



SHORT COMMUNICATION

Neuroendocrine predictors of emotional and behavioral adjustment in boys: Longitudinal follow-up of a community sample

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Summary

Background: Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis has been observed in adults and children with mood and anxiety disorders and is thought to be involved in the pathogenesis of these disorders. We recently studied a diverse community sample of boys and found associations of behavioral problems, including symptoms of depression and anxiety, with basal and stress-induced cortisol concentrations. Here we examine cortisol–emotional/behavioral associations at a two-year follow-up and test whether initial cortisol is predictive of worsening emotional/behavioral problems two years later.

Method: Seventy-eight 10–14-year-old boys and their mothers completed a battery of psychosocial assessments, provided morning and afternoon saliva samples, and participated in a home visit involving mildly stressful tasks and saliva collection for cortisol assay during a two-year follow-up assessment.

Results: Consistent with the findings from our time 1 assessment, greater declines in cortisol across the home-visit challenge task were significantly associated with internalizing and externalizing behaviors as well as attention problems and social problems at the two-year follow-up. In

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addition, morning and afternoon cortisol concentrations at the initial assessment were significant positive predictors of the later development of child depressive symptoms at follow-up after controlling for initial depressive symptoms.

Conclusion: These findings demonstrate that children in the community with internalizing and externalizing behavior problems have altered patterns of HPA axis stress reactivity. In addition, our prospective findings suggest that elevated cortisol concentrations may influence the later development of emotional/behavioral problems in boys.

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1. Introduction

Excessive activation of the hypothalamic–pituitary–adrenal (HPA) axis is seen in a substantial proportion of adults and children with depressive and anxiety disorders (Guerry and Hastings, 2011; Pervanidou, 2008). Youth with subsyndromal symptoms of depression and anxiety (internalizing symptoms) also show increased basal and provoked cortisol concentrations in several studies, whereas externalizing behaviors have been linked to blunted cortisol concentrations and responses in some reports. Other studies have documented elevated cortisol concentrations with externalizing behaviors, possibly due to the co-occurrence of internalizing and externalizing behaviors (Tyrka et al., 2010).

HPA axis dysfunction may lead to behavioral symptoms because glucocorticoid receptors (GRs), are widely distributed in limbic brain regions involved in the regulation of stress responses, cognition, affect, and behavior. Animal studies show that excessive or prolonged activation of GRs results in neurostructural changes in these regions (McEwen, 2007). Therefore, longitudinal studies of the association between cortisol activity and the later development of psychopathology are of great interest. Statistically controlling for the contemporaneous association between cortisol and emotional/behavioral problems at the initial assessment is necessary to show that cortisol is linked to the *later* development of emotional/behavioral problems.

Only a few prior investigations have conducted such prospective analyses, but the findings are suggestive of an effect of cortisol on the later development of emotional/behavior problems. In a study of 4.5-year-old boys, higher afternoon cortisol predicted the development of greater internalizing behaviors and social wariness in Kindergarten, after controlling for initial behavior (Smider et al., 2002). Elevated morning saliva cortisol in a sample of 13-year-olds predicted depressive symptoms at age 16, after controlling for initial depressive symptoms (Halligan et al., 2007). In a 6-month follow-up study of 7–17-year-old clinic-referred children, cortisol reactivity to a parent–child conflict discussion task was inversely predictive of later internalizing and externalizing behaviors, but positively predictive of anxiety disorders (Granger et al., 1996). Finally, in adolescents at-risk for psychopathology, high morning cortisol predicted onset of a major depressive episode in the following 12 months (Goodyer et al., 2000).

While most prior work on this topic has involved white, middle-class samples of preschool or adolescent-aged boys and girls, we recently found, in a study of boys, aged 8–11 from diverse backgrounds, that internalizing problems were linked to higher basal afternoon cortisol concentrations (Tyrka et al., 2010). Additionally, a greater decline in cortisol

concentrations across a home-visit challenge task was seen in boys with internalizing behaviors and social problems, as well as attention and thought problems. Here we report a two-year follow-up assessment in this sample to determine whether these associations persist. In addition, we hypothesized a prospective relationship between initial cortisol concentrations and later increases in emotional/behavioral problems at the two-year follow-up.

2. Methods

2.1. Participants

Boys aged 8–11 ($M = 9.10$, $SD = 0.73$) were recruited from several New York City area public schools and followed two years later (range = 1.5–2.5 years) when they were aged 10–14 ($M = 12.3$, $SD = 0.72$). One hundred two of these participants provided data for analyses of the association between cortisol measures and emotion/behavior at the time 1 assessment and were thus included in our initial report on this topic (Tyrka et al., 2010). The current study includes seventy-eight boys with follow-up cortisol and emotional/behavioral measures, including 61 who were included in our initial report (Tyrka et al., 2010), and an additional 17 who participated in the study initially but did not have complete cortisol and emotional/behavioral data at the initial assessment for inclusion in the prior report. The prospective analyses included 69 of these boys who had both the follow-up cortisol and emotional/behavioral measures as well as the initial emotional/behavioral measure that was controlled in the analysis.

The follow-up sample did not differ from the initial cohort at time 1 (T_1) on the demographic measures (Table 1). Those who did not participate at follow-up had higher self-reported depressive symptoms at T_1 ($p < .05$), but did not differ on any of the other emotional/behavioral measures.

2.2. Procedures

Methodological details are described in Tyrka et al. (2010). Briefly, boys and their mothers participated in a home visit stress paradigm involving four challenging tasks with saliva sampling at baseline and after each task. These visits took place between 4:00 and 8:00 PM ($M = 5:32$ PM, $SD = 1:16$). In addition, mothers and boys completed questionnaires and collected saliva samples at home on two consecutive days: a morning saliva sample immediately upon awakening and before brushing teeth or consuming anything, and an afternoon sample upon return from school. Cortisol concentrations from the two sampling days were averaged for the AM and the

Table 1 Demographics T_1 and follow-up.

Demographics (range)	Initial M (SD)	Follow-up M (SD)
Age (8–11 initial, 10–14 follow up)	9.13 (0.74)	12.28 (0.69)
SES: Combined Family Hollingshead (8–66 T_1 , 4–63 follow up)	36.50 (13.89)	30.12 (16.86)
Puberty: Tanner stage	One: 77 (77%) Two: 19 (18.6%)	One: 4 (4.6%) Two: 21 (24.1%) Three: 31 (35.6%) Four: 28 (32.2%) Five: 3 (3.4%)
Race	Black: 46 (45%) White: 33 (32%) Hispanic: 23 (23%)	White: 32 (41%) Black: 29 (37.2%) Hispanic: 17 (21.8%)

PM samples. Procedures used at T_1 and the follow-up assessment were identical except for the use of an age-appropriate cognitive task during the home visit: Object Assembly task from the WISC (Wechsler, 1991) at T_1 , and the Mazes task (Wechsler, 1974) at follow-up. In addition, while salivettes were used to collect saliva during home visits, boys provided free spit using a straw at follow-up for morning and afternoon samples. Cortisol values using these two methods are nearly identical (Sarstedt, 2007). Procedures were approved by the IRB of Teachers College at Columbia University.

2.2.1. Cortisol assay

Saliva cortisol (Table 2) was assayed in duplicate using radioimmunoassay procedures outlined by Kirschbaum and colleagues (Diagnostic Products Company), with a lower detection limit of 0.02 $\mu\text{g}/\text{dl}$, and intra- and inter-assay coefficients of variation less than 5% and 3%, respectively.

2.3. Measures

2.3.1. Child report of depressive and anxiety symptoms

Boys completed the Children's Depression Inventory (CDI; Kovacs, 1985) ($\alpha = .81$ and $\alpha = .85$ for T_1 and follow-up assessments, respectively) and the anxiety subscale of the Hopkins Symptom Checklist (HSCL; Rickels et al., 1976) ($\alpha = .79$ and $\alpha = .90$).

2.3.2. Parent report of behavior problems

To assess behavior problems, we used the internalizing ($\alpha = .84$ and $\alpha = .88$ at T_1 and follow-up assessments, respectively) and externalizing ($\alpha = .88$ and $\alpha = .87$) subscales of the widely used parent-report Child Behavior Checklist (CBCL; Achenbach, 2001). We also included the CBCL subscales social problems, thought problems, and attention problems

Table 2 Behavioral measures and cortisol at time 1 and follow-up.

	Initial	Follow-up
Behavioral measures		
<i>Parent report (t-scores range)</i>		
CBCL internalizing symptoms	37.6–76.7	39.3–79.2
CBCL externalizing symptoms	38.6–77.9	39.4–78.0
CBCL social problems	40.4–77.1	41.5–79.3
CBCL thought problems	41.8–81.5	43.1–79.5
CBCL attention problems	39.2–77.1	39.3–78.1
<i>Child report (M, SD)</i>		
Child Depression Inventory (CDI) (0–52)	5.7 (4.6)	6.2 (5.5)
HSCL anxiety subscale (1–5)	1.5 (0.6)	1.4 (0.5)
Cortisol concentrations (M, SD)		
AM cortisol ($\mu\text{g}/\text{dl}$)	0.413 (0.278)	0.358 (0.191)
PM cortisol ($\mu\text{g}/\text{dl}$)	0.103 (0.101)	0.118 (0.097)
Home visit sample 1 ($\mu\text{g}/\text{dl}$)	0.092 (0.074)	0.115 (0.105)
Home visit sample 2 ($\mu\text{g}/\text{dl}$)	0.060 (0.037)	0.084 (0.062)
Home visit sample 3 ($\mu\text{g}/\text{dl}$)	0.059 (0.045)	0.076 (0.061)
Home visit sample 4 ($\mu\text{g}/\text{dl}$)	0.055 (0.044)	0.066 (0.050)
Home visit sample 5 ($\mu\text{g}/\text{dl}$)	0.062 (0.074)	0.060 (0.046)
Home visit Δscore ($\mu\text{g}/\text{dl}$) (HV 1–HV 5)	0.030 (0.061)	0.050 (0.058)

Note: CBCL is the Child Behavior Checklist; HSCL refers to the Hopkins Symptom Checklist.

($\alpha = .70$, $.65$, and $.79$ at T_1 , and $\alpha = .66$, $.53$, and $.79$, at the follow-up assessment, respectively).

2.4. Statistical analyses

Data were analyzed using SPSS for Mac version 19, all tests were two-tailed, and α was set to $.05$. For cortisol measures, mean morning and afternoon cortisol concentrations were computed from samples collected on two consecutive days. Outliers, three standard deviations from the mean, were identified and Winsorized to equal the next highest value. For home visit cortisol, at both assessments the first saliva sample yielded the highest cortisol concentration, suggesting that anticipatory adrenocortical activation and degree of subsequent decline in activation was indicative of HPA reactivity in this context. Therefore, a home visit change score was calculated by subtracting the value of the last cortisol sample from the first cortisol sample.

Time of sample was controlled in all analyses to account for the diurnal variation in cortisol concentrations. Correlation coefficients and analysis of variance were computed to determine whether any of the emotional/behavioral measures were correlated with the following potential covariates: age, race (Black, Hispanic and White non-Hispanic), socioeconomic status (SES, assessed with the Combined Family Hollingshead Score) and pubertal development. For the prospective analyses, these tests assessed associations between the potential covariates reported at T_1 with emotional/behavioral measures at follow-up. In both concurrent and prospective analyses, SES was identified as a significant covariate for the CBCL externalizing scale. In addition, Tanner stage was correlated with CBCL social problems in the concurrent analysis only. These demographic variables were included as covariates in the relevant analyses. Finally, given the mixed findings for externalizing behaviors in prior studies and that internalizing and externalizing behaviors co-vary ($r = .57$ and $r = .59$, at T_1 and follow-up assessments, respectively), we controlled for internalizing scores in any analyses with significant findings for externalizing behaviors.

Partial correlations, controlling for time of cortisol sample and significant covariates, tested for associations between the follow-up cortisol and emotional/behavioral measures. For the prospective hypotheses, general linear models were used to test for associations between T_1 cortisol measures and the follow-up emotional/behavioral measures, controlling for T_1 time of sample, the T_1 emotional/behavioral measure, and any other significant covariates.

3. Results

3.1. Concurrent analyses of cortisol and emotional/behavioral measures at follow-up

3.1.1. Home visit cortisol change score

The follow-up home visit cortisol change score was significantly associated with CBCL internalizing behaviors ($r(72) = .29$, $p = .011$), externalizing behaviors ($r(71) = .31$, $p = .007$), social problems ($r(72) = .32$, $p = .006$), and attention problems ($r(72) = .26$, $p = .027$), and there was a trend for thought problems ($r(72) = .20$, $p = .09$). For each of these subscales, higher levels of behavior problems were associated

with greater declines in saliva cortisol during the home visit. The cortisol change score was not significantly correlated with anxiety symptoms or CDI score. Externalizing behaviors were no longer significant after controlling for internalizing behaviors ($r(70) = .18$, $p = .14$).

3.1.2. Morning and afternoon cortisol

Partial correlations testing for associations of behavior and symptoms with morning or afternoon cortisol concentrations at the follow-up were not significant. There was a trend-level negative association between externalizing behaviors and morning cortisol concentrations ($r(73) = -.21$, $p = .07$), which remained after controlling for internalizing behaviors ($r(72) = -.26$, $p = .052$).

3.2. Prospective analysis of T_1 cortisol measures and behavior/emotion at follow-up

3.2.1. Home visit cortisol change score

There were no significant effects of T_1 home visit cortisol on the later development of emotional/behavioral problems. There were trend level effects of T_1 cortisol change score on follow-up anxiety symptoms ($F(1, 57) = 3.9$, $p = .053$) and thought problems ($F(1, 63) = 3.6$, $p = .061$), after controlling for T_1 symptoms and time of sample.

3.2.2. Morning and afternoon cortisol

Both T_1 morning cortisol and afternoon cortisol were positive predictors of CDI score at follow-up, controlling for T_1 CDI score and time of sample [$F(1, 56) = 5.2$, $p = .027$, and $F(1, 58) = 4.2$, $p = .045$, respectively]. None of the other emotional/behavioral measures was predicted by T_1 morning or afternoon cortisol concentrations.

4. Discussion

Our concurrent findings of associations between change in home visit cortisol and internalizing, externalizing, social, and attention problems are consistent with our prior findings for internalizing and social problems when boys were 8–11 years of age. Our prospective analyses showed that both T_1 morning and afternoon cortisol concentrations predicted later depressive symptoms (and greater declines in home visit cortisol at T_1 predicted the development of thought problems and anxiety at trend level). These findings extend previous work on this topic to a diverse community sample of young adolescent boys. Taken together, the findings raise the possibility that elevated cortisol concentrations may lead to changes in brain regions implicated in mood, anxiety, and cognition.

Although basal cortisol concentrations predicted an increase in CDI score (and we previously found concurrent associations between basal cortisol and behavior at T_1 , Tyrka et al., 2010), we did not find concurrent associations at follow-up between behavior and morning or afternoon basal cortisol concentrations, possibly due to power limitations. The present sample is limited by the modest size due to attrition as well as missing data for some who did participate in the follow-up assessment. Although our follow-up sample did not differ from those who did not continue with respect to most emotional/behavioral measures or the demographic characteristics, they did have lower self-reported depressive

symptoms on the CDI at T_1 and this may have influenced our findings.

It is important to note that we did not assess effects of adverse experiences which are linked to altered adrenocortical responding as well as behavioral problems (Cicchetti et al., 2010). We also acknowledge that the home visit cortisol measure did not reflect a rise in cortisol, but rather different degrees of decline across the home visit, so that it remains unclear whether these findings would be replicated with a classic stress-induced cortisol response. In addition, this is a non-clinical community sample with emotional/behavioral problems and cortisol concentrations in the normative range. Only a few participants were above the clinical threshold for depressive symptoms on the CDI, however, several participants met the clinical thresholds for internalizing and externalizing at the initial assessment ($n = 10$ and 10 , respectively) and at follow-up ($n = 16$ and 13 , respectively). The findings for internalizing and externalizing remained unchanged using this categorical variable in logistic regression models (data not shown). Whether these findings reflect processes related to clinical conditions remains to be determined, but it should be noted that the literature on cortisol in children and youth with major depression has been inconsistent (Guerry and Hastings, 2011).

In conclusion, our results confirm and extend findings from the few existing studies on the prospective link between cortisol levels and the later development of internalizing problems in youth. Given the literature on the effects of glucocorticoids on brain regions implicated in cognition, mood, and anxiety, our findings are consistent with the possibility that elevated cortisol concentrations could be causally implicated in the development of these symptoms.

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Conflict of interest

None declared.

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