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HPA-axis stress reactivity in youth depression: evidence of impaired regulatory processes in depressed boys

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Abstract

Given the link between youth depression and stress exposure, efforts to identify related biomarkers have involved examinations of stress regulation systems, including the hypothalamic–pituitary–adrenal (HPA) axis. Despite these vast efforts, the underlying mechanisms at play, as well as factors that may explain heterogeneity of past findings, are not well understood. In this study, we simultaneously examined separate components of the HPA-axis response (e.g. activation intensity, peak levels, recovery) to the Socially Evaluated Cold-Pressor Test in a targeted sample of 115 youth (age 9–16), recruited to overrepresent youth with elevated symptoms of depression. Among youth who displayed a cortisol response to the task, depression symptoms were associated with higher peak responses but not greater rate of activation or recovery in boys only. Among those who did not respond to the task, depression symptoms were associated with greater cortisol levels throughout the visit in boys and girls. Results suggest that depression symptoms are associated with a more prolonged activation of the axis and impaired recovery to psychosocial stressors primarily in boys. We discussed two potential mechanistic explanations of the link between depression symptoms and the duration of activation: (1) inhibitory shift (i.e. point at which the ratio of inhibitory and excitatory input into the axis shifts from greater excitatory to greater inhibitory input) or (2) inhibitory threshold (i.e. level of cortisol exposure required to activate the axis' feedback inhibition system).

Keywords

Adolescents; children; cortisol; depression; HPA; stress

Introduction

Exposure to life stressors is a strong predictor of depression in youth (Franko et al., 2004; Tram et al., 2000). Thus, efforts to identify related biological mechanisms have involved examinations of stress regulation systems, including the hypothalamic–pituitary–adrenal axis (HPA-axis) (Lopez-Duran et al., 2009b). In youth, atypical HPA-axis functioning

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precedes depression onset (Adam et al., 2010; Vrshek-Schallhorn et al., 2013), and is associated with depression caseness (Guerry & Hastings, 2011), and course (Rao et al., 2010). Yet, questions remain about the underlying mechanisms at play and factors that contribute to heterogeneity of past findings.

Identifying the aspect of the HPA-axis that may be dysregulated in youth depression has been elusive. Studies using direct biological probes point toward *inhibitory* processes as the most likely source of dysregulation. Specifically, depressed youth tend to have an atypical response to the Dexamethasone Suppression Test (DST) (Lopez-Duran et al., 2009b), suggesting atypical functioning of inhibitory corticoid receptors (i.e. inhibitory feedback). Unfortunately, mixed DST results led to early doubts about HPA-axis involvement in youth depression (Birmaher & Heydl, 2001) and examinations of this effect ended before the functional relevance of DST findings to psychosocial stress regulation could be determined. Studies examining HPA-axis responses to psychosocial stressors have been equivocal. Some have linked *greater* post-stress cortisol with depression symptoms (Booij et al., 2013) and diagnosis (Rao et al., 2008). Yet, whether such findings reflect excitatory (more intense/prolonged activation) or inhibitory factors (e.g. poor recovery) are unclear because common analytic approaches to examining post-stress cortisol have precluded the proper dissection of these effects (Lopez-Duran et al., 2014). In addition, other studies have linked *blunted* stress responses with depression symptoms (Dieleman et al., 2010) and diagnosis (Luby et al., 2003; Luby & Mrakotsky, 2004; Spies et al., 2011; Suzuki et al., 2013). These contradictory findings could be partially attributed to methodological differences, including the nature of the stress tasks (Spies et al., 2011), heterogeneity of samples (Harkness et al., 2011) or a failure to acclimate to some laboratory settings (Suzuki et al., 2013).

Conflicting findings may also be due to developmental and/or sex effects. Internalizing symptoms have been linked to HPA hypo-reactivity in childhood but to hyper-reactivity in adolescence (Hankin et al., 2010). Likewise, studies linking depression to blunted reactivity included only pre-adolescent children (Luby et al., 2003; Luby & Mrakotsky, 2004; Suzuki et al., 2013), while those finding hyper-reactivity have included mostly adolescents (Rao et al., 2008). Furthermore, there is growing evidence of a male-specific HPA effect in youth depression. Some studies have linked depressive symptoms with atypical HPA functioning in boys only (Dietrich et al., 2013; Hartman et al., 2013). Likewise, elevated morning cortisol predicted depression onset in boys, but not girls in a large population sample (Owens et al., 2014). Furthermore, there is recent evidence that males may have a more delayed recovery of the stress response (Lopez-Duran et al., 2014), which may underlie the HPA-depression link in males.

In summary, the main objective of this study was to probe different aspects of the HPA-axis response to psychosocial stress in youth depression with the goal of identifying specific areas of dysregulation and to probe age and sex effects. We hypothesized that depressive symptoms would be associated with impaired recovery, but not intensity of activation. We further hypothesized that evidence of impaired recovery of the stress response would increase in strength by age, and would be observed primarily in males.

Methods

Participants

Participants were 115 children ($M_{\text{age}} = 12.79$, $SD_{\text{age}} = 2.26$; range: 9–16; 45% females), recruited to represent the full range of internalizing symptoms with an overrepresentation of children with clinical levels of depressive symptoms. Participants were recruited through online and printed advertisements placed in local newspapers and community centers seeking “healthy” youth, as well as through targeted advertisement seeking youth with significant depression symptoms or a current depression diagnosis. Exclusion criteria included: (1) having evidence of mental retardation, any pervasive developmental disorder or a major systemic medical condition (e.g. diabetes); (2) having a comorbid diagnoses of Obsessive Compulsive Disorder, Post-traumatic Stress Disorder (PTSD), Bipolar Spectrum Disorder or a history of psychosis as determined by the diagnostic evaluation (see below). The racial composition of the full sample was 78% Caucasian, 6.3% African American, 2.4% Asian American, 2.4% Latino and 10.2% biracial children. Three participants were taking oral contraceptive medications but the results presented below did not change if these participants were deleted from the analysis.

Measures and procedures

Protocol summary—Children arrived to the laboratory between 1300 and 1600 h. After consent, children relaxed in a child-friendly waiting room for 30 min (adaptation phase), while their parents completed the child’s diagnostic interview in a separate room. The children then completed a psychosocial stress task, and then watched a calming child-appropriate film for 60 min (recovery phase). After the film, the children underwent a diagnostic interview and completed several questionnaires (Figure 1). This protocol was reviewed and approved by the University of Michigan Institutional Review Board.

Depression symptoms and diagnostic procedures—Symptoms of depression were quantified via the child-completed Children’s Depression Inventory (CDI) (Kovacs & MHS Scaff, 2003). Children rated 27 items on a 0–2 point scale regarding experiencing standard depression symptoms during the past 2 weeks. The CDI has excellent internal consistency (alpha in current sample = 0.92), and is one of the most widely validated and commonly used self-report measures of depression for children and adolescents (Timbremont et al., 2004). In addition, we obtained psychiatric diagnoses via the semi-structured, multi-informant Interview Schedule for Children and Adolescents-Diagnostic Version, which is an extension of the Interview Schedule for Children and Adolescents (Sherrill & Kovacs, 2000). The interviews were conducted by fully trained advanced doctoral students in clinical psychology who received weekly clinical supervision by the Principal Investigator (PI) of the study, who is a licensed clinical psychologist and coordinates the diagnostic assessment training of a clinical psychology doctoral program at a large research university. All diagnostic interviews (100%) were reviewed during weekly diagnostic meetings that included all clinicians and the PI. Final diagnoses were derived by the clinical team via consensus using the best-estimate procedures (Maziade et al., 1992) based on the child and parent report, family history and other self-report symptom checklists.

Demographics, family history and day log—Parents completed a pencil and paper questionnaire including questions about demographics, family finances and living situation, child developmental and caregiver history, parental demographics, child education, child social relationships, child psychiatric history, history of major life events, current medications and physical health information. In addition, participants and their parents completed a day of activities in which they reported waking time, food consumption during the 90 min prior to the assessment and any unusual events that may have led to increased stress.

Psychosocial stress task and regulation—We used the Socially Evaluated Cold Pressor Test (SE-CPT) (Schwabe et al., 2008), designed specifically for eliciting a cortisol stress response by combining thermal stress and social evaluation (Schwabe et al., 2008). It has been validated as an effective activator of HPA-axis in both individual and group administration (Minkley et al., 2014; Schwabe et al., 2008). Participants were told that they would be videotaped and that the videos would be used to examine their facial expressions. Children then sat next to a bucket of ice water (1–3 °C) and were asked to stare into a video camera while putting their non-dominant hand in the water. A research assistant stared stoically and redirected the participants' attention toward the camera if needed. If participants removed their hands before 10 s, they were instructed to place it back in the water until at least 30 s of immersion was completed. Anyone who kept the hand in the water for 3 min was asked to remove their hand from the water. Participants stared at the camera for 3 min regardless of total hand immersion time. The entire task takes approximately 5 min, which helps reduce individual variability in peak times that can add “noise” during statistical modeling (Lopez-Duran et al., 2014), while still activating the stress response (Schwabe et al., 2008; Schwabe & Wolf, 2010a,b). Following the SE-CPT, participants watched a calming documentary by National Geographic for 60 min in a separate room (e.g. The Appalachian Trail).

Saliva samples—Eight samples were collected via passive drool at 30 min before the task (–30), immediately before the task (0) and at 25, 35, 45, 55, 65 and 75 min after the *start* of the task. These times were selected because they maximize our ability to capture the cortisol peak levels in saliva, which are observed between 25 and 45 min post-stress initiation (Dickerson & Kemeny, 2004; Lopez-Duran et al., 2009a, 2014). Samples were stored at –20 °C until assayed at the Core Assay Facility of the University of Michigan Psychology Department using an enzyme immunoassay kit (Salimetrics™). The inter-assay and intra-assay coefficients of variability were 5% and 9%, respectively.

Analytic strategy—Extreme cortisol samples at the upper 2% of the distribution (19 samples of 916 samples) were winsorized (i.e. the top 2 percentile values were recorded and set at the 98 percentile value). All samples were box-transformed to normalize their distribution (Miller & Plessow, 2013) using the following formula (skewness = 6.84 pre- and 0.53 post-transformation):

$$X' = \frac{X^{26} - 1}{0.26}.$$

We first used a repeated measures framework via random effects modeling to examine how depression (symptoms and diagnoses), sex, age and their interaction impact overall cortisol levels throughout the visit. We used random effects as opposed to ANOVA in order to model the correct covariance structure of the repeated data and to better model unbalanced data (i.e. different group sizes) (Littell et al., 2006). We then examined the impact of these variables on specific aspects of the HPA-axis response using a two-piece multilevel growth curve modeling (GCM) with landmark registration as applied to neuroendocrine data (Lopez-Duran et al., 2014). This approach has been shown to be more sensitive than traditional methods (rANOVA, examinations of AUC, etc.) in the identification of subtle differences in distinct aspects of the response (intensity of activation, recovery capacity, etc.), facilitates the partitioning of variance due to amplitude (e.g. peak magnitude) and timing effects (timing of peaks), and to more accurately reflect the underlying differences in ACTH and plasma cortisol (Lopez-Duran et al., 2014). This approach involved three steps. First, individual post-stress peaks are identified from a visual analysis of the individual curves using Lopez-Duran et al. (2014) peak identification procedure. Peaks were visually identified from smoothed curves and were defined as the first identifiable point in the upward curve that was at least 20% higher than baseline and was followed by either a decline or a plateau. If the peak was followed by a plateau, none of the samples in the plateau could be more than 10% higher than the peak, otherwise the higher sample was considered peak. Participants with peaks that met the above definition were labeled “responders”, while those without peaks were labeled “non-responders”. Second, the timing of each individual peak was identified and used to create a new time axis reflecting minutes from peak. This entails the adjustment of the curves so that each peak falls on the same time point. For those without an identifiable peak, we used the +35 time point (the mode peak time) as their expected, but not observed, “peak time” in order to model their non-response. Finally, we created two spline time variables to represent minutes before (TimeBeforePeak) and after the peak (TimeAfterPeak). We then conducted a multilevel random effects model of the cortisol pre- and post-peak trajectory with peak levels as the intercept. The unconditional fixed effects model was defined as:

$$\text{Cortisol} = \beta_0 + (\beta_1 \times \text{Time Before Peak}) + (\beta_2 \times \text{Time After Peak}) + e,$$

where β_0 is the intercept (peak), β_1 is the activation slope and β_2 is the recovery slope. Pre-task levels and anxiety comorbidity were added to all models as covariates. All models included random intercepts and slopes using an AR(1) covariance structure, which was selected based on model fit. We used the Kenward–Roger adjustment to account for the unbalance nature of our data (Littell et al., 2006) when examining the effects of diagnostic categories. All analyses were conducted in SAS v9 (Cary, NC) via PROC MIXED.

Results

Sample characteristics

Depression symptoms and diagnoses—Current depression symptoms (CDI scores) ranged from 0 to 32 ($M = 6.63$, $SD = 7.30$, skewness = 1.55). Only 12% of the sample ($N = 14$) reported no depression symptoms (CDI = 0), and 20% ($N = 25$) endorsed at least

moderate levels ($CDI > 13$). Results from the diagnostic interview revealed that 70% of the sample ($N = 80$; $M_{age} = 12.37$, $SD_{age} = 2.22$, 45% females) did not have a history of anxiety or depression difficulties. A smaller group of 35 youth ($M_{age} = 13.75$, $SD_{age} = 2.11$, 57% females) was diagnosed with a current or past depressive disorder (MD group), including Major Depression (69%), Dysthymia (14%), or Depressive Disorder – Not Otherwise Specified (17%). Of this MD group, 18 were currently experiencing a major depressive episode and 17 were in remission. Approximately 34% of the MD group ($N = 12$) had a comorbid anxiety disorder. Because of the heterogeneity and relatively small size of the MD group, hypotheses were tested using only current depressive symptoms (CDI scores) in continuous format. However, we also replicated all results using diagnosis status (i.e. MD vs. Comparison Group).

Cortisol response and covariates—Salivary cortisol levels increased significantly in response to the task, time effect $F(6, 671) = 28.46$, $p < 0.001$, with cortisol levels increasing on average about 60% from baseline to peak. The task produced a noticeable stress response in 51.30% ($N = 59$) of the sample. This response rate is consistent with those found using more standard stress tasks with children (e.g. Trier Social Stress Test; Gunnar et al., 2009) although, as is common in children, lower than the 70% rate observed in adults (Foley & Kirschbaum, 2010; Schwabe et al., 2008). The responder group displayed a robust 200% increase in cortisol levels from start to peak. They displayed the expected cubic trajectory with an initial increase in cortisol, time $b = 0.05$, $t(58) = 5.38$, $p < 0.001$, followed by a decline, time² $b = -0.001$, $t(234) = -4.25$, $p < 0.001$, and then by a deceleration of this decline, time³ $b = 0.0001$, $t(234) = 3.21$, $p = 0.001$. However, the non-responders displayed a linear declining trajectory throughout the visit, time $b = -0.016$, $t(56) = -11.36$, $p < 0.001$. Specifically, cortisol declined by 50% from the start to the end of the visit. This decline was steeper than expected from the diurnal slope during the afternoon (Edwards et al., 2001), suggesting that the non-responder group was likely recovering from stress experienced prior to the start of the task (e.g. arrival to the laboratory). Therefore, the cortisol responses of responders and non-responders followed two different trajectories that likely reflect different phenomena. Because we are interested in modeling specific features of the stress response (e.g. activation intensity, peak magnitude, regulatory capacity), modeling responders and non-responders together would add unnecessary statistical noise as the non-responders do not have variability across the indices of interest. GCM also assumes that the underlying trajectories (parameters) are equivalent for all individuals and subgroups and results will be biased if subgroups with two drastically different trajectories are modeled simultaneously, as if they were equivalent (Curran et al., 2010). Furthermore, the non-responders may well provide important and unique information about group and individual differences in cortisol trajectory during the visit. Therefore, we modeled responders and non-responders in two separate models.

Responders and non-responders did not differ in depression symptoms (CDI), diagnostic status (MD vs. comparison), age, sex distribution, time from awakening to laboratory session, time of laboratory session or whether they ate prior to the session (Table 1). However, responders had significantly lower pre-task cortisol levels. In order to control for the impact of arrival (time -30) and pre-task levels (time 0), we averaged both samples and

used it as a covariate. Yet, we tested the models controlling for arrival and time 0 levels separately and the results presented below were unchanged. Furthermore, we identified age and time from awakening as potential covariates impacting cortisol reactivity among responders and these were included in subsequent models. Finally, water immersion time did not impact the stress response among responders or non-responders, and was not included in subsequent models, but results were unchanged if included.

Impact of depression symptoms on neuroendocrine response among responders

Overall cortisol levels via repeated measures—There was no main effect of symptoms, age or sex (all $ps > 0.20$). The impact of depression symptoms on cortisol levels over time was not moderated by age, age $\times$ CDI $\times$ time $F(6, 287) = 0.31$, $p = 0.93$. However, sex moderated the impact of symptoms on cortisol levels over time, sex $\times$ CDI $\times$ time $F(6, 294) = 2.79$, $p < 0.01$. Specifically, greater CDI was associated with higher cortisol levels at 55 and 65 min post-stress among males only, $t(294) = 2.95$, $p = 0.003$, and $t(294) = 2.11$, $p = 0.04$, respectively. No such effect was observed in girls, $t(294) = 1.01$, $p = 0.31$, and $t(294) = 1.51$, $p = 0.13$. Figure 2 displays the estimated cortisol levels during the task (from pre-task baseline) for prototypical groups of males and females with high and low depression scores.

Reactivity, recovery and peak response via GCM with landmark registration—Depression symptoms did not directly impact activation slope, peak levels or recovery slope (Table 2). Age did not moderate the link between symptoms and activation slopes, peak levels or recovery slopes (all $ps > 0.20$). Sex moderated the link between symptoms and peak levels, sex $\times$ CDI $F(1, 42) = 7.79$; $p = 0.007$. Higher depressive symptoms were linked to higher peak levels but only in boys, $b = 0.04$, $t(42) = 2.43$, $p = 0.01$. No such effect was observed in girls, $b = -0.01$, $t(42) = -1.38$, $p = 0.17$. Figure 3 displays the estimated trajectories to and from peak for prototypical groups of males and females with high and low depression scores.

Impact of depression symptoms on neuroendocrine functioning among non-responders

Since non-responders displayed a linear cortisol trajectory, repeated measures and GCM produce arguably redundant information. Thus, we present only results of the growth curve model. Depression symptoms were associated with greater starting levels, CDI $b = 0.02$, $t(199) = 2.19$, $p = 0.03$, but symptoms did not impact the declining slope, CDI $\times$ time $b = -0.001$, $t(199) = -0.03$, $p = 0.97$. The main effects were not moderated by age or sex (all $ps > 0.20$).

Discussion

In this study we examined separate aspects of the neuroendocrine stress response in a sample of youth with a broad range of depressive symptoms. We did not find evidence that depression symptoms were associated with greater intensity of activation (activation slope). Instead, depression symptoms were associated with a more *sustained activation* of the axis in response to stress, potentially due to sustained excitatory input into the axis and/or higher threshold of inhibitory feedback, and *impaired recovery* of the stress response.

Among male participants who responded to the task, depressive symptoms were associated with greater cortisol peak levels, which are consistent with evidence of hypercortisolemia in depressed youth (Lopez-Duran et al., 2009b). However, peak magnitude is a function of where the activation starts (baseline), the rate of activation (slope) and the *duration* of the activation. Because we modeled peak levels and activation slope simultaneously (i.e. controlling for each other), and we also controlled for pre-task levels (baseline), higher peaks associated with depression symptoms in boys may be due to a more *prolonged*, but not more “intense” activation of the axis (see Lopez-Duran et al., 2014). In fact, estimation of peak times after adjusting for covariates (Singer & Willett, 2003) indicated that boys with a history of depression symptoms peaked about 5 min after their non-affected peers (31 vs. 26 min post-stress).

Our data do not speak to factors that may explain such prolonged activation of the axis, but we provide two speculative interpretations to guide further examination. First, corticoid output can be extended via *continuous* excitatory signaling into the axis, such as during continuous exposure to a stressor (Dhabhar et al., 1997). Corticoid output eventually diminishes when excitatory signals decline or when inhibitory signals are sufficiently strong to modulate the corticoid response (Dhabhar et al., 1997). Therefore, the *ratio* of excitatory and inhibitory input can impact the duration of the activation, such that prolonged excitatory input can delay the shift of the ratio from greater excitatory to greater inhibitory signals. We call this the *inhibitory shift* hypothesis. In our case, all participants were exposed to the same length of stressor. In addition, adrenal and hypothalamic sensitivity, which can increase excitatory signals, appear to be intact in youth depression (Birmaher et al., 1996; Dorn et al., 1996; Kaufman et al., 1997; Ronsaville et al., 2006). Thus, factors at the pre-pituitary levels may be responsible for the observed prolonged activation. For example, biased perception of the stressor as more intense or poor emotion regulation and coping behaviors (e.g. brooding rumination, attentional biases) in response to the stressor could prolong the activation of the axis. In fact, rumination has been found to prolong HPA activation in depressed adults (LeMoult & Joormann, 2014) and delay recovery in depressed teens (Stewart et al., 2013). Thus, future studies could test this hypothesis by examining whether differences in excitatory factors (rumination, attention biases, etc.) mediate the link between depression and the *duration* of HPA activation.

Second, engagement of the axis’ inhibitory feedback is under the control of a complex interplay between mineralo-corticoid (MR) and glucocorticoid receptors (GR) (De Kloet et al., 2005). Regulation of the axis after acute stress occurs when corticoids saturate MR receptors and are able to occupy *sufficient* GR receptors to initiate the down regulation of CRH and vasopressin synthesis (De Kloet et al., 2005). Therefore, prolonged activation of the axis can be the result of atypically high threshold for GR-mediated inhibition, such as in the case of reduced sensitivity, or numbers of GR receptors in key brain regions. We call this the *inhibitory threshold* hypothesis. Thus, boys showing higher symptoms of depression may require greater cortisol levels to initiate GR-mediated inhibition. This is consistent with studies directly probing the axis’ inhibitory feedback using the DST (Lopez-Duran et al., 2009b), and a recent study suggesting that parental behaviors that have been associated with improved GR-mediated regulatory functioning in animals (Meaney et al., 2007) can have a long-term impact on the *duration* of HPA activation to stress in children (Kuhlman et al.,

2014). Thus, future studies could examine whether differences in GR receptor sensitivity (DST non-suppression) or density (e.g. via methylation) explains the prolonged HPA activation observed in depression.

Furthermore, depression symptoms were not associated with post-peak recovery slopes, which could be misinterpreted as indicating no difference in regulatory capacity. Yet, peak levels and recovery slopes are highly correlated such that higher peaks lead to more intense recovery in healthy samples (Lopez-Duran et al., 2014). Therefore, the failure to show steeper regulation slopes despite higher peak levels associated with depression symptoms may actually reflect problems with regulatory capacity. Likewise, depression symptoms were associated with higher overall cortisol levels throughout the task among non-responders. We argue that the decline of cortisol among non-responders during the task likely reflected recovery to the stress of coming to a novel environment because such decline is more intense than expected from diurnal changes during the afternoon (Edwards et al., 2001). Therefore, these findings suggest a link between depression symptoms and atypical regulatory processes. Whether this is due to sustained excitatory signaling, dysregulation of inhibitory feedback (e.g. GR sensitivity) or both are unknown.

Notably, we found a symptoms-HPA link in boys only. Although this is inconsistent with previous hypotheses about sex differences in depression (Kuehner, 2003), it is consistent with growing evidence linking atypical HPA and depression in boys and young adults only (Binder et al., 2009; Dietrich et al., 2013; Grant et al., 2007; Hartman et al., 2013; Hinkelmann et al., 2012; Owens et al., 2014). There were no sex differences on pre-task cortisol levels; thus, it is unlikely that the sex effect is due to higher arrival levels in females leading to a blunted response. Our data do not speak to potential mechanisms, but previous examinations of sex-specific impact of corticoids on behavior and cognition can provide some guidance for future exploration. Corticoids appear to have a greater impact on emotional memory in males than in females (Zorawski et al., 2005). In addition, male and female emotional learning are governed by different hormonal processes, with corticoids impacting males and estrogen impacting females (Cheung et al., 2013; Wood et al., 2001). Thus, HPA-axis involvement in depression in males may reflect the sex-specific impact of corticoids on emotional memories. Alternatively, there are known sex differences in the phenomenology of depression, including its presentation (Baji et al., 2009) and cognitive vulnerabilities (Abela et al., 2002; Nolen-Hoeksema et al., 1991) that may contribute to the male only effect.

Finally, we did not find evidence of an age moderation effect, which may reflect the relatively narrow age range of our sample (9–16 years). Blunted HPA reactivity has been linked to depressive symptoms in much younger samples (Hankin et al., 2011; Luby et al., 2003; Suzuki et al., 2013), and thus by middle childhood and early adolescence the more common adult-like picture of hyper-reactivity of the axis in depression may be fully formed. This would not be surprising given that there are important developmental HPA changes during the pubertal transition (Del Giudice et al., 2011), which may interact with increased psychosocial challenges associated with puberty to increase the risk for depressive disorders (Hankin et al., 2010).

There are important limitations to this study. Our sample size was relatively small, which may have reduced power especially when examining age and sex differences. For example, varying levels of corticosteroid binding globulin in females can impact salivary cortisol (Kudielka et al., 2009) and potentially make it more difficult to find effects in girls. However, the trend among depressed females was in the opposite direction than in males, and thus it is unlikely that the observed male-specific effect is due to power limitations. Small samples may also increase the risk for false positive findings (Button et al., 2013), although we found evidence of dysregulation in both responders and non-responders groups, making false positive findings unlikely. In addition, our sample was heterogeneous in regard to diagnostic status preventing us from examining differences between these three groups. Yet, we conducted additional analysis merging the currently depressed and remitted participants and compared them to their non-affected peers, which replicated the CDI findings (results available online as a supplementary table). We decided against presenting such data given that it is unclear whether merging currently depressed and remitted participants in HPA-axis studies is appropriate (Ahrens et al., 2008; Zobel et al., 2001). Nonetheless, our results provide guidance for future studies with larger clinical samples. We did not have menstrual cycle and pubertal stage information for the participants. This may have affected our sex-specific findings if there were systematic biases in menstrual cycle or pubertal stage timing among females with high and low depression symptoms. Finally, the observed cortisol values are in the low range of mid-afternoon values, potentially due to assay sensitivity limits that may impact the ability to see very subtle cortisol changes.

In conclusion, our study provides preliminary evidence of atypical regulation of the HPA response to psychosocial stressors is associated with depressive symptoms, but primarily in boys. Our findings expand extant data linking atypical HPA and depression in boys (Dietrich et al., 2013; Hartman et al., 2013; Owens et al., 2014) by identifying potential sources of dysregulation: the duration of post-stress activation and recovery capacity. We also propose two distinct hypotheses to explain variability in the duration of HPA activation; namely: sustained imbalance of greater excitatory to inhibitory signals (inhibitory shift hypothesis) and/or higher threshold of inhibitory feedback (inhibitory threshold hypothesis). Although these two hypotheses reflect processes that are not independent (inhibitory threshold impacts inhibitory shift), identifying what is primarily responsible for a prolonged HPA activation can help identify the underlying mechanisms of dysregulation in youth depression and similar disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Stress protocol summary.

Figure 2.

Estimated adjusted means of post-stress cortisol as impacted by depressive symptoms (CDI) and sex for responders only ($N = 59$; 29 girls). Groups represent males and females at different levels of depression created using 1 SD below and above the CDI mean. Boys with higher CDI scores have greater cortisol than boys with lower CDI scores during the recovery samples (time 55 and 65). No such effect was observed in girls. Standard error bars provided for males only for ease of interpretation.

Figure 3.

Estimated saliva cortisol response trajectories before and after peak response to the SE-CPT among responders after controlling for pre-task levels ($N = 59$; 29 girls). Groups represent males and females at different levels of depression created using 1 SD below and above the CDI mean. For boys only, higher depression scores were associated with higher peak levels (time 0). Depression did not affect peaks in females. Depression did not affect activation or recovery slopes in males or females.

Table 1
Differences between responders and non-responders across covariates of the cortisol response.

	Non-responder <i>n</i> = 56		Responder <i>n</i> = 59		<i>T</i> / <i>χ</i> ²	ES
	<i>M</i>	SD	<i>M</i>	SD		
Age	12.83	2.24	12.74	2.3	0.22	0.04
Hand immersion time (s)	65.2	57.72	85.04	61.15	-1.82	-0.33
Time from waking (h)	6.87	2.51	7.44	2.72	-1.14	-0.22
Time of Task (24 h)	15.57	1.55	15.98	1.37	-1.51	-0.28
Pre-task cortisol levels (time 0, box-transformed)	-2.9	0.99	-3.55	1.19	3.14 ^{**}	0.60
Arrival cortisol levels (time -30, box-transformed)	-2.8	1.28	-3.17	1.15	1.66	0.30
Sex (% males)	48.15		55.17		0.55	0.07
Ate within 1 h (% yes)	33.93		22.03		2.02	-0.13
CDI	6.79	7.99	6.67	7.01	0.08	0.93
+MD status (% MD participants)	26.79		33.9		0.69	0.08

CDI, Children's Depression Inventory.

^{**} $p < 0.01$, ES = Cohen's d or Phi.

Table 2

Estimates of depression symptoms predicting cortisol response to stress task.

	<i>b</i>	SE	<i>t</i>	<i>p</i>
<i>Direct effects model</i>				
Intercept (peak)	−3.355	0.106	−31.69	<0.001
Activation slope	0.024	0.008	3.18	0.00
Regulation slope	−0.021	0.007	−3.13	0.00
CDI scores impact on peak	−0.005	0.010	−0.51	0.61
CDI scores impact on activation slope	0.000	0.001	−0.38	0.70
CDI scores impact on regulation slope	0.000	0.001	−0.53	0.60
<i>Moderation by sex</i>				
Intercept (peak)	−3.454	0.205	−14.60	<0.001
Activation slope	0.023	0.010	2.340	0.020
Regulation slope	−0.030	0.009	−3.470	0.001
CDI impact on peak (males)	0.042	0.017	2.430	0.019
CDI impact on activation slope (males)	0.000	0.001	0.320	0.751
CDI impact on regulation slope (males)	0.001	0.001	0.570	0.568
Sex (females) impact on peak	0.110	0.198	0.560	0.581
Sex (females) impact on activation slope	0.002	0.014	0.180	0.859
Sex (females) impact on regulation slope	0.018	0.013	1.360	0.174
CDI × sex impact on peak	−0.059	0.021	−2.790	0.008
CDI × sex impact on activation slope	−0.001	0.001	−0.750	0.451
CDI × sex impact on regulation slope	−0.001	0.001	−0.980	0.328

CDI, Children's Depression Inventory.