K., Winslow, J.T., O'Brien, D., Gutman, D.A., Hoffman, J.M., Kilts, C.D., 2001. Neural correlates of maternal separation in rhesus monkeys. Biological Psychiatry 49, 146–157. http://dx.doi.org/10.1016/S0006-3223(00)00977-X11164761.

Risbrough, V.B., Stein, M.B., 2006. Role of corticotropin releasing factor in anxiety disorders: a translational research perspective. Hormones and Behavior 50, 550–561. http://dx.doi.org/10.1016/j.yhbeh.2006.06.01916870185.

Schneider, S., Brassen, S., Bromberg, U., Banaschewski, T., Conrod, P., et al., 2012. Maternal interpersonal affiliation is associated with adolescents' brain structure and reward processing. Translational Psychiatry 2, e182. http://dx.doi.org/10.1038/tp.2012.11323149446.

Schwartz, E.B., Granger, D.A., Susman, E.J., Gunnar, M.R., Laird, B., 1998. Assessing salivary cortisol in studies of child development. Child Development 69, 1503–1513. http://dx.doi.org/10.2307/11321289914636.

Shea, A., Walsh, C., MacMillan, H., Steiner, M., 2005. Child maltreatment and HPA axis dysregulation: relationship to major depressive disorder and post traumatic stress

disorder in females. Psychoneuroendocrinology 30, 162–178. http://dx.doi.org/10.1016/j.psyneuen.2004.07.00115471614.

Shin, L.M., Liberzon, I., 2010. The neurocircuitry of fear, stress, and anxiety disorders. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology 35 (1), 169–191. http://dx.doi.org/10.1038/npp.2009.8319625997.

Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., et al., 2004. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 23 (Suppl 1), S208–S219. http://dx.doi.org/10.1016/j.neuroimage.2004.07.05115501092.

Smith, S.M., 2002. Fast robust automated brain extraction. Human Brain Mapping 17, 143–155. http://dx.doi.org/10.1002/hbm.1006212391568.

Song, S.K., Sun, S.W., Ramsbottom, M.J., Chang, C., Russell, J., Cross, A.H., 2002. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 17 (3), 1429–1436. http://dx.doi.org/10.1006/nimg.2002.126712414282.

Stark, R., Wolf, O.T., Tabbert, K., Kagerer, S., Zimmermann, M., et al., 2006. Influence of the stress hormone cortisol on fear conditioning in humans: evidence for sex differences in the response of the prefrontal cortex. NeuroImage 32 (3), 1290–1298. http://dx.doi.org/10.1016/j.neuroimage.2006.05.04616839780.

Suárez, M.M., Maglianesi, M.A., Perassi, N.I., 1998. Involvement of the anterodorsal thalami nuclei on the hypophysoadrenal response to chronic stress in rats. Physiology & Behavior 64, 111–116. http://dx.doi.org/10.1016/S0031-9384(98)00028-69661990.

Suárez, M., Molina, S., Rivarola, M.A., Perassi, N.I., 2002. Effects of maternal deprivation on adrenal and behavioural responses in rats with anterodorsal thalami nuclei lesions. Life Sciences 71 (10), 1125–1137. http://dx.doi.org/10.1016/S0024-3205(02)01830-112095534.

Tarullo, A.R., Gunnar, M.R., 2006. Child maltreatment and the developing HPA axis. Hormones and Behavior 50 (4), 632–639. http://dx.doi.org/10.1016/j.yhbeh.2006.06.01016876168.

Thomason, M.E., Hamilton, J.P., Gotlib, I.H., 2011. Stress-induced activation of the HPA axis predicts connectivity between subgenual cingulate and salience network during rest in adolescents. Journal of Child Psychology and Psychiatry, and Allied Disciplines 52 (10), 1026–1034. http://dx.doi.org/10.1111/j.1469-7610.2011.02422.x21644985.

Twardosz, S., Lutzker, J.R., 2010. Child maltreatment and the developing brain: a review of neuroscience perspectives. Aggression and Violent Behavior 15, 59–68. http://dx.doi.org/10.1016/j.avb.2009.08.003.

Wang, R., Benner, T., Sorensen, A.G., Wedeen, V.J., 2007. Diffusion toolkit: a software package for diffusion imaging data processing and tractography. Proceedings of the International Society for Magnetic Resonance in Medicine 15, 3720–3725.

Wade, T.J., Cairney, J., Pevalin, D.J., 2002. Emergence of gender differences in depression during adolescence: national panel results from three countries. Journal of the American Academy of Child and Adolescent Psychiatry 41 (2), 190–198. http://dx.doi.org/10.1097/00004583-200202000-0001311837409.

Weinfield, N.S., Ogawa, J.R., Egeland, B., 2002. Predictability of observed mother–child interaction from preschool to middle childhood in a high-risk sample. Child Development 73 (2), 528–543. http://dx.doi.org/10.1111/1467-8624.0042211949907.

Whittle, S., Yap, M.B., Sheeber, L., Dudgeon, P., Yücel, M., et al., 2011. Hippocampal volume and sensitivity to maternal aggressive behavior: a prospective study of adolescent depressive symptoms. Development and Psychopathology 23 (1), 115–129. http://dx.doi.org/10.1017/S095457941000068421262043.

Willette, A.A., Coe, C.L., Colman, R.J., Bendlin, B.B., Kastman, E.K., et al., 2012. Calorie restriction reduces psychological stress reactivity and its association with brain volume and microstructure in aged rhesus monkeys. Psychoneuroendocrinology 37 (7), 903–916. http://dx.doi.org/10.1016/j.psyneuen.2011.10.00622119476.

Wittchen, H.U., Knäuper, B., Kessler, R.C., 1994. Lifetime risk of depression. British Journal of Psychiatry 26, 16–22.

Wolf, O.T., Convit, A., de Leon, M.J., Caraos, C., Qadri, S.F., 2002. Basal hypothalamo-pituitary-adrenal axis activity and corticotropin feedback in young and older men: relationships to magnetic resonance imaging-derived hippocampus and cingulate gyrus volumes. Neuroendocrinology 75 (4), 241–249. http://dx.doi.org/10.1159/00005471511979054.

Yap, M.B., Whittle, S., Yücel, M., Sheeber, L., Pantelis, C., et al., 2008. Interaction of parenting experiences and brain structure in the prediction of depressive symptoms in adolescents. Archives of General Psychiatry 65 (12), 1377–1385. http://dx.doi.org/10.1001/archpsyc.65.12.137719047524.