Published in final edited form as:

Horm Behav. 2011 January ; 59(1): 123–132. doi:10.1016/j.yhbeh.2010.10.015.

Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviors in youth: Longitudinal and concurrent associations with cortisol

Paula L. Ruttle, M.A.^{a,*}, Elizabeth A. Shirtcliff, Ph.D.^b, Lisa A. Serbin, Ph.D.^a, Dahlia Ben-Dat Fisher, Ph.D.^a, Dale M. Stack, Ph.D.^a, and Alex E. Schwartzman, Ph.D.^a

^a Concordia University, Psychology Department, Center for Research in Human Development, 7141 Sherbrooke St. West, PY-170, Montreal, Quebec, Canada, H4B 1R6

^b University of New Orleans, Department of Psychology, Geology/Psychology Bldg Lakefront Campus, 2000 Lakeshore Drive, New Orleans, LA, 70148

Abstract

Research examining cortisol dysregulation is seemingly contradictory with studies showing that both internalizing and externalizing behaviors are related to high and low cortisol. One extant theory to explain divergent findings in the stress literature is that both hypo- and hyper-arousal of the hypothalamic-pituitary-adrenal (HPA) axis may be present depending on time since onset of the stressor. This theory may extend to the onset of internalizing and externalizing behaviors. Data from 96 youth participating in a longitudinal project were used to examine this possibility. Composite measures of internalizing and externalizing behaviors at both childhood and early adolescence were formed using mother and teacher reports. Multiple salivary cortisol samples were also collected over two consecutive days during early adolescence. Problematic behaviors were associated with cortisol and the direction of the association was dependent on amount of time passed since onset of the behaviors. When examined concurrently in adolescence, youth with more internalizing behaviors had *higher* morning cortisol; however, when examined longitudinally, youth with more internalizing behaviors in childhood had *lower* morning cortisol levels as adolescents. Youth with more externalizing behaviors in childhood had flattened diurnal cortisol rhythms as adolescents, and this finding persisted when examined in adolescence. Cortisol dysregulation was greatest in children with the most severe behavior problems. Findings support the theoretical model of blunting of the HPA axis over time. While the HPA axis may show hyper-arousal when youth first display behaviors, long-term exposure may lead to a hypo-arousal of the HPA axis which culminates in a dysregulated diurnal rhythm.

Keywords

diurnal cortisol; internalizing behavior; externalizing behavior; blunted; hierarchical linear modeling

Behavior problems are often debilitating with profound social, emotional, and psychological ramifications. Children who exhibit problematic behaviors often perform more poorly at school (Ansary and Luther, 2009; Aunola et al., 2000), are more socially rejected by their peers (Hymel et al., 1990; Pederson et al., 2007), have more strained relationships with their parents and siblings (Richmond and Stocker, 2006), and lower self-esteem (Aunola et al.,

*Corresponding author: Paula L. Ruttle, plruttle@gmail.com, Telephone: 1-514-848-2424 x.7561, Fax: 1-514-848-2515.

2000). Children's problem behaviors typically fall into two broad categories: internalizing problems (e.g. depression and anxiety) and externalizing problems (e.g. aggression and oppositional behaviors) and often these disorders are co-morbid (Angold et al., 1999). Given that children who demonstrate higher levels of internalizing and externalizing behaviors are at an increased risk of developing clinical level-disorders (Kroes et al., 2002; Mesman and Koot, 2002; Petty et al., 2008), it is critical to identify mechanisms associated with such behaviors before they develop into more severe disorders.

While there is little doubt that both internalizing and externalizing problems are etiologically heterogeneous, promising research in the area of psychobiology has helped provide a window into the onset and persistence of these types of behaviors. Sustained exposure to unhealthy levels of stress and/or an accumulation of daily hassles has been related to the development of various psychopathologies (Monroe and Hadjiyannakis, 2002; van Praag et al., 2004). Activity of the hypothalamic-pituitary-adrenal (HPA) axis, frequently measured noninvasively via the stress hormone cortisol, is elicited by a variety of stressors (Dickerson and Kemeny, 2004). While a certain level of stress is healthy and necessary for an organism's survival, stress becomes unhealthy when it occurs for a prolonged period of time, at a high intensity, or involves the accumulation of many minor stressors (Sapolsky, 1992). Thus, dysregulated HPA axis activity may provide a window into the exposure to life stressors or accumulation of daily hassles over time and serve as a psychobiological mechanism whereby stressors manifest as a risk for the onset and persistence of mental health problems (van Praag et al., 2004).

In addition to its role as a hormone that helps individuals cope with a stressful situation, cortisol is also present in the body at resting levels, with basal activity modulated largely by different receptor subtypes than cortisol reactivity. Basal levels of cortisol are greatly influenced by the sleep/wake cycle and, as such, follow a diurnal rhythm. The diurnal pattern is characterized by a peak in cortisol levels approximately 30 minutes post-awakening which is followed by a decline throughout the course of the day (Ice et al., 2004; Kirschbaum and Hellhammer, 1994; Klimes-Dougan et al., 2001). A surge in morning cortisol followed by a steady decline helps initiate waking activities and primes the body for the demands of the pending day (Klimes-Dougan et al., 2001; Smyth et al., 1997). The morning peak appears to be biologically influenced while afternoon or evening levels are largely influenced by environmental and individual factors such as eating, sleeping and mood (Schreiber et al., 2006). The robust diurnal rhythm (and slight increases in response to a stressor) is thought to be a healthy cortisol pattern; in contrast, extremely low morning or high evening cortisol may signify stress dysregulation (Shirtcliff and Essex, 2008) or a mismatch between an individual's biorhythm and social context (Stetler et al., 2004).

Both internalizing and externalizing behaviors have been linked to dysregulated cortisol levels in youth. The findings in the externalizing literature are somewhat mixed. While the majority of research suggests that externalizing problems in middle childhood and adolescence are associated with low levels of basal or diurnal cortisol, both concurrently (Kariyawasam et al., 2002; Moss et al., 1995; Pajer et al., 2001; Shirtcliff et al., 2005) and longitudinally (Fairchild et al., 2008; McBurnett et al., 2000; Popma et al., 2007) some studies suggest that there no association (Azar et al., 2004; Klimes-Dougan et al., 2001; van Bokhoven et al., 2005) or even a positive association (Gerra et al., 1997; McBurnett et al., 2005; van Bokhoven et al., 2005). However, it must be noted that the studies reporting positive or null associations typically measured externalizing/aggressive behavior or cortisol in an alternative form than the one used in this study (e.g. induced aggression, reactive cortisol).

While the association between cortisol and externalizing behaviors appears to be somewhat consistent in older children and adolescents, the association between cortisol and internalizing and/or depressive behaviors has not been as consistent. In the concurrent literature some studies report that higher cortisol levels are associated with internalizing behaviors (Cicchetti and Rogosh, 2001; Kaufman, 1991; Perez-Edgar et al., 2008), while others suggest that lower cortisol levels are associated with internalizing behaviors (De Bellis et al., 1996; Granger et al., 1998). The longitudinal literature is much clearer with nearly all of the longitudinal studies reporting that elevated basal cortisol levels precede the onset of later internalizing symptoms in children (Smider et al., 2002) and depression in adolescents (Goodyer et al., 1996, 2000, 2001; Halligan et al., 2007). One exception is a longitudinal study that found high levels of anxiety to be related to high levels of cortisol secretion (Greaves-Lord et al., 2007); however these divergent findings are most likely because they controlled for symptoms of depression. The adult depression literature shows a similar pattern with mostly divergent findings in the concurrent literature (Board et al., 1956; Gold et al., 1988; Peeters et al., 2004; Posener et al., 2004; Sachar et al., 1973) but a positive association longitudinally (Harris et al., 2000). Taken together, these studies suggest that the association between cortisol and internalizing problems may differ depending on the time lag between when cortisol is measured and the onset of depression. At least one prior study (Shirtcliff and Essex, 2008) illustrated that the association between cortisol and general mental health symptoms within the same sample differed depending on whether it was examined concurrently or longitudinally. Nevertheless, the authors point out that the developmental mechanism that links low concurrent cortisol, yet high cortisol over an extended period of time, with mental health is not yet fully understood. The present study will provide a unique contribution to the literature as it involves examining a different sequence: the occurrence of internalizing and externalizing behaviors before and then at the same time as diurnal cortisol.

One theory in the stress literature which may possibly extend to, and account for, the divergent findings in the internalizing and depression literature is that low or declining basal cortisol levels may be the product of a “counter-regulatory” or “down regulation” response due to long-term exposure to excessive cortisol levels (Fries et al., 2005; Miller et al., 2007). The HPA axis is a regulatory system; therefore, it modulates the amount of cortisol released to achieve a favorable physiological state (Koob and Le Moal, 2001; McEwen, 2000; Sapolsky, 2003). Miller and colleagues (2007) suggest that recent exposure to a severe stressor may initially result in elevated cortisol, but that after extended exposure to severe stress, the HPA axis develops a counter-regulatory response whereby cortisol rebounds below normal (Koob and Le Moal, 2001; Miller et al., 2007, pp.26). This theory is groundbreaking in the stress literature but has only linked stress, not behavior problems in particular, to blunting of the HPA axis.

Although basic research such as this is essential for establishing theory and helping develop our general understanding of various phenomena, it is crucial to extend these ideas to applied research in order to explore how basic mechanisms function in real life settings. While it seems plausible that internalizing and externalizing behaviors may be implicated in this theory, it has yet to be sufficiently empirically investigated. Miller, Chen and Zhou (2007) conducted a meta-analysis which explored various factors that could potentially influence the HPA axis to produce either elevated or blunted levels of cortisol after experiencing an extreme stressor. They found a negative association between the amount of time since the onset of the stress and cortisol secretion (Miller et al., 2007). More specifically, individuals who had experienced a recent stressful situation had higher morning and overall average cortisol levels; however, individuals who had experienced a stressor for a prolonged period of time had lower morning and overall average cortisol levels. Miller and colleagues suggest that the cross-sectional literature on cortisol and depression may only

seem contradictory because the studies were assessing participants at different time frames from stressor onset. Since these results were not specific to children with internalizing behaviors, in an attempt to better explain ambiguous findings in the youth internalizing literature, it is worthwhile to explore this avenue that a similar mechanism may link behavior-onset with HPA axis functioning. We propose that internalizing and externalizing behaviors may also be implicated in this theory as a developmental mechanism, either as an outcome of stress or acting as the stressors themselves.

The present paper was designed around the observation that the cross-sectional literature examining internalizing behaviors and cortisol provides mixed results, but there appears to be a consistent longitudinal pattern of elevated morning cortisol levels in individuals at risk for expressing later internalizing behaviors or depression. Following the theory put forth by Miller and colleagues (2007), we expected that *higher* cortisol would be associated with the development of new internalizing behaviors when examining cross-sectional findings. However, when internalizing behaviors have been present for a prolonged period of time we anticipated that high levels of internalizing behaviors would be related to *low* cortisol levels due to the subsequent down-regulation of the HPA axis and corresponding re-organization of the HPA axis set point in response to persistently elevated cortisol levels. Demonstrating both hypo- and hyper-arousal of the HPA axis within the same population over time provides a strong test of Miller and colleagues (2007) model. Our focus was largely on internalizing behaviors because it had more bearing on the hypothesis by Miller and colleagues (2007), and because the literature findings are more ambiguous when examining internalizing rather than externalizing behaviors. Nevertheless, we felt it important to extend our analyses to include both externalizing and comorbid behaviors due to the high rates of comorbidity of internalizing and externalizing behaviors within youth (Angold et al., 1999). Studying the possibility of comorbidity in children and adolescents is particularly important because developmental trajectories are still in the process of being established and therefore, behaviors are likely to manifest as comorbid (Boyce and Ellis, 2005; Boyce et al., 2002). Similarly, we also determined if youth with the most behavior problems had the greatest evidence for distinct diurnal cortisol patterns.

Method

Participants

The participants were part of the Concordia Longitudinal Risk Project, a three generation study. The project began in 1976 and examined a community-based sample of over 4000 French speaking children (and their families) in grade 1, 4, or 7 at public schools, serving economically disadvantaged neighborhoods in Montreal, Quebec, Canada. For a complete description of the original sample population and procedures, see Ledingham (1981) and Schwartzman and colleagues (1985).

Current sample—Participants were the offspring of original participants, were primarily Caucasian, and spoke French as their first language. The data were collected at two time points. For the purpose of this paper these will be referred to as “childhood” and “early adolescence”. Youth and their legal guardians gave informed, written consent before participation and were reimbursed for their time and involvement in the study. Although 109 participants agreed to engage in the saliva sampling procedure, only those who were able to provide sufficient saliva to assay for cortisol were included in the present analyses ($n=96$). The 96 youth included 56 females and 40 males. During the childhood testing period, participants ranged in age from 6.3–10.8 yrs ($M=7.7$, $SD=0.91$) and during early adolescence they ranged from 9.3–13.5 yrs ($M=10.87$; $SD=0.90$). The majority of participants were able to provide samples over two days ($n=91$) with an average of 8.43 samples per day. The data

used in these analyses was collected as part of a larger study that was approved by the Institutional Review Board of Concordia University.

Measures

Written measures—All of the measures used in the current study were written in French. Translated versions of English measures were used if original French versions were not available.

Children's internalizing and externalizing behaviors at childhood and early adolescence: Both the mother- and teacher- report versions of the Child Behavior Checklist (CBCL/TRF; Achenbach, 1991a, 1991b, respectively) were used to measure children's internalizing and externalizing behaviors at childhood and early adolescence which was approximately three years apart. These sub-scales are normed for both age and gender, so children were rated in relation to same sex peers. Total scores were calculated for internalizing and externalizing subscales with scores above 60 indicating a clinical level of symptomology. When scores were only available for one informant (childhood: $n=12$; early adolescence: $n=19$), that score was used in analyses; however, when participants were missing both mother and teacher report forms (childhood: $n=5$; adolescence: $n=0$) missing values were mean replaced according to participant's sex and age. Although mean replacement is not ideal, it is biased towards a more normative score and thus would decrease the probability of obtaining significant findings (Engels and Diehr, 2003). During childhood 28.1% of participants ($n=27$) demonstrated clinical levels of behaviors (internalizing: $n=9$; externalizing: $n=9$; comorbid: $n=9$). During early adolescence 32.3% of youth ($n=31$) demonstrated clinical levels of behaviors (internalizing: $n=20$; externalizing: $n=6$; comorbid: $n=5$). Of the participants who, in childhood, scored above the clinical cut-off in a particular subscale, twelve youth remained above the clinical cut-off for the same subscale as adolescents and nine remained above a clinical cut-off in at least one subscale, but not the same one as in childhood.

In order to create a variable that best captured a child's internalizing behaviors, both the total scores from the mother's and the teacher's reports were examined in a factor analysis. This principle component analysis extracted the shared variability across informants separately from the informant bias following Kraemer and colleagues (2003). See Table 1 for more information. This strategy was employed to produce a measure that more accurately reflects the child's behaviors and reduces informant bias. This PCA produced two factors: one measuring the trait of internalizing behaviors and one measuring rater differences. Only the factor measuring the trait of internalizing behaviors was used in subsequent analyses. The same process was used to create a variable that best captured the child's externalizing behaviors.

Salivary cortisol samples in early adolescence: Participants were instructed to provide saliva samples over two consecutive days at specified time periods: upon awakening, 20 minutes post-awakening and then every two hours until bedtime, while refraining from eating within the 30 minutes prior to sampling. Saliva samples were collected via salivettes. Youth were instructed to remove the cotton swab from a plastic vial, chew on it for 30–45 seconds until it was saturated with saliva, and then place it back in the vial, trying to touch it as little as possible. Saliva samples were kept frozen until they were assayed for cortisol at the Douglas Hospital Research Laboratories (DHRL). The detection limit of the assay was .01 $\mu\text{g/dL}$ and the mean intra-assay and inter-assay variability coefficients were 4.0% and 4.6% respectively. In order to normalize cortisol scores, a log transformation was performed. Remaining outlying scores ($n=9$) were winsorized to within three standard deviations of the mean. In conjunction with the saliva sampling, participants completed

daily diaries for each of the two days of sampling that recorded time of saliva sampling, mood, stress, health, food consumption and time of eating, exercise, time of awakening, and bedtime. Medication intake ($n=14$) was also recorded with over half of this subsample ($n=8$) taking methylphenidate (e.g. Ritalin, Concerta).

Analytical Strategy

Exploring comorbidity of internalizing and externalizing behaviors—

Internalizing and externalizing behaviors have a high rate of co-occurrence in children and adolescents (Angold et al., 1999) which can raise challenges when trying to understand the contribution of each symptom type. Although the comorbidity of behaviors is acknowledged, many studies have examined the association between cortisol and internalizing or externalizing disorders in childhood and adolescence individually but not comorbidly (Shirtcliff and Essex, 2008). In the present sample, internalizing and externalizing behaviors were correlated in both childhood ($r=.60, p<.001$) and in adolescence ($r=.35, p<.001$). In order to fully examine comorbidity, two factors were created. In order to obtain a measure of the total number of internalizing and externalizing behaviors, *Severity*, was created by obtaining the average of both of the factors of internalizing and externalizing behaviors. In order to determine whether there was a preponderance of externalizing over internalizing behaviors (or vice versa), a second measure, *Directionality*, was created by obtaining the half difference of the internalizing and externalizing scores. For the purpose of this study, higher scores on directionality indicate a preponderance of externalizing behaviors. (For a more complete review of this process see Essex et al., 2003, 2006 and Shirtcliff and Essex, 2008). Severity and directionality variables were calculated from the internalizing and externalizing factors for both time points.

Additionally, standardized residuals were computed to measure change in participants' behaviors over time (Llabre et al., 1991). Residuals were created for internalizing and externalizing behaviors as well as severity and directionality. Furthermore, quadratic functions of these variables were computed and examined to determine if children with more extreme behavior problems had different diurnal cortisol trajectories.

Hierarchical linear modeling—A three-level hierarchical linear model separated within-the-day ($n=1578$ samples) day-to-day ($n=187$ days), and between individual ($N=96$ participants) sources of cortisol variability (see Bryk and Raudenbush, 1992 for more information). Level 1 included Time Since Waking as a within the day predictor capturing the diurnal rhythm. The intercept thereafter reflects the level of cortisol upon awakening. Also, since the diurnal rhythm is steeper in the morning than afternoon, this model included quadratic and cubic functions of Time Since Waking to allow for the examination of curvature in the slope. Another variable representing Cortisol Awakening Response (CAR), was also included at level 1 as a dummy code ($1=\text{CAR}$, $0=\text{not CAR}$), to distinguish the CAR from cortisol level or slope. All time-related variables were found to be significant in the model (See Table 2.). Additional Level 1 predictors were entered into the model to explore if mood, stress, health, food intake and exercise influenced cortisol levels; however, none of these variables had a significant effect on cortisol (See Table 2).

Level 2 captures the day-to-day variability in cortisol levels upon awakening and the diurnal slope. The model showed substantial variability in cortisol intercept levels between the two days, $\chi^2(91)=141.27, p<.001$, as well as variability in slope from one day to another, $\chi^2(91)=135.09, p<.01$. Youth reported whether they had taken medication on each day of cortisol assessment. Medication (coded as a yes/no for the day), was significantly related to cortisol slopes such that youth who were on medications had a less steep slope on the day they took medications ($B=.016, t=3.071, p<.01$).

Level 3 captures between individual variation, or the stability in cortisol levels across time. Cortisol intercept levels varied across individuals, $\chi^2(95)=227.47, p<.001$ and the slopes varied linearly, $\chi^2(95)=156.98, p<.001$, as well as quadratically, $\chi^2(95)=146.99, p<.001$, suggesting that each individual had their own diurnal slope, and the degree to which the morning decline was steeper than the afternoon decline was different from one person to another. Predictors of variability between individuals were entered at Level 3. First, we entered the control variables of age and sex into the model to assess if these variables influenced cortisol. There was no effect of sex on either intercept or any of the slope variables. There was a significant effect of age on cortisol slope ($B=.005, t=2.316, p<.05$) such that older children had less steep slopes.

According to this base model, day-to-day fluctuations accounted for 10.0% of the total variance in the level of cortisol, within-the-day fluctuations accounted for 60.1% of the total variance, and between individual fluctuations accounted for 29.9% of the total variance in cortisol levels.

As described below, *Severity* and *Directionality* of behaviors were then examined as Level 3 predictors of cortisol levels and the diurnal rhythm. Next we entered childhood and early adolescence measures of internalizing and externalizing behaviors and their residual scores in an attempt to clarify the severity and directionality findings; however due to brevity only the most pertinent of these results will be presented. After each set of analyses we examined quadratic functions of the aforementioned predictor variables to see if youth with more extreme behavior problems had distinct cortisol profiles. Unless otherwise mentioned, all variables were entered independently to predict the overall cortisol intercept and various aspects of diurnal slope. Additionally, cortisol intercept will now be referred to as morning cortisol level.

Results

Longitudinal Findings: Behaviors in Childhood Predicting Morning Cortisol Levels in Early Adolescence

Upon examining total internalizing and externalizing behaviors in childhood (i.e. severity) as a predictor of cortisol in early adolescence, results suggest that youth with a greater number of behavior problems in childhood developed lower levels of morning cortisol in adolescence, particularly when using the severity standardized residual which controlled for concurrent behavior severity during adolescence ($B=-0.044, t=-2.23, p<.03$).

To determine if this finding was specific to one behavior type, we next examined internalizing and externalizing behaviors separately. When using the standardized residual that controlled for internalizing behaviors in adolescence, youth with more internalizing behaviors in childhood developed *lower* morning cortisol levels by the time they reached adolescence ($B=-0.042, t=-2.11, p<.04$). In contrast, there was no relation between externalizing behaviors in childhood and morning cortisol levels ($B=-.035, t=-1.55, p<.20$). These findings suggest that the association between severity of problem behavior in childhood and the development of blunted morning cortisol levels by adolescence was driven largely by internalizing behaviors.

Directionality scores (in which higher scores indicate a preponderance of externalizing behaviors regardless of severity of behaviors) were not associated with any either morning levels of cortisol or diurnal cortisol slope, either longitudinally or concurrently and thus will not be mentioned in future subsections.

Concurrent Findings: Adolescent Behaviors Predicting Concurrent Morning Cortisol Levels

Severity of adolescent behaviors was not a significant predictor of adolescent morning cortisol. However, when controlling for internalizing behaviors in childhood, we found that youth who had more internalizing behaviors as adolescents had *higher* concurrent morning cortisol levels ($B=0.039$, $t=1.95$, $p=.05$). This relation did not exist with concurrent externalizing behaviors and morning cortisol in adolescence ($B=0.013$, $t=0.46$, $p<.70$). When coupled with the longitudinal association between internalizing and morning cortisol, these findings suggest that internalizing behaviors are influential in helping to shape morning cortisol levels in adolescence.

To help disentangle whether hypo- or hyper-arousal of the HPA axis was more robust, both internalizing behaviors at childhood and adolescence were entered simultaneously. Greater internalizing behaviors in childhood longitudinally predicted the development of low morning cortisol by adolescence ($B=-0.045$, $t=-2.103$, $p<.04$), but greater concurrent internalizing behaviors in adolescence were associated with higher morning cortisol (at a trend level) ($B=0.043$, $t=1.911$, $p<.06$).

Longitudinal Findings: Behaviors in Childhood Predicting Cortisol Slope in Early Adolescence

When examining the *longitudinal* association between a greater number of total behaviors (i.e. severity) and amount of decline in cortisol secretion across the day, or *slope*, youth with a greater number of total behaviors in childhood developed flatter diurnal cortisol slopes by adolescence ($B=0.006$, $t=2.437$, $p<.02$). To determine if the predictions of the slope were driven by internalizing or externalizing behaviors, the model was re-examined with behavior type separately tested. Youth with externalizing behaviors in childhood had a flatter diurnal cortisol slope by adolescence ($B=0.006$, $t=2.62$, $p<.01$); however, there was no relation between internalizing behaviors in childhood and diurnal cortisol slopes ($B=.003$, $t=1.63$, $p<.20$).

Concurrent Findings: Concurrent Behaviors Predicting Cortisol Slope in Early Adolescence

Although there was no association between concurrent severity of behaviors and cortisol slope in adolescence, upon examining internalizing and externalizing behaviors separately we found that the only significant associations were found with externalizing behaviors. Adolescents with more externalizing behaviors had flatter concurrent diurnal cortisol slopes ($B=.005$, $t=2.209$, $p<.03$) which parallels the longitudinal association between externalizing behaviors and diurnal cortisol. When externalizing behaviors at both time points were entered into the model, externalizing behaviors at childhood predicted the amount of decline in cortisol across the day years later when the child was an adolescent ($B=.005$, $t=2.02$, $p<.05$), but externalizing behaviors at adolescence did not ($B=.002$, $t=0.62$, $p<.60$). This suggests that the relation between the slope of diurnal cortisol and externalizing behaviors may be primarily due to behaviors that were established earlier in childhood.

Associations between Cortisol and Behaviors after Including Control Variables

Next, we determined if associations of interest persisted after including control variables. Although there was a significant main effect of age, including age in the models did not significantly change the findings. For gender, there was no main effect on cortisol levels or slope and controlling for gender did not substantially change models.

After controlling for medications at Level 2, the significant association between morning cortisol levels and internalizing behaviors persisted ($p<.05$), although the association

between severity of behaviors (which was primarily driven by internalizing behaviors) decreased to a trend level ($p < .07$). In contrast, the associations between slope of cortisol and externalizing behaviors diminished ($ps > .16$ to $ps > .25$), although there was still a trend for high externalizing behaviors in childhood to predict flatter slopes in adolescence, $p < .08$. While medications may have influenced the concurrent association between externalizing behavior and decline of cortisol across the day, the persistence of the longitudinal association between externalizing behavior and cortisol slope (at a trend level) after controlling for medications suggests that this association was not entirely eliminated by the inclusion of medication in the model.

Longitudinal Associations between Cortisol and Behaviors when Examining Youth with the Most Extreme Behavior Problems

Next, we wanted to determine if participants who had the most behavior problems had distinct diurnal cortisol trajectories. Linear and quadratic functions of the predictor variables were entered into the model simultaneously which allowed for examination of HPA trajectories belonging to the participants with the most extreme behavior problem scores. Given the large number of predictors, non-significant effects were removed through backwards elimination procedures to arrive at parsimonious models.

To determine if youth with the most internalizing problems in childhood had a distinct diurnal cortisol pattern in adolescence, the quadratic function of internalizing behavior was examined. Adolescents with the most extreme internalizing behaviors had a trend for the lowest morning cortisol levels ($B = -.085$, $t = -2.40$, $p < .02$ for linear internalizing, $B = 0.018$, $t = 1.93$, $p < .06$ for quadratic internalizing). Compared to youth with average internalizing symptoms in childhood, youth with the most extreme internalizing behaviors had less steep diurnal slopes ($B = .042$, $t = 2.45$, $p < .02$ for linear internalizing, $B = -.013$, $t = -2.98$, $p < .004$ for quadratic internalizing), as well as a greater curvature to their diurnal slope ($B = -.008$, $t = -2.95$, $p < .005$ for linear internalizing, $B = .003$, $t = 3.64$, $p < .001$ for quadratic internalizing), and less of a cubic curve to their diurnal slope ($B = .0003$, $t = 3.25$, $p < .002$ for linear internalizing, $B = -.0001$, $t = -3.86$, $p < .001$ for quadratic internalizing) which culminated in the flattest overall slopes for youth with the most internalizing behaviors in childhood (See Figure 1). Controlling for medication usage had no significant effect on the abovementioned associations between internalizing problems and diurnal cortisol profile.

To determine if youth with the most externalizing problems in childhood had a distinct diurnal cortisol pattern in adolescence, linear and quadratic functions of externalizing behavior were examined and non-significant predictors were eliminated. The parsimonious model continued to find that adolescents with more externalizing behaviors in childhood had lower morning cortisol levels ($B = -.055$, $t = -2.31$, $p < .02$), but this was not more pronounced in the adolescents with the most extreme externalizing behaviors. Nonetheless, adolescents with the most extreme externalizing behaviors in childhood had less steep diurnal slopes at some points in the day ($B = .051$, $t = 3.09$, $p < .003$ for linear externalizing, $B = -.016$, $t = -3.09$, $p < .003$ for quadratic externalizing), greater quadratic curvature to their diurnal slope ($B = -.007$, $t = -2.68$, $p < .009$ for linear externalizing, $B = .003$, $t = 2.90$, $p < .005$ for quadratic externalizing), and less of a cubic curvature to their diurnal slope ($B = .0001$, $t = 2.61$, $p < .01$ for linear externalizing, $B = -.0003$, $t = -2.89$, $p < .004$ for quadratic externalizing) which culminated in the flattest overall slopes for youth with the most extreme externalizing behaviors in childhood (See Figure 2). Contrary to the findings of the previous linear model where adding medication decreased the association between externalizing behavior and diurnal cortisol slope, including medication in the quadratic model had no significant effect on the abovementioned associations between externalizing problems and any aspect of the diurnal cortisol profile.

Similarly to the linear findings, there was no effect of the quadratic function of *Directionality* either concurrently or longitudinally.

Concurrent Associations between Cortisol and Behaviors when Examining Youth with the Most Extreme Behavior Problems

To determine if youth with the most internalizing problems in adolescence had distinct concurrent diurnal cortisol patterns, the linear and quadratic functions of internalizing were examined. Quadratic effects of internalizing problems were not significant and did not emerge after eliminating all non-significant effects, culminating in the linear model as described above (see Figure 3). Adding medication to the model did not significantly decrease the significance of the association between internalizing and cortisol.

To determine if youth with the most externalizing problems in adolescence had a distinct concurrent diurnal cortisol pattern, linear and quadratic functions of externalizing behavior were entered. A parsimonious model revealed a significant quadratic effect of externalizing on the linear cortisol slope ($B = 0.022$, $t = 2.83$, $p < .006$), quadratic cortisol slope ($B = -0.003$, $t = -2.79$, $p < .007$), and cubic cortisol slope ($B = 0.000$, $t = 2.92$, $p < .004$), such that youth with the most extreme behavior problems had a distinct, flat diurnal rhythm (See Figure 4). Controlling for medication usage eliminated the significant quadratic effect of externalizing behavior on the linear slope ($p < .12$) and decreased the effects on the quadratic and cubic cortisol slopes to trend levels ($p < .08$ and $p < .06$, respectively).

Conclusions & Discussion

For many years, research has suggested the existence of altered HPA activity in the presence of internalizing and externalizing behaviors, although the directions of the effects have been inconsistent and the developmental mechanisms that alter HPA axis activity remained elusive. The present investigation revealed systematic longitudinal and concurrent associations between HPA activity and problematic behaviors which may be associated with the development of later mental health problems. Even within the same sample, evidence for both hypo- and hyper-arousal of HPA axis functioning was found. Comorbidity of internalizing and externalizing behaviors is often present in youth as was the case in this sample; therefore, we examined whether the total number of behaviors in general, or specifically a preponderance of one type of behavior, influenced cortisol levels and the diurnal rhythm. The total number of behaviors was important, but closer inspection revealed that internalizing and externalizing behaviors predicted separate, but not necessarily opposing, components of the HPA axis. Internalizing behaviors were particularly influential for morning levels of cortisol whereas diurnal cortisol slope reflected the influence of externalizing behaviors. Interestingly, the stability and direction of these associations over time was different for internalizing behaviors depending on whether they had been present for an extended period of time or only appeared more recently. Furthermore, examination of the youth with the most behavior problems (internalizing, externalizing, or combined) revealed that these youth had distinct cortisol patterns, particularly the youth who had extreme levels of symptoms for a prolonged period of time.

For decades, research has linked internalizing behaviors and altered HPA functioning with varying success. The present study found that higher internalizing behaviors in children longitudinally predicted lower morning cortisol later when youth were adolescents. Yet, when internalizing and cortisol were measured concurrently in adolescence, an increased number of internalizing behaviors were expressed in adolescents with higher levels of morning cortisol. These seemingly opposing findings may be best understood through the theory proposed by Miller and colleagues (2007). Stress exposure may initially cause hypercortisolism, but after prolonged exposure to elevated levels of stress (and thus

persistently elevated cortisol levels), the HPA axis down-regulates, resulting in a blunting effect or hypocortisolism. In their meta-analysis, Miller and colleagues illustrated that the findings from cross-sectional studies initially appeared divergent. Yet, separating the studies by time since onset of stress revealed that the majority of the studies focusing on recent and ongoing stress typically displayed higher levels of cortisol in stressed individuals, whereas individuals whose stress exposure began several years beforehand typically displayed lower cortisol levels. Our findings are in line with this notion such that more recent internalizing behaviors were related to a higher level of morning cortisol. This pattern was particularly evident after controlling for previous behaviors, a statistical practice which would essentially exclude individuals who had developed mental health problems earlier in childhood. Conversely, higher levels of internalizing behaviors in childhood (controlling for the effects of current levels of internalizing behaviors in adolescence) were related to the development of lower cortisol levels approximately three years later when the children were adolescents.

These findings may fit into Miller and colleagues' theory of "down-regulation" through two possible psychobiological mechanisms. First, internalizing behaviors may, in and of themselves, be operating as a stressor. This may be evident especially for individuals who recently developed behaviors which may precede the onset of a subsequent disorder, events which are commonly included on subjective stressful life events checklists (Rudolph and Clark, 2001; Rudolph et al., 2006). The present study examined internalizing behaviors and not necessarily clinical diagnoses. Approximately 30% of our sample demonstrated clinical levels of behaviors, these youth and those within a more moderate range may interpret internalizing emotions as a stressor which may lead to the development of a variety of internalizing behaviors and subsequent dysregulated morning cortisol levels.

The second explanation is not mutually exclusive with the first. Individuals may experience an objective stressor which manages to dysregulate their HPA axis; then, this altered physiological state (combined with the stressful situation) induces feelings of depression, particularly if the stressor triggered mixed internalizing emotions (Rudolph et al., 2006). The result may include heightened internalizing behaviors and heightened HPA axis activity around the time of the stressor (Miller et al., 2007) or the first experience with heightened depression symptoms. Regardless of the original pathway, both environmental forces would be expected to lead to blunting of the HPA axis over time (Koob and Le Moal, 2001). In the present study, internalizing, and not externalizing, behaviors mapped onto the blunting theory. These findings may help refine the model so that it applies primarily to internalizing symptomology. Alternatively, it is possible that this same mechanism occurs (or had already occurred) in youth with externalizing problems, but our study did not capture it. One possible explanation for this, discussed below, may be due to the typical developmental period when externalizing behaviors first present.

The presence of high cortisol followed by blunting over time in individuals with internalizing behaviors may be specific to morning cortisol levels for several reasons. Individuals with environmental or personality characteristics that predispose them to developing depressive symptoms have been shown to possess high levels of morning cortisol (Ockenfels et al., 1995; Polk et al., 2005; Portella et al., 2005; Pruessner et al., 2003; Schulz et al., 1998; Steptoe et al., 2005). Cortisol levels are at their peak in the morning part of the diurnal rhythm, and thus, occupy the most cortisol receptors at this time point, especially mineralocorticoid (MR) receptors. If individuals with predispositions for developing internalizing problems (e.g. high levels of stress) produced an excessive amount of morning cortisol, it could chronically exceed the capacity of the MR receptors in particular, potentially straining glucocorticoid receptor binding as well. Over time, elevated morning cortisol in youth experiencing stress may no longer be able to sustain hyper-arousal

of the HPA axis. Our study provides some evidence that expression of internalizing behaviors leads to the development of more severe or persistent internalizing behaviors and the down-regulation of the HPA axis.

When examining the relation between cortisol *slope* and behavior, youth with more externalizing behaviors had flattened diurnal cortisol rhythms as adolescents, regardless of whether they had expressed these behaviors as children or as adolescents. While the finding appeared stable over time, the longitudinal extension from childhood appeared slightly stronger than the concurrent findings. Although certain studies have not found this association (Azar et al., 2004; Klimes-Dougan et al., 2001; McBurnett et al., 2005; van Bokhoven et al., 2005), our findings are consistent with the majority of the literature which suggests that older children and adolescents with externalizing behaviors display lower basal or diurnal HPA activity both concurrently (Kariyawasam et al., 1995; Pajer et al., 2001; Shirtcliff et al., 2005; Snoek et al., 2004) and longitudinally (McBurnett et al., 2000). The pattern of low morning cortisol levels in youth with high externalizing behaviors was also present in our data, though the association was not significant. Our findings are also consistent with two previous studies (Fairchild et al., 2008; Popma et al., 2007) which found high levels of externalizing behaviors to be associated with a flattened cortisol slope when this relation was examined longitudinally.

Initially, the model proposed by Miller and colleagues (2007) does not seem to apply to externalizing behaviors. However, it may be possible that blunting of cortisol over time is also applicable to youth with externalizing disorders (Susman, 2006). A meta-analysis by Alink and colleagues showed that externalizing problems are related to higher cortisol levels in very young children but not older children (Alink et al., 2008; Bakermans-Kranenburg et al., 2008; see also Kestler and Lewis, 2009). As alluded to above, externalizing behaviors often emerge very early in development (particularly compared to internalizing behaviors). Therefore, elevated levels of cortisol may only exist in children with externalizing problems when they are very young (Alink et al., 2008). This notion is supported by studies which have found an inverse or null association between cortisol and externalizing behaviors, depending on the stage of this psychobiological developmental process (Pérez-Edgar et al., 2008; Spinrad et al., 2009). In sum, it may be that children with externalizing behaviors experience blunting of the HPA axis earlier in development than those with internalizing behaviors due to the timing of behavior onset. Given that our study examined processes in mid- to late- childhood and early adolescence, we would not have been able to assess initial HPA hyper-arousal because we did not study children early enough to capture the developmental stage in which hyperarousal first emerged.

Again, multiple psychobiological mechanisms may be operating at this earlier developmental point. A temperamental predisposition to aggression may act as a stressor which produces initially elevated levels of cortisol very early in development and consequently results in later externalizing behavior problems which exposes a person to more and more stressors (Rudolph and Hammen, 1999; Rudolph et al., 2000). Alternatively, high levels of oppositional or defiant behaviors and activities may cause emotional distress due to the negative consequences of those behaviors. This emotional distress may initially produce elevated cortisol levels; however, further exposure to high levels of cortisol may then result in a blunted HPA system linked with the manifestation of externalizing behavior problems which is consistently seen in late childhood and throughout adolescence (Kariyawasam et al., 2002; McBurnett et al., 2000; Moss et al., 1995; Pajer et al., 2001; Shirtcliff et al., 2005). Excessive blunting of cortisol levels in externalizing youth, when developmentally evident for a longer period of time, may continue to exert its toll on the HPA axis. Further dysregulation may be demonstrated as lack of a typical diurnal decline over the course of the day. It is anticipated that this would especially be true for individuals

who had evidence of HPA axis dysregulation for a longer period of time across early development (Susman, 2006). Although it still needs to be empirically validated, this perspective would serve as a good model to explain the preponderance of low cortisol levels typically associated with externalizing behaviors expressed persistently across child development and into adolescence (Caspi et al., 1995; Moffitt, 1990).

Further examination of the youth with the most extreme levels of behavior problems revealed the existence of additional dysregulation of the HPA axis, such that the greatest dysregulation was present in youth who demonstrated the most extreme symptoms, particularly when they were younger. It must be noted that this is not simply an additive effect whereby youth who experience severe symptoms for an extended period of time demonstrate the same altered profile as youth who experience symptoms for a prolonged period of time, just to a greater degree. Rather, these youth demonstrate a distinct diurnal cortisol profile not demonstrated in the other participants with lower levels of symptoms. Youth with the most symptoms in childhood demonstrate discernible profiles in adolescence, identifiable by low morning levels, atypical diurnal curves throughout the day, and elevated evening levels. Such a profile seems to be a product of an HPA axis that is not able to self-regulate in order to produce healthy levels of cortisol at nearly any point in the day. While the additional dysregulation may be a function of excessive exposure to cortisol which can occur at any point in the lifespan, it is possible that HPA dysregulation is a function of exposure to greater levels of behavior problems in a developmentally time-sensitive period. Although a normal pattern of diurnal cortisol production is typically established within the first year of life (de Weerth et al., 2003), the HPA axis continues to develop into early childhood and may continue to be sensitive to external influences during the formative early years. Behaviors or stress during this period may have a substantially greater influence on the development of diurnal cortisol patterns thus producing dysregulation of the HPA axis in childhood not seen in later developmental time periods.

Secondary analyses revealed a significant effect of medication on the association between externalizing behavior and cortisol levels. Although the initial association between externalizing behaviors and a less steep slope remained at trend level for the longitudinal association after controlling for medication, this effect disappeared when examining the relation concurrently with medication in the model. This may be because stimulant medications used to control externalizing behaviors can sometimes increase cortisol levels (Hibel et al., 2007; Kariyawasam et al., 2002). Since children typically take this medication in the morning, by including the effect of medication in the model it may have resulted in more elevated morning levels of cortisol which would then produce a steeper slope. However, although taking medication may have been associated with a less steep cortisol slope when examining the larger sample, it had a limited association with the diurnal cortisol profiles of youth with more severe levels of behavior problems. Use of medication only reduced the association between concurrent quadratic externalizing behavior and linear diurnal cortisol slope in adolescence; however, it only reduced the quadratic and cubic slopes to a trend level. Furthermore, it had no significant effect on any longitudinal associations involving quadratic functions of behavior problems. This suggests that internalizing behaviors in both childhood and adolescence and extreme levels of both types of behavior problems in childhood have stronger influences on the diurnal cortisol profile in adolescence than the medications youth were taking in adolescence.

While this study provides an important starting point for future research to continue studying the possibility of blunting of the HPA axis in response to behavior problems, it is not without its limitations. The design employed in the current study could have included additional waves of assessment to be ideal. Furthermore, researchers who wish to examine this association should employ a design that that involves at least two time points, one

shortly after symptom onset and one after symptoms have been experienced for a prolonged period of time, at which both cortisol and behavior problems are measured. Although examining this association in a community sample is important, studying this phenomenon in a larger, more at-risk population may help clarify ambiguities.

Further research needs to be conducted in younger children to establish whether or not blunting occurs in response to externalizing behaviors or if it is specific to internalizing behaviors. Furthermore, research is needed to determine if blunting and additional dysregulation is due to cumulative exposure to symptoms regardless of developmental period or if it only occurs early on in development. Blunting of the HPA axis may have initially served as a protective mechanism for youth who would otherwise be subjected to chronically high cortisol levels; however, research suggests that atypical cortisol levels (e.g. too high or too low) are detrimental to the brain (Lupien et al., 2005). More research is required to determine the potential costs and/or benefits of hypo-regulation of the HPA axis (blunting) or hyper-regulation of the HPA axis as a function of behavior problems, and their potential effects on future physical and psychological health. In summary, this study helped disentangle the complex association between cortisol and internalizing and externalizing behaviors. More specifically, it complimented basic research findings by adding empirical support to the theoretical notion of blunting of the HPA axis over time in relation to internalizing behaviors and added new findings to support the notion of lowered HPA activity in youth with externalizing behavior.

Acknowledgments

This research was supported through funding by the Social Sciences and Humanities Research Council of Canada. The authors would like to thank Claude Senneville for his statistical expertise as well as the participants for their continued involvement.

References

- Achenbach, TM. Manual for the Child Behavior Checklist/4-18 and 1991 Profile. Burlington, VT: University of Vermont Department of Psychiatry; 1991a.
- Achenbach, TM. Manual for the Teacher's Report from and 1991 Profile. Burlington, VT: University of Vermont Department of Psychiatry; 1991b.
- Alink LR, van IJzendoorn MH, Bakermans-Kranenburg MJ, Mesman J, Juffer F, Koot HM. Cortisol and externalizing behavior in children and adolescents: mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Dev Psychobiol.* 2008; 50:427–450. [PubMed: 18551461]
- Angold A, Costello EJ, Erkanli A. Comorbidity. *J Child Psychol Psych.* 1999; 40:57–87.
- Ansary NS, Luther SS. Distress and academic achievement among adolescents of affluence: A study of externalizing and internalizing problem behaviors and school performance. *Dev Psychopathol.* 2009; 21:319–341. [PubMed: 19144236]
- Aunola K, Stattin H, Nurmi J. Adolescents' achievement strategies, school adjustment, and externalizing and internalizing problem behaviors. *J Youth Adolescence.* 2000; 29:289–306.
- Azar R, Zoccolillo M, Paquette D, Quiros E, Baltzer F, Tremblay RE. Cortisol levels and conduct disorder in adolescent mothers. *J Am Acad Child Adolesc Psychiatry.* 2004; 43:461–472. [PubMed: 15187806]
- Bakermans-Kranenburg MJ, van IJzendoorn MH, Mesman J, Alink LR, Juffer F. Effects of an attachment-based intervention on daily cortisol moderated by dopamine receptor D4: a randomized control trial on 1- to 3-year-olds screened for externalizing behavior. *Dev Psychopathol.* 2008; 20:805–820. [PubMed: 18606032]
- Board F, Persky H, Hamburg DA. Psychological stress and endocrine functions. *Psychosom Med.* 1956; 18:324–333. [PubMed: 13350458]

- Boyce WT, Ellis BJ. Biological sensitivity to context. I. An evolutionary- developmental theory of the origins and functions of stress reactivity. *Dev Psychopathol.* 2005; 17:271–301. [PubMed: 16761546]
- Boyce WT, Essex MJ, Woodward HR, Measelle JR, Ablow JC, Kupfer DJ. The confluence of mental, physical, social, and academic difficulties in middle childhood. I. Exploring the “head waters” of early life morbidities. *J Am Acad Child Psych.* 2002; 41:580–587.
- Bryk, AS.; Raudenbush, SW. *Hierarchical Linear Models: Applications and Data Analysis Methods.* Sage Publications; Thousand Oaks, CA: 1992.
- Caspi A, Henry B, McGee RO, Moffitt TE, Silva PA. Temperamental origins of child and adolescent behavior problems: From age three to fifteen. *Child Dev.* 1995; 66:55–68. [PubMed: 7497829]
- Cicchetti D, Rogosch FA. The impact of child maltreatment and psychopathology on neuroendocrine functioning. *Dev Psychopathol.* 2001; 13:783–804. [PubMed: 11771908]
- De Bellis MD, Dahl R, Perel J, Birmaher R. Nocturnal ACTH, cortisol, growth hormone, and prolactin secretion in prepubertal depression. *J Am Acad Child Psych.* 1996; 35:1130–1138.
- de Weerth C, Zijl RH, Buitelaar JK. Development of cortisol circadian rhythm in infancy. *Early Hum Dev.* 2003; 73:39–52. [PubMed: 12932892]
- Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychol Bull.* 2004; 130:355–391. [PubMed: 15122924]
- Engels JM, Diehr P. Imputation of missing longitudinal data: a comparison of methods. *J Clin Epidemiol.* 2003; 56:968–976. [PubMed: 14568628]
- Essex MJ, Klein MH, Cho E, Kraemer HC. Exposure to maternal depression and marital conflict: Gender differences in children’s later mental health symptoms. *J Am Acad Child Psych.* 2003; 42:728–737.
- Essex MJ, Kraemer HC, Armstrong JM, Boyce WT, Goldsmith HH, Klein MH, Woodward H, Kupfer DJ. Exploring risk factors for the emergence of children’s mental health problems. *Arch Gen Psychiat.* 2006; 63:1246–1256. [PubMed: 17088505]
- Fairchild G, Van Goozen SH, Stollery SJ, Brown J, Gardiner J, Herbert J, Goodyer IM. Cortisol diurnal rhythm and stress reactivity in male adolescents with early-onset or adolescence-onset conduct disorder. *Biol Psychiat.* 2008; 64:599–606. [PubMed: 18620338]
- Fries E, Hesse J, Hellhammer J, Hellhammer DH. A new view on hypocortisolism. *Psychoneuroendocrino.* 2005; 30:1010–1016.
- Gerra G, Zaimovic A, Avanzini P, Chittolini B, Giucastro G, Caccavari R, Palladino M, Maestri D, Monica C, Delsignore R, Brambilla F. Neurotransmitter-neuroendocrine responses to experimentally induced aggression in humans: influence of personality variable. *Psychiatry Res.* 1997; 66:33–44. [PubMed: 9061802]
- Gold PW, Goodwin FK, Chrousos GP. Clinical and biochemical manifestations of depression in relation to the neurobiology of stress. *New Engl J of Med.* 1988; 319:413–420. [PubMed: 3041279]
- Goodyer IM, Hebert J, Altham PM, Pearson J, Secher SM, Shiers HM. Adrenal secretion during major depression in 8- to 16-year-olds. I. Altered diurnal rhythms in salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. *Psychol Med.* 1996; 26:245–256. [PubMed: 8685281]
- Goodyer IM, Hebert J, Tamplin A, Altham PM. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *Brit J Psychiat.* 2000; 177:499–504.
- Goodyer IM, Park RJ, Herbert J. Psychosocial and endocrine features of chronic first-episode major depression in 8–16 year olds. *Biol Psychiat.* 2001; 50:351–357. [PubMed: 11543738]
- Granger DA, Serbin LA, Schwartzman AE, Lehoux PM, Cooperman JM, Ikeda S. Children’s salivary cortisol, internalizing behavior problems, and family environment: Results from the Concordia Longitudinal Risk Project. *Int J Behav Dev.* 1998; 23:707–728.
- Greaves-Lord K, Ferdinand RF, Oldehinkel AJ, Sondeijker FEPL, Ormel J, Verhulst FC. Higher cortisol awakening response in young adolescents with persistent anxiety problems. *Acta Psychiatr Scand.* 2007; 116:137–144. [PubMed: 17650276]

- Halligan SL, Herbert J, Goodyer I, Murray L. Disturbances in morning cortisol secretion in association with maternal postnatal depression predict subsequent depressive symptoms in adolescents. *Biol Psychiat*. 2007; 62:40–46. [PubMed: 17188253]
- Harris TO, Borsanyi S, Messari S, Stanford K, Cleary SE, Shiers HM, Brown GW, Hebert J. Morning cortisol secretion as a risk factor for subsequent major depression in adult women. *Brit J Psychiat*. 2000; 177:505–510.
- Hibel LC, Granger DA, Cicchetti D, Rogosch F. Salivary biomarker levels and diurnal variation: Associations with medications prescribed to control children's problem behavior. *Child Dev*. 2007; 78:927–937. [PubMed: 17517013]
- Hymel S, Rubin KH, Rowden L, LeMare L. Children's peer relationships: Longitudinal prediction of internalizing and externalizing problems from middle to late childhood. *Child Dev*. 1990; 61:2004–2021.
- Ice GH, Katz-Stein A, Himes J, Kane RL. Diurnal cycles of salivary cortisol secretion in older adults. *Psychoneuroendocrino*. 2004; 29:355–370.
- Kariyawasam SH, Zaw F, Handley SL. Reduced salivary cortisol in children with comorbid attention deficit hyperactivity disorder and oppositional defiant disorder. *Neuroendocrinol Lett*. 2002; 23:45–48. [PubMed: 11880861]
- Kaufman J. Depressive disorders in maltreated children. *J Am Acad Child Psych*. 1991; 30:257–265.
- Kestler LP, Lewis M. Cortisol response to inoculation in 4-year-old children. *Psychoneuroendocrino*. 2009; 34:743–751.
- Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrino*. 1994; 19:313–333.
- Klimes-Dougan B, Hastings PD, Granger DA, Usher BA, Zahn-Waxler C. Adrenocortical activity in at-risk and normally developing adolescents: Individual differences in salivary cortisol basal levels, diurnal variation, and responses to challenges. *Dev Psychopathol*. 2001; 13:695–719. [PubMed: 11523855]
- Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharm*. 2001; 24:97–129.
- Kraemer HC, Measelle JR, Ablow JC, Essex MJ, Boyce WT, Kupfer DJ. A new approach to integrating data from multiple informants in psychiatric assessment and research: Mixing and matching context and perspectives. *Am J Psychiat*. 2003; 160:1566–1577. [PubMed: 12944328]
- Kroes M, Kalff AC, Steyaert J, Kessels AG, Feron FJ, Hendeiksen JGM, van Zeben TMC, Troost J, Jolles J, Vles JSH. A longitudinal community study: Do psychosocial risk factors and Child Behavior Checklist Scores at 5 years of age predict psychiatric diagnoses at a later age? *J Am Acad Child Psych*. 2002; 41:955–963.
- Ledingham JE. Developmental patterns of aggressive and withdrawn behaviour in childhood: A possible method for identifying preschizophrenics. *J Abnorm Child Psych*. 1981; 9:1–22.
- Llabre MM, Spitzer SB, Saab PG, Ironson GH, Schneiderman N. The reliability and specificity of delta versus residualized change as measures of cardiovascular reactivity to behavioral challenges. *Psychophysiology*. 1991; 28:701–711. [PubMed: 1816598]
- Lupien SJ, Fiocco A, Wan N, Maheu F, Lord C, Schramek T, Tu MT. Stress hormones and human memory function across the lifespan. *Psychoneuroendocrino*. 2005; 30:225–242.
- McBurnett KM, Lahey BB, Rathouz PJ, Loeber R. Low salivary cortisol and persistent aggression in boys referred for disruptive behaviour. *Arch Gen Psychiat*. 2000; 57:38–43. [PubMed: 10632231]
- McBurnett K, Raine A, Stouthamer-Loeber M, Loeber R, Kumar AM, Kumar M, Lahey BB. Mood and hormone responses to psychological challenge in adolescent males with conduct problems. *Biol Psychiatry*. 2005; 57:1109–1116. [PubMed: 15866550]
- McEwen BS. Allostasis and allostatic load: Implications for neuropsychopharmacology. *Neuropsychopharmacology*. 2000; 22:108–124. [PubMed: 10649824]
- Mesman J, Koot HM. Early preschool predictors of preadolescent internalizing and externalizing DSM-IV diagnoses. *J Am Acad Child Psych*. 2001; 40:1029–1036.
- Miller GE, Chen E, Zhou E. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenal axis in humans. *Psychol Bull*. 2007; 133:25–45. [PubMed: 17201569]

- Moffitt TE. Juvenile delinquency and attention deficit disorder: Boys' developmental trajectories from age 3 to 15. *Child Dev.* 1990; 61:893–910. [PubMed: 2364762]
- Monroe, SM.; Hadjiyannakis, K. The social environment and depression: Focusing on severe life stress. In: Gotlib, H.; Hammen, C., editors. *Handbook of Depression*. Guilford Press; London: 2002. p. 314–340.
- Moss HB, Vanyukov MM, Martin CS. Salivary cortisol responses and the risk for substance abuse in prepubertal boys. *Biol Psychiat.* 1995; 38:547–555. [PubMed: 8562667]
- Ockenfels MC, Porter L, Smyth J, Kirschbaum C, Hellhammer DH, Stone AA. Effect of chronic stress associated with unemployment on salivary cortisol: Overall cortisol levels, diurnal rhythm, and acute stress reactivity. *Psychosom Med.* 1995; 57:460–467. [PubMed: 8552737]
- Pajer K, Gardner W, Rubin RT, Perel J, Neal S. Decreased levels of cortisol in adolescent girls with conduct disorder. *Arch Gen Psychiat.* 2001; 58:297–302. [PubMed: 11231837]
- Pederson S, Vitaro F, Barker ED, Borge AIH. The timing of middle-childhood peer rejection and friendship: Linking early behavior to early-adolescent adjustment. *Child Dev.* 2007; 78:1037–1051. [PubMed: 17650124]
- Peeters F, Nicolson NA, Berkof J. Levels and variability of daily life cortisol secretion in major depression. *Psychiat Res.* 2004; 126:1–13.
- Perez-Edgar K, Schmidt LA, Henderson HA, Schulkin J, Fox NA. Salivary cortisol levels and infant temperament shape developmental trajectories in boys at risk for behavioral maladjustment. *Psychoneuroendocrino.* 2008; 33:916–925.
- Petty CR, Rosenbaum JF, Hirshfeld-Becker DR, Henin A, Hubley S, LaCasse S, Faraone SV, Biederman J. The Child Behavior Checklist broad-band scales predict subsequent psychopathology: A five-year follow-up. *J Anxiety Disord.* 2008; 22:532–539. [PubMed: 17521868]
- Polk DE, Cohen S, Doyle WJ, Skoner DP, Kirschbaum C. State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrino.* 2005; 30:261–272.
- Popma A, Doreleijers TAH, Jansen LMC, Van Goozen SHM, Van Engeland H, Vermeiren R. The diurnal cortisol cycle in delinquent male adolescents and normal controls. *Neuropsychopharmacology.* 2007; 32:1622–1628. [PubMed: 17228341]
- Portella MJ, Harmer CJ, Flint J, Cowen P, Goodwin GM. Enhanced early morning salivary cortisol in neuroticism. *Am J Psychiat.* 2005; 162:807–809. [PubMed: 15800161]
- Posener JA, DeBattista C, Veldhuis JD, Province MA, Williams GH, Schatzberg AF. Process irregularity of cortisol secretion in men with major depressive disorder. *Psychoneuroendocrino.* 2004; 29:1129–1137.
- Pruessner M, Hellhammer DH, Pruessner JC, Lupien SJ. Self-reported depressive symptoms and stress levels in healthy young men: Associations with the cortisol response to awakening. *Psychosom Med.* 2003; 65:92–99. [PubMed: 12554820]
- Richmond MK, Stocker CM. Associations between family cohesion and adolescent siblings' externalizing behavior. *J of Fam Psychol.* 2006; 20:663–669. [PubMed: 17176202]
- Rudolph KD, Clark AG. Conceptions of relationships in children with depressive and aggressive symptoms: Social-cognitive distortion or reality? *J Abnorm Child Psych.* 2001; 29:41–56.
- Rudolph, KD.; Hammen, C.; Daley, SE. Mood disorders. In: Wolfe, DA.; Mash, EJ., editors. *Behavioral and Emotional Disorders in Adolescents: Nature, Assessment, and Treatment*. Guilford; New York: 2006. p. 300–342.
- Sachar EJ, Hellman L, Roffwarg HP, Halpern FS, Fukunishima DK, Gallagher TF. Disrupted 24-hour patterns of cortisol secretion in psychotic depression. *Arch Gen Psychiat.* 1973; 28:19–24. [PubMed: 4683141]
- Sapolsky, RM. Neuroendocrinology of the stress response. In: Becker, JB.; Breedlove, M.; Crews, D., editors. *Behavioral Endocrinology*. The MIT Press; Cambridge, M.A: 1992. p. 287–324.
- Sapolsky RM. Neurochemistry: Taming stress. *Sci Am.* 2003; 289:88–98.
- Schreiber JE, Shirtcliff E, Van Hulle C, Lemery-Chalfant K, Klein MH, Kalin NH, Essex MJ, Goldsmith HH. Environmental influences on family similarity in afternoon cortisol levels: Twin and parent-offspring designs. *Psychoneuroendocrino.* 2006; 31:1131–1137.

- Schulz P, Kirschbaum C, Prüsner J, Hellhammer DH. Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress Medicine*. 1998; 14(2):91–97.
- Schwartzman AE, Ledingham J, Serbin LA. Identification of children at risk for adult schizophrenia: A longitudinal study. *Int J Appl Psychol*. 1985; 34:363–380.
- Shirtcliff EA, Essex MJ. Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Dev Psychobiol*. 2008; 50:690–703. [PubMed: 18726897]
- Shirtcliff EA, Granger DA, Booth A, Johnson D. Low salivary cortisol levels and externalizing behaviour problems in youth. *Dev Psychopathol*. 2005; 17:167–184. [PubMed: 15971765]
- Snoek H, Van Goozen SHM, Matthys W, Buitelaar JK, Van Engeland H. Stress responsivity in children with externalizing behavior disorders. *Dev Psychopathol*. 2004; 16:389–406. [PubMed: 15487602]
- Smider NA, Essex MJ, Kalin NH, Buss KA, Klein MH, Davidson RJ, Goldsmith HH. Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: A prospective study. *Child Dev*. 2002; 73:75–92. [PubMed: 14717245]
- Smyth JM, Ockenfels MC, Gorin AA, Catley D, Porter LS, Kirschbaum C, Hellhammer DH, Stone AA. Individual differences in the diurnal cycle of cortisol. *Psychoneuroendocrino*. 1997; 22:89–105.
- Spinrad TL, Eisenberg N, Granger DA, Eggum ND, Sallquist J, Haugen RG, Kupfer A, Hofer C. Individual differences in preschoolers' salivary cortisol and alpha-amylase reactivity: Relations to temperament and maladjustment. *Horm Beh*. 2009; 56:133–139.
- Steptoe A, Brydon L, Kunz-Ebrecht S. Changes in financial strain over three years, ambulatory blood pressure, and cortisol responses to awakening. *Psychosom Med*. 2005; 67:281–287. [PubMed: 15784795]
- Stetler C, Dickerson SS, Miller GE. Uncoupling of social zeitgebers and diurnal cortisol secretion in clinical depression. *Psychoneuroendocrino*. 2004; 29:1250–1259.
- Susman EJ. The relation of relative hormonal levels and physical development and social-emotional behavior in young adolescents. *J Youth Adolescence*. 1985; 14:245–264.
- Susman EJ. Psychobiology of persistent antisocial behavior: Stress, early vulnerabilities and the attenuation hypothesis. *Neurosci Biobehav Rev*. 2006; 30:376–389. [PubMed: 16239030]
- van Bokhoven I, Van Goozen SHM, van Engeland H, Schaal B, Arseneault L, Séguin JR, Nagin DS, Vitaro F, Tremblay RE. Salivary cortisol and aggression in a population-based longitudinal study of adolescent males. *J Neural Transm*. 2005; 112:1083–1096. [PubMed: 15583952]
- van Praag, HM.; de Kloet, R.; van Os, J. *Stress, the Brain, and Depression*. Cambridge University Press; Cambridge, UK: 2004.

Figure 1. Longitudinal association between level of internalizing behaviors in childhood and diurnal cortisol in early adolescence.

Figure 2.
Longitudinal association between level of externalizing behaviors in childhood and diurnal cortisol in early adolescence.

Figure 3.
Concurrent association between level of internalizing behaviors and diurnal cortisol in early adolescence.

Figure 4.
Concurrent association between level of externalizing behaviors and diurnal cortisol in early adolescence.

Table 1

Percentage of Variability Accounted for by Factors

Factor	Shared informant variability	Informant bias variability
Childhood Internalizing	53.8	39.0
Childhood Externalizing	59.5	36.3
Adolescence Internalizing	53.9	38.2
Adolescence Externalizing	62.1	33.5

Table 2

Descriptive Statistics of Level 1 Predictors

Predictor	<i>B</i>	<i>t</i>	<i>p</i>
TSW	−0.086	−22.63	<0.001
TSW ²	−0.065	2.37	0.02
TSW ³	−.00	−1.93	0.05
CAR	0.111	4.59	<0.001
Mood	0.002	0.12	.902
Stress	−0.002	−0.22	.828
Health	−0.004	−0.38	.703
Food	0.008	0.23	.854
Exercise	0.005	0.19	.892