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Examining Child Sex as a Moderator of the Relationship Between Cortisol Reactivity and Symptoms Over Time

Andrew R. Daoust
The University of Western Ontario

Supervisor
Hayden, Elizabeth P.
The University of Western Ontario

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Abstract

Females' reactivity to stress appears to be closely tied to internalizing symptoms, while males who are under-reactive may be at risk for externalizing problems. Little is known about when such differences emerge, despite possible implications for early prevention. Cortisol reactivity to a laboratory stressor was assessed in 409 three-year-old children along with children's parent-reported internalizing and externalizing symptoms, which were re-collected at child ages 5 and 8. Multilevel modelling was used to investigate whether the relationship between cortisol reactivity and symptoms differed between boys and girls longitudinally. Over time, girls with lower cortisol reactivity showed a decrease in depressive symptoms while girls with higher reactivity showed relatively elevated symptoms. Boys with higher cortisol reactivity showed a decrease in externalizing problems; boys with lower reactivity remained relatively stable in such symptoms. Findings suggest sex differences in children's stress reactivity, with implications for the later manifestation of symptoms across childhood.

Keywords

cortisol, HPA axis, stress reactivity, psychopathology, children, sex differences, longitudinal

Co-Authorship Statement

This thesis contains materials submitted for publication to the academic journal "Comprehensive Psychiatry," with Dr. Katie R. Kryski, Dr. Yuliya Kotelnikova, Dr. Haroon Sheikh, Dr. Shiva Singh, and Dr. Elizabeth Hayden listed as co-authors. As this study was a secondary data analysis, Dr. Kryski, Dr. Kotelnikova, and Dr. Hayden are credited for their parts in the previous data collection. Dr. Sheikh and Dr. Singh were responsible for the chemical analysis of cortisol samples in an associated biology laboratory. All parties were offered the chance to review and comment on the manuscript before its submission for publication.

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1. Introduction

Understanding the network of causal factors in psychopathology is key to informing theory and intervention. However, developing precise causal models has proven challenging as biological and environmental factors show both interactional and transactional relationships. Furthermore, given that many causal factors can be studied using diverse methodologies and vantage points, it is important to identify the level of analysis that is the most informative. Given its dynamic nature and the capacity to assess relevant constructs using diverse tools, the study of individual differences in response to stress and how these interact with the environment is a prime example of these issues. In this thesis, I focus on the hypothalamic-pituitary-adrenal (HPA) axis as but one vantage point from which variations in stress reactivity can be understood. Current study goals are to understand how activity of this system relates to the developmental psychopathology of internalizing and externalizing symptoms, and whether any patterns of associations differ for boys versus girls.

1.1. The Hypothalamic-Pituitary-Adrenal Axis

The HPA axis is a complex system which activates in response to physical or psychological stress, particularly to stressors involving social evaluation and uncontrollability (Dickerson & Kemeny, 2004), mobilizing the body's resources to handle these threats. In response to a stressor, the hypothalamus releases corticotropin-releasing hormone, which stimulates the pituitary gland to secrete adrenocorticotropic releasing hormone, which acts on the adrenal glands, leading to the release of the glucocorticoid hormone cortisol. As a downstream product of HPA axis functioning, cortisol is a useful measure of the HPA axis system's functioning. Cortisol circulates

through the body in the bloodstream, binding notably to glucocorticoid and mineralocorticoid receptors in the brain (e.g., the amygdala and hypothalamus; Erickson, Higley & Schulkin, 2008) and key organs throughout the body, with the unbound hormone having downstream physiological and psychological effects including reduced glucose metabolism in the brain (Erickson, Drevets & Schulkin, 2003), immune system suppression, reduced inflammation (Katsu & Iguchi, 2016), as well as changes in mental states, such as enhanced memory for emotionally charged stimuli and impaired recall of neutral information (Erickson, Drevets & Schulkin, 2003; Lupien et al., 2005). In healthy individuals, the binding of cortisol to glucocorticoid and mineralocorticoid receptors causes a negative feedback loop, preventing excessive cortisol release and allowing for the lowering of cortisol concentrations as the hormone is broken down and filtered out of the blood (Gunnar & Talge, 2008; Pariante, 2006). Concentrations of unbound (i.e., active) cortisol in the bloodstream were initially primarily measured via blood plasma, but this process is necessarily invasive, which can be problematic for use with sensitive populations. Other less invasive measures of cortisol in the body, such as hair or urine concentrations, reflect more chronic HPA axis functioning, which can be less useful for some research pursuits (Gunnar & Talge, 2008). In contrast, the concentration of cortisol in the bloodstream can be accurately and acutely indexed using saliva samples, as cortisol readily and reliably diffuses between the two mediums (Kirschbaum & Hellhammer, 1989). Further, salivary cortisol concentrations are accessible and minimally invasive, are unaffected by an individual's rate of salivation, have been shown to be highly correlated with unbound cortisol concentrations in the blood (Kirschbaum & Hellhammer, 1989), and are easy to use with young children (Gunnar & Talge, 2008), the

latter of which facilitates the study of the stress response in developmental psychopathology.

1.2. HPA Axis Maladaptation

While HPA axis activation is adaptive in the context of stress, exposure to persistently elevated cortisol resulting from activation may have neurotoxic effects (Sapolsky, 1996; Starkman, Giordani, Berent, Schork & Schteingart, 2001; Levernez et al., 1999) and is associated with mental health problems (Kirschbaum, Wolf, May, Wippich & Hellhammer, 1996; Pruessner, Hellhammer, Pruessner & Lupien, 2003; Starkman et al., 2001; Takahashi et al., 2005; Vrshek-Schallhorn et al., 2013). The neurotoxic effects of cortisol have been linked to difficulty responding to stressors (Boyer, 2000), producing anxious- and depressive-like behaviours in animals (David et al., 2009; Murray, Smith & Hutson, 2008), and prenatal exposure to elevated cortisol is implicated in infant negative reactivity (Davis et al., 2007) and difficult behaviour (de Weerth, van Hees & Buitelaar, 2003), with some studies finding negative effects on cognitive development in humans (Davis & Sandman, 2010). Longitudinally, elevated neonatal cortisol is associated with increased cortisol reactivity to novel situations in school-age children (Gutteling, de Weerth & Buitelaar, 2005), and maladaptive diurnal cortisol functioning in pre-adolescence (O'Connor et al., 2005). Thus, early elevated cortisol activity has long-term implications for well-being.

On the other hand, maladaptively low cortisol may also indicate problems responding effectively to stress, and has been linked to emotional, social, and behavioural problems (Österberg, Karlson & Hansen, 2009; Ouellet-Morin et al., 2011; Pruessner, Hellhammer & Kirschbaum, 1999). Indeed, decreased cortisol stress reactivity is

associated with aggression (McBurnett, Lahey, Rathouz & Loeber, 2000; Yang, Shin, Noh & Stein, 2007), callous-unemotional traits (Stadler et al., 2011), insensitivity to punishment (van Honk, Schutter, Hermans & Putman, 2003), and concurrent externalizing disorders (Freitag et al., 2009; King, Barkley & Barrett, 1998). Thus, both hyper- and hypoactive cortisol stress reactivity have been tied to maladaptation.

1.3. Sex and Developmental Differences in HPA Axis Function

Sex and developmental influences may affect how cortisol relates to maladaptation. Sex differences in cortisol stress reactivity emerge in adolescence (De Bellis et al., 2001; Feingold, 1994; Gershon & Gershon, 2002; Maughan, Rowe, Messer, Goodman & Meltzer, 2004; Twenge & Nolen-Hoeksema, 2002), and may play a role in accounting for the well-established sex differences in some psychiatric disorders. Specifically, across multiple domains including the cortisol stress response, young and adult women tend to show relatively heightened stress reactivity compared to men (Hankin, Mermelstein & Roesch, 2007; Nolen-Hoeksema, 2001) while also showing stronger ties between stress reactivity and internalizing (e.g., depressive, anxious) symptoms. For example, rumination after adverse life events is associated with depression in girls but not boys (Abela, Hankin, Sheshko, Fishman, & Stolow, 2012), and girls' emotional reactivity to negative life events is related to later depressive symptoms (Charbonneau, Mezulis & Hyde, 2009). Thus, heightened stress reactivity in females may mark particular vulnerability to internalizing (e.g., depressive and anxious) disorders (Altemus 2006; Bekker & van Mens-Verhulst, 2007; Nolen-Hoeksema, 1987). However, when this difference first emerges is unclear as much of this work has focused on older youth and adults.

Conversely, males low in stress reactivity may be at heightened vulnerability to externalizing symptoms. Low stress reactivity, measured through cortisol reactivity, has been linked to behavioural expressions of lower punishment sensitivity and increased reward sensitivity (van Honk et al., 2003), as well as callous-unemotional traits (Hawes, Brennan & Dadds, 2009), all of which are associated with antisocial behavior, which in turn tends to be higher in men. Adolescent boys with lower salivary cortisol show increased externalizing behaviours (Shirtcliff, Granger, Booth & Johnson, 2005) and aggression (McBurnett et al., 2000). However, other studies have found either no association between cortisol and externalizing symptoms in boys (Alink et al., 2008; Pérez-Edgar, Schmidt, Henderson, Schulkin & Fox, 2008) or that heightened cortisol reactivity predicts increased externalizing symptoms (van Bokhoven et al., 2005).

Thus, past work suggests adolescent and adult sex differences in the implications of both heightened and relatively low stress reactivity, but little is known about when such patterns may emerge. While extant work suggests that young boys and girls do not differ in mean cortisol responses to stress (Dettling, Gunnar & Donzela, 1999; Lewis & Ramsay, 2002), the lack of sex differences in reactivity does not preclude the possibility that distinct patterns of reactivity (i.e., relatively low versus relatively high) may have unique implications for young boys' versus girls' psychopathology risk (i.e., a sex-by-reactivity interaction predicting internalizing versus externalizing symptoms). To test this possibility, I examined whether sex moderated the association between early cortisol stress reactivity and children's symptoms over time. I examined this question in a community sample of children characterized on cortisol stress reactivity at age 3 who were then followed up multiple times during childhood. I hypothesized that girls'

heightened cortisol reactivity would have greater relevance for their emerging internalizing symptoms. Given that relevant past work is less conclusive, I tentatively hypothesized that boys' lower cortisol reactivity would be associated with emerging externalizing symptoms.

2. Methods

2.1. Participants

409 children (201 boys) with a mean age of 3.43 years (*SD* = .30) were recruited at baseline. Each child was recruited along with one primary caregiver (382 mothers, 27 fathers), who had a mean age of 34.00 years (*SD* = 4.85). Participants were recruited through a combination of locally posted advertisements and a psychology department participant database. Eligible children resided with at least one biological parent and had no health-related conditions that would prevent them from engaging in the assessment tasks, nor any serious psychological disorder. Families were primarily Caucasian (93%; Asian = 2%, African-Canadian = 0.5%, Hispanic = 1.7%, Other = 2.4%) and varied in socioeconomic status (4% < $20,000, 11% = $20,000-$40,000, 24% = $40,001-$70,000, 30% = $70,001-$100,000, 31% > $100,000). The Peabody Picture Vocabulary Test – Fourth Edition (PPVT) (Dunn & Dunn, 2007), showed that children were, on average, within the normal range (*M* = 112.00, *SD* = 14.05) of cognitive ability.

We obtained measures of child symptoms at baseline and at two follow-up assessments, with the first follow-up (T2) at child age 5 (*M* = 5.49 years, *SD* = 1.58) and the second follow-up (T3) at age 8 (*M* = 8.60 years, *SD* = .74). 379 children (92.7%) participated at T2 and 364 (89.0%) at T3. Between the first and third time points, 89% of the sample was retained. Non-white children were less likely to participate at T3 (χ^2 (1)

= 3.96, p = .047), but not T2 (χ^2 (1) = .71, p = .400). Full information maximum likelihood estimation was used to retain participants with incomplete survey data, excluding only 4 children who did not provide any cortisol data at T1. The resulting sample contained 1140 observations across 405 children.

2.2. Measures

2.2.1. Child Behavior Checklist (CBCL). The CBCL (Achenbach & Rescorla, 2001) is a 113-item parent-report checklist of child behavioural and emotional problems rated as "0 = Not True (as far as you know)", "1 = Somewhat or Sometimes True", or "2 = Very True or Often True.". Reports were collected at all three waves of the study. The primary caregiver's responses were aggregated according to the approach of Lengua, Sadowski, Freidrich, and Fisher (2001), which aligns CBCL items following symptoms drawn from the DSM-IV diagnostic categories (American Psychiatric Association, 1994). As I was interested predicting children's internalizing and externalizing symptoms, the Depression (M_α = .63, α_{range} = .52 - .71), Anxiety (M_α = .67, α_{range} = .61 - .76), Oppositional-Defiant (M_α = .70, α_{range} = .62 - .74), and Attention Problems and Hyperactivity (M_α = .72, α_{range} = .68 - .75) subscales were used. As I am using these as measures of child symptomatology, I refer to these scales following their associated disorder (i.e., Depression, Anxiety, ODD, and ADHD respectively).

2.2.2. Stress Task. At T1, children's cortisol stress responses were assessed during a home visit (see Kryski, Smith, Sheikh, Singh & Hayden, 2011). All home visits occurred between the hours of 12:00 PM and 3:30 PM to reduce the influence of diurnal variation on collected cortisol concentrations. Prior to participating in the stress task, children participated in a thirty-minute period of quiet play with an experimenter with

whom they were familiar from previous study activities with the intention of negating any increases in cortisol due to the arrival of the assessment team at the house. A baseline saliva sample was taken after this acclimation period.

To introduce the task, the experimenter asked each child to choose a prize that they would try to win during the upcoming task. The child was seated in front of a poster board containing multiple picture icons of cartoon bears and frogs and was told the task was for them to match each animal with a specific colored game piece (i.e., a ball) as quickly as possible. After an opportunity to practice matching to ensure comprehension, the child was shown a toy traffic light that had been adapted to ostensibly indicate how much time children had to complete the matching task; the green light indicated that the child had "plenty of time left," while the yellow light indicated that the child was "running out of time," and the red light (accompanied by a buzzer sound) indicated that the child had run out of time. The experimenter used a remote control to switch from one light color to the next to ensure that completion was impossible; children were given three minutes to perform the task, with the time limit shortened if the child were skilled enough to complete the task before then. To further increase the stressful nature of the task, the child was also informed that this was an easy task to complete (that "little kids" could finish on time), and that they would have to complete the task within the time limit to receive their chosen prize. The task was repeated in this way three times before the child was informed that the stoplight was "broken", that they had performed well on the task, and that they could collect their chosen prize. The entirety of the task, including instruction, lasted approximately fifteen minutes, after which the child was allowed to interact with their primary caregiver and engage in quiet play with the experimenter for

another fifty minutes. In support of the task's validity, Kryski and colleagues (2011) showed that children's negative affectivity increased and positive affectivity decreased during the task, and that the task elicited a significant and reliable cortisol response.

2.3. Salivary Cortisol

2.3.1. Sampling. At each collection point, the child was asked to chew on a cotton dental roll until it was wet with saliva. To increase compliance, the experimenter presented the activity as a game, "racing" the child in collecting flavoured drink crystals from a cup using the cotton dental roll; the use of flavored crystals does not negatively affect cortisol collection or assay (Schwartz, Granger, Susman, Gunnar & Liard, 1998; Talge, Donzella, Kryzer, Gierens & Gunnar, 2005). Each child was also rewarded with stickers after each completed sample. As noted previously, the baseline saliva sample was taken after the thirty-minute acclimation period. The subsequent saliva sample was taken ten minutes after the end of the stress task, with additional samples taken every successive ten minutes following this for a total of six samples. Afterwards, the cotton rolls were sealed in microtubes and frozen at -20^{o} C for later analysis. Rates of non-compliance in salivary cortisol sampling were minimal. Of the 2454 samples attempted, only 51 were not collected (2.1%). Of the 409 children, 392 provided all six samples, with 17 children missing at least one sample. Four children refused to participate entirely in sampling and were excluded from analyses.

2.3.2. Analysis. Saliva samples were analyzed in an associated laboratory for cortisol concentration, measured in micrograms per decalitre (μ/dL). Using an expanded range, high sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, PA, USA), samples were assayed in duplicate. No pair of duplicate samples were found to differ in

concentration by more than 5%. Optical density was read on a standard plate reader at 450 nm and corrected at 650 nm (Molecular Devices, Sunnyvale, CA, USA). Enzyme immunoassays were performed according to manufacturer instructions, with average intra-assay coefficients of 3.5% and inter-assay coefficients of 5.1%.

The cortisol concentration values over the course of each visit were used to calculate the area under the curve with respect to ground (AUC_G), described by the equation:

$AUC_G = \sum_{i=1}^{n-1} \frac{(m_{(i+1)}+m_i)}{2}$ (Pruessner, Kirschbaum, Meinschmid & Hellhammer, 2003)

This summary value, measured in concentration over time (μ/dL/hour), reflects both an individual's baseline cortisol output and their reactivity to the stress task. As the collected cortisol values were positively skewed, a natural log transformation was applied to normalize the data, allowing for analysis with parametric statistics (Gunnar & Talge, 2008).

3. Results

3.1. Correlations between study variables

Correlations between variables are presented in Table 1. Child sex was significantly associated with ADHD symptoms at all time points, ODD symptoms at T2, and anxious symptoms at T1, with boys showing more externalizing symptoms and girls showing more symptoms of anxiety. Age at T1 was negatively associated with T1 ODD and ADHD symptoms. PPVT scores were negatively correlated with externalizing symptoms across all time points, with the exception of ODD symptoms at T3. T2 and T3 ADHD symptoms were not significantly correlated with the T1 depression; otherwise, all child symptom scales at all time points were significantly intercorrelated. Cortisol

Table 1. Correlations among variables.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.
1. Age T1	-																		
2. Age T2	.89**	-																	
3. Age T3	.47**	.45**	-																
4. Child Sex†	.06	.09	.09	-															
5. PPVT Score	.05	.04	.02	.07	-														
6. Race‡	.06	.06	.08	.06	.02	-													
7. AUCG§	-.07	-.10	.22**	-.00	-.00	.11*	-												
8. Lengua ADHD T1	-.12*	-.12*	-.01	-.12*	-.13**	-.04	.10*	-											
9. Lengua ADHD T2	-.15**	-.13*	-.12*	-.15**	-.13*	-.02	.05	.50**	-										
10. Lengua ADHD T3	-.08	-.09	-.08	-.21**	-.12*	-.03	.04	.42**	.60**	-									
11. Lengua ODD T1	-.10*	-.11*	-.01	.04	-.12*	.05	.10	.49**	.29**	.24**	-								
12. Lengua ODD T2	-.20**	-.18**	-.09	-.13*	-.12*	.03	.02	.40**	.58**	.41**	.50**	-							
13. Lengua ODD T3	-.01	-.02	-.04	-.09	-.09	.08	-.01	.29**	.40**	.54**	.40**	.57**	-						
14. Lengua Depression T1	-.02	-.06	.06	.10	-.02	.01	.03	.27**	.09	.10	.36**	.17**	.20**	-					
15. Lengua Depression T2	-.15**	-.15**	.01	.02	-.00	.04	.06	.24**	.32**	.19**	.26**	.45**	.25**	.37**	-				
16. Lengua Depression T3	-.11*	-.12*	.01	.05	-.06	.09	.11*	.25**	.29**	.44**	.30**	.35**	.48**	.38**	.57**	-			
17. Lengua Anxiety T1	-.01	-.00	.05	.11*	.05	.11*	.13*	.24**	.08	-.06	.28**	.11*	.07	.35**	.23**	.19**	-		
18. Lengua Anxiety T2	-.03	-.052	-.05	.06	.07	.08	.03	.17**	.14**	.10	.21**	.20**	.16**	.26**	.36**	.29**	.38**	-	
19. Lengua Anxiety T3	.04	.021	.01	.00	.05	.05	.05	.20**	.20**	.33**	.22**	.19**	.36**	.24**	.27**	.52**	.24**	.50**	-

†Child sex: male = 0, female = 1; ‡Race: white = 0, other = 1; §AUCG: "Area under the curve with respect to ground", a measure of cortisol reactivity during the stress task.
T1 = Time 1, T2 = Time 2, T3 =Time 3; * p < .05, ** p < .01

reactivity to the stress task at T1 was correlated with concurrent symptoms of ADHD and anxiety, as well as with identifying as a non-white race.

3.2. Growth modelling

Multi-Level Modelling (MLM) was performed in MPlus 8 (Muthén & Muthén, 2017). MLM allows for the investigation of longitudinal change across multiple waves of data, estimating both an intercept, which reflects symptoms at the baseline assessment, and a slope, which reflects the rate of change in symptoms over time. The unconditional model suggested a linear decrease in ADHD (b = -.13, p < .001) and ODD (b = -.06, p < .001) symptoms, consistent with well-established, normative developmental increases in self-control and compliance that characterize the period between early and middle childhood (Markus & Nurius, 1984), but not for symptoms of anxiety (b = .02, p = .323) or depression (b = -.03, p = .148). The unconditional model did show significant variability in intercepts and slopes of all symptoms over time, allowing us to explore an explanation for these variance components (Singer & Willet, 2003). Children's ages at all waves of the study were centered around the grand mean of the children's age at T1 (i.e., 3.43 years) to create a starting point for the growth model.

The Level 1 model consisted of specific symptom measures at each time point (i.e., T1, T2, T3), which was nested within each participant, the Level 2 variable. Cortisol expression over the course of the stress task (AUC_G) and child sex were Level 2 between-subjects predictors. Furthermore, whether the child's sex moderated the influence of cortisol on symptoms was tested via an interaction term between centered AUC_G and dummy-coded sex (i.e., male = 0, female = 1). A linear equation was constructed to examine the effects of Level 2 variables on the slope and intercept of symptoms over

time, with an independent model analyzed for each symptom measure. When interaction terms were significant, the constructed equations were plotted on a graph to assist in interpretation, with AUC_G recentered at +1 SD above and -1 SD below the mean (Aiken & West, 1991). Simple slopes were calculated to further aid in the interpretation of results.

3.2.1. Depressive Symptoms. A model predicting children's parent-reported symptoms of depression, with cortisol reactivity to the stress task, child sex, and the interaction between cortisol reactivity and child sex as predictors was constructed. Overall, girls had more depressive symptoms than boys at baseline at a trend level (b = .250, p = .094). A significant interaction of sex and cortisol on slope was found (b = .585, p = .050; Figure 1). Tests of simple slopes showed that girls with higher reactivity had relatively high and stable parent-reported symptoms over time (b = .041, p = .402), while girls with lower reactivity decreased in parent-reported symptoms over time (b = -.124, p = .002), approximately to the level of boys. Boys had relatively stable and low symptoms of depression over time, regardless of whether they were high (b = -.006, p = .831) or low (b = -.019, p = .529) in cortisol reactivity.

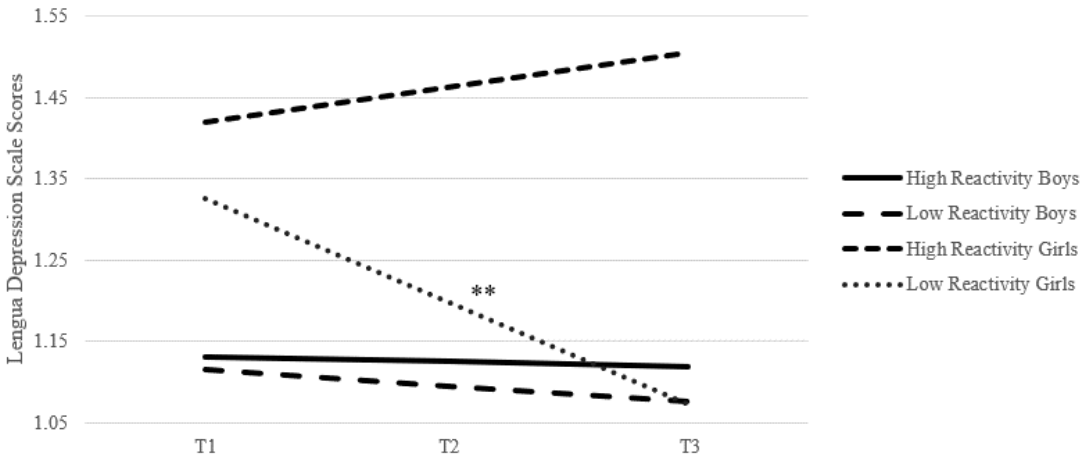

Figure 1. Lengua Depression scale scores over time in high and low reactivity boys and girls. ** = p < .01

3.2.2. Anxious Symptoms. Next, I constructed a model using cortisol reactivity to the stress task, child sex, and the interaction between cortisol reactivity and child sex as predictors of anxious symptoms. Both girls (b = .36, p = .014) and individuals with higher cortisol reactivity (b = 1.58, p = .009) had significantly higher parent-reported anxiety than boys or individuals with lower reactivity at baseline. Cortisol reactivity had a significant effect on slope (b = -.33, p = .047), with lower reactivity associated with a greater increase in anxious symptoms over time. Child sex also had a trend-level effect on slope (b = -.08, p = .070), with boys showing an increase in symptoms over time compared to girls. No significant interaction between sex and cortisol reactivity on slope was found (b = .41, p = .141).

3.2.3. ODD Symptoms. I then used cortisol reactivity to the stress task, child sex, and the interaction between cortisol reactivity and child sex to predict parent-reported oppositional-defiant symptoms. A significant effect of sex on slope was found (b = -.08, p = .020), with girls showing a significantly greater decrease in ODD

symptoms over time. Cortisol reactivity also had a trend-level effect on slope (b = -.39, p = .057), suggesting that individuals with higher cortisol reactivity had a greater decrease in symptoms over time. The interaction between sex and cortisol reactivity had a trend-level effect on slope (b = .52, p = .074; Figure 2). Simple slopes indicated that boys with low cortisol reactivity did not show a significant change in ODD symptoms over time (b = -.04, p = .349), maintaining similar symptoms over time; in contrast, girls higher (b = -.15, p < .001) and lower (b = -.19, p <.001) in reactivity, as well as boys higher in reactivity (b = -.14, p < .001) showed significant decreases in ODD symptoms over time.

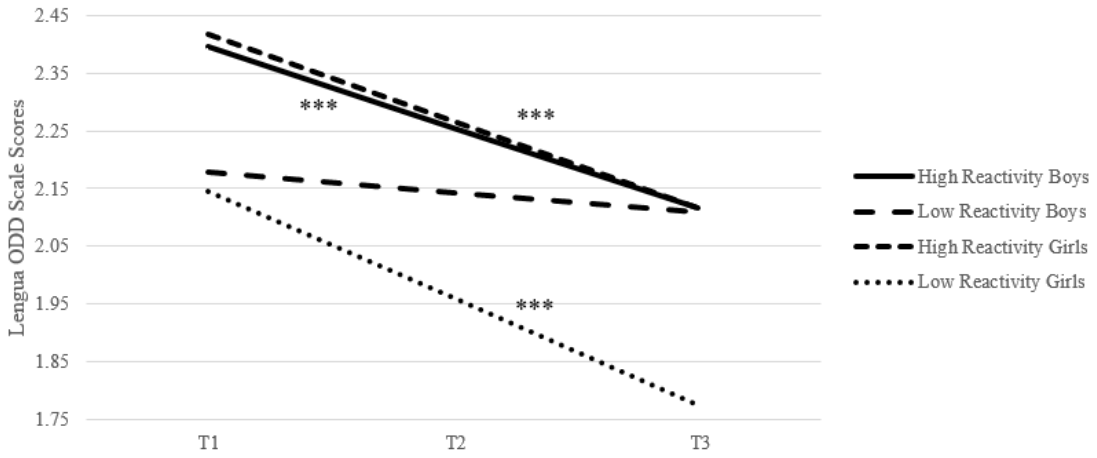

Figure 2. Lengua ODD scale scores over time in high and low reactivity boys and girls. *** = p < .001

3.2.4. ADHD Symptoms. Finally, a model predicting children's parent-reported ADHD symptoms, with cortisol reactivity to the stress task, child sex, and the interaction between cortisol reactivity and child sex as predictors was constructed. Sex was found to have an effect on intercept (b = -.31, p = .019), with males showing significantly more ADHD symptoms at baseline. Sex was also found to have a significant effect on slope (b = -.07, p = .021), with girls showing a greater decrease in symptoms over time. Cortisol

reactivity was also found to have an effect on slope at a trend-level (b = -.29, p = .066), with higher reactivity generally being associated with a greater decrease in symptoms over time. A significant interaction of sex and cortisol reactivity was found on slope (b = .45, p = .049; Figure 3). Both high reactivity girls (b = -.07, p = .007) and low reactivity girls (b = -.11, p < .001) were found to have a significant decrease in attention problems and hyperactivity symptoms over time. Boys with high reactivity had symptoms which decreased at a trend level of significance (b = -.06, p = .051), while the symptoms of boys with low reactivity did not change significantly over time (b = .01, p = .661).

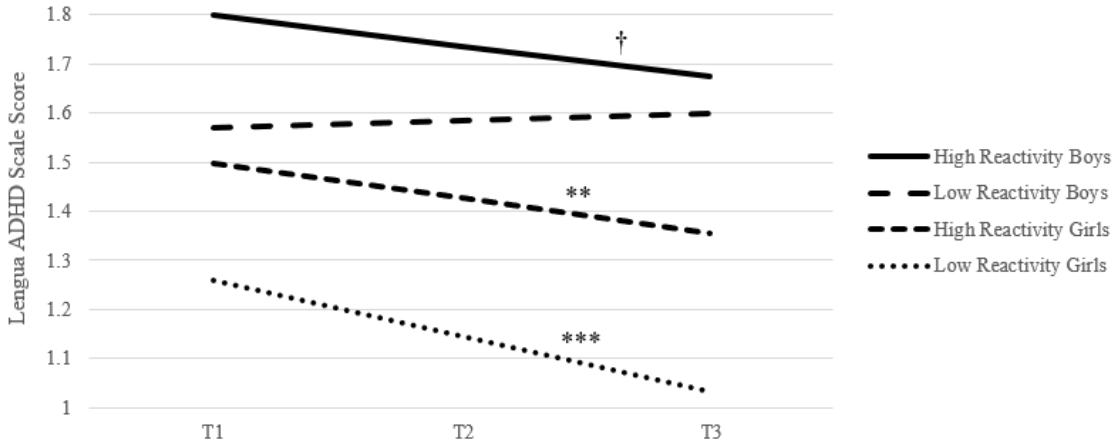

Figure 3. Lengua ADHD scale scores over time in high and low reactivity boys and girls. † = p < .10, ** = p < .01, *** = p < .001

4. Discussion

4.1. Conclusions

Previous research has focused on sex differences in cortisol reactivity in adolescence and adults, and how such reactivity relates to risk. Here, I provide new data concerning when cortisol stress reactivity begins to show associations with symptoms, and whether associations differed for boys and girls, and between internalizing and

externalizing symptoms. I found that boys with higher cortisol reactivity decreased in ODD and ADHD symptoms over time, while boys with lower reactivity did not. I also found that girls with lower cortisol reactivity decreased in depressive symptoms over time, compared to girls with higher reactivity.

The predisposition of boys with lower cortisol reactivity to show stably higher externalizing symptoms may be linked to deficits in passive avoidance learning, in which individuals normally learn to associate specific behaviours with punishment. In early adolescent populations, lower cortisol reactivity has previously been associated with callous-unemotional or "psychopathic" traits (Stadler et al., 2011; Hawes, Brennan & Dadds, 2009; Loney, Butler, Lima, Counts & Eckel, 2006), which are more common in males (Cale & Lilienfeld, 2002; Essau, Sasagawa & Frick, 2006; Levenson, Kiehl & Fitzpatrick, 1995). Individuals high in these traits show deficits in passive avoidance learning in the context of competing rewards (Blair et al., 2004, Newman & Kosson, 1986). While we did not measure relevant traits in this sample, taken in conjunction with these findings, such boys may show both trait-like and psychophysiological markers that reflect difficulty attending to cues of threat, thereby persisting in behavior that manifests itself as externalizing symptoms.

These findings for girls align with past work implicating heightened stress reactivity in internalizing psychopathology, particularly for females. However, these findings indicate that this association develops much earlier than past research indicates (Altemus, 2006; Bekker & van Mens-Verhulst, 2007; Nolen-Hoeksema, 1987, Shirtcliff et al., 2005) given that extant work has focused on adolescence and adulthood. I used cortisol stress reactivity as the marker of stress responding, which is especially useful in

examining this issue in young children for whom other aspects of stress responding may prove challenging to assess (e.g., cognitive stress reactivity). Should these findings prove robust, cortisol stress reactivity may serve as a useful, developmentally sensitive marker of heightened vulnerability to internalizing symptoms that can be assessed early in development, thereby informing early prevention and intervention.

4.2. Future Directions

Children in the current study were young, and it will be important for future work to integrate indices of cortisol stress responding with other hormonal systems that interact with cortisol and are implicated in the development of psychopathology. Adolescence in particular is a crucial stage for developing more complex models of gonadal hormones and HPA axis stress responding in predicting adjustment. For example, past work indicates that aggression is related to the ratio of testosterone to cortisol (Montoya, Terbug, Bos & van Honk, 2012), such that higher endogenous cortisol reduces the testosterone to cortisol ratio, promoting withdrawal and reduced aggression. Further, while the hypothalamic-pituitary-gonadal and HPA axes reciprocally inhibit one another (Viau, 2002), the magnitude of this relationship may differ based on sex differences in testosterone production (Montoya et al., 2012). Weiss, Longhurst & Mazure (1999) speculate that estrogen may also play a role in the sensitization of the HPA axis to stress, leading to elevated cortisol reactivity in women. These hormonal interactions may act as a pathway through which sex differences in symptoms become more pronounced throughout adolescence and adulthood.

While it is commonplace to refer to “hyper” and “hypo” cortisol stress reactivity in studies of all age groups, the lack of normative data on the development of the cortisol

stress response seriously limits our understanding of when cortisol stress responding can be understood as maladaptively low or high. This is in large part due to the proliferation of laboratory paradigms used to elicit a cortisol stress response in children. While efforts have been made towards developing a set of normative data characterizing typical cortisol functioning and reactivity in different populations (see Kobayashi & Miyazaki, 2015; McCarthy et al., 2009; Tollenaar, Jansen, Beijers, Riksen-Walraven & de Weerth, 2010), the field would benefit greatly from establishing a battery of valid, developmentally sensitive paradigms to map change in cortisol stress responding during childhood and beyond.

In addition to informing our understanding of the development of normative cortisol stress reactivity, a battery of developmentally informed paradigms would permit cross-lagged analyses testing reciprocal influences of cortisol on symptoms over time. A wide variety of tasks have previously been used to elicit stress responses in participants (e.g., the Trier Social Stress Task [Kirschbaum, Pirke & Hellhammer, 1993], viewing emotionally charged film clips [Eisenberg et al., 1988], or the cold pressor task [Walsh, Schoenfeld, Ramamurthy, Hoffman, 1989]). These tasks generate different kinds of stress (e.g., social, physical), leading to possible differences in the participant's stress response between tasks. The stress task used in this study (Kryski et al., 2011) hinges on social evaluation and self-criticism, which may be more relevant to risk for internalizing disorders, given that they are characterized by sadness and intropunitive behavior (Eisenberg et al., 2003; Tandon, Cardeli & Luby, 2011). In future studies, using stress tasks in which participants avoid potential punishment in the context of reward may relate more closely to externalizing symptoms, given that these disorders are

characterized by impulsivity and insensitivity to punishment (Eisenberg et al., 2003; van Honk et al., 2003). Relatedly, we cannot exclude the possibility that childhood cortisol stress responding is simply a concomitant marker of children's current symptoms, an important limitation of the current study. While strengths of the current study include the longitudinal design with impressive retention of a large sample, as well as the use of a validated, well-controlled stress paradigm, it is unclear whether these findings will generalize to children from higher-risk populations or to more diverse samples.

It is unlikely that cortisol stress reactivity can serve as a viable marker of risk in isolation; however, in conjunction with other markers of maladaptive stress responding, it may inform the development of interventions designed to reduce children's future psychopathology risk. For example, mindfulness techniques may reduce cortisol responses to stressors (Brand, Holsboer-Trachsler, Naranjo & Schmidt, 2012; Matousek, Dobkin & Pruessner, 2010); thus, girls who show elevated stress responding across multiple domains might benefit from training in these and related techniques as a means of reducing risk for internalizing symptoms following stress exposure. Conversely, rather than targeting stress response systems directly, boys who are under-responsive to threat may benefit more so from interventions designed to enhance inhibitory control (Riggs, Greenberg, Kusché & Pentz, 2006; Raver et al., 2011). Having said that, it is possible that low cortisol reactivity to stress might ultimately help identify boys at highest risk for externalizing symptoms due to impairments in the capacity to recognize cues for punishment. In addition to methodological work focused on developmentally appropriate, valid assessment approaches to mapping the cortisol stress response across childhood, preventative work that integrates cortisol stress responding as a screening

component will help verify its potentially causal role in boys' and girls' development of psychopathology.

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Appendix A: Ethics approval form

Western Research

Research Ethics

Western University Health Science Research Ethics Board
NMREB Annual Continuing Ethics Approval Notice

Date: May 31, 2015
Principal Investigator: Prof. Elizabeth Hayden
Department & Institution: Social Science\Psychology,Western University

NMREB File Number: 5246
Study Title: Gene-Environment Interplay and the Development of Child Temperament - 15121S
Sponsor: Canadian Institutes of Health Research

NMREB Renewal Due Date & NMREB Expiry Date:
Renewal Due -2016/05/31
Expiry Date -2016/06/11

The Western University Non-Medical Research Ethics Board (NMREB) has reviewed the Continuing Ethics Review (CER) form and is re-issuing approval for the above noted study.

The Western University NMREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), Part 4 of the Natural Health Product Regulations, the Ontario Freedom of Information and Protection of Privacy Act (FIPPA, 1990), the Ontario Personal Health Information Protection Act (PHIPA, 2004), and the applicable laws and regulations of Ontario.

Members of the NMREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The NMREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000941.

[redacted]

Ethics Officer, on behalf of Prof. Riley Hinson, NMREB Chair

Ethics Officer to Contact for Further Information

___ Erika Basile ebasile@uwo.ca	✓ Grace Kelly grace.kelly@uwo.ca	___ Moa Mekhail mmekhail@uwo.ca	___ Vikki Tran vikki.tran@uwo.ca

This is an official document. Please retain the original in your files.

CURRICULUM VITAE

Andrew Daoust

EDUCATION

Sep. 2016 – Present	Master of Science in Clinical Psychology University of Western Ontario Supervisor: Dr. Elizabeth Hayden
Sep. 2011 – Apr. 2015	Honours Bachelor of Science in Life Sciences (High Distinction), University of Toronto Majors: Psychology, Biology for Health Science Minor: Philosophy of Science

PUBLICATIONS

1. Daoust, A. R., Kotelnikova, Y., Kryski, K. R., Sheikh, H. I., Singh, S. M. & Hayden, E. P. (2018). Examining child sex as a moderator of the relationship between cortisol reactivity and symptoms over time. Manuscript submitted for publication.

2. Daoust, A. R., Thakur, A., Sheikh, H. I., Kleiber, M. L., Singh, S. M., & Hayden, E. P. (2018). Associations between children's telomere length and vulnerabilities in the early environment. Manuscript in preparation.

3. MacDougall, M. J., Tabor, N. J., Woodhead, J., Daoust, A. R., & Reisz, R. R. (2017). The unique preservational environment of the Early Permian (Cisuralian) fossiliferous cave deposits of the Richards Spur locality, Oklahoma. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 475, 1-11.

CONFERENCE PRESENTATIONS

1. Daoust, A. R., Kotelnikova, Y., Kryski, K. R., Sheikh, H. I., Singh, S. M. & Hayden, E. P. *The role of child sex in the stability of cortisol stress reactivity and its associations with symptoms.* Poster presentation: Annual Meeting of the Society for Research in Psychopathology; 2018 Sept 20-23; Indianapolis, IN.

2. Vandermeer, M. R. J., Daoust, A. R., Mohamed Ali, O., Joanisse, M. F., Barch, D. M. & Hayden, E. P. *Associations between children's resting state functional connectivity and maternal history of depression*. Poster presentation: Annual Meeting of the Society for Research in Psychopathology; 2018 Sept 20-23; Indianapolis, IN.

3. Gabel, L. N., Salisbury, M. R., Daoust, A. R., Grahn, J. A., Durbin, C. E. & Hayden, E. P. *Development and validation of a developmentally appropriate battery of emotionally evocative stimuli for use with young children.* Poster presentation: Annual Meeting of the Society for Research in Psychopathology; 2018 Sept 20-23; Indianapolis, IN.

4. Vandermeer, M. R. J., Daoust, A. R., Mohamed Ali, O., Joanisse, M. F., Barch, D. M., & Hayden, E. P. *Gray matter concentrations in never-depressed children at risk for depression*. Poster presentation: Flux Congress; 2018 Aug 30 – Sept 1; Berlin, Germany.

5. Daoust, A. R., Thakur, A., Sheikh, H. I., Kleiber, M. L., Singh, S. M., & Hayden, E. P. *Associations between children's telomere length and vulnerabilities in the early environment. Poster presentation*: Annual Meeting of the Society for Research in Psychopathology; 2017 Sept 14-17; Denver, CO.

6. Vandermeer, M. R. J., Joanisse, M. F., Daoust, A. R., Mohamed Ali, O., Salisbury, M., Barch, D. M., & Hayden, E. P. *Resting state functional connectivity in children at high risk for depression*. Poster presentation: Annual Meeting of the Society for Research in Psychopathology; 2017 Sept 14-17; Denver, CO.

7. Andersen, J.P., Papazoglou, K., Pitel, M, Weerasinghe, A., & Daoust A. R. *Examining the role of physiological reactivity as a way to predict performance among SWAT team officers*. Oral presentation: Toronto Forensic Research Exchange; 2016 Jun 6; Toronto, ON.

8. Andersen, J.P., Papazoglou, K., Pitel, M, Weerasinghe, A., & Daoust A. R. *Examining the role of physiological reactivity as a way to predict performance among SWAT team officers.* Paper presentation: Academy of Criminal Justice Sciences, 2016 Mar 29 - Apr 2; Denver, CO.

9. Andersen, J.P., Papazoglou, K., Pitel, M, Weerasinghe, A., & Daoust A. R. *Examining the role of physiological reactivity as a way to predict performance among SWAT team officers.* Poster presentation: American Psychosomatic Society, 2016 Mar 9 – 12; Denver, CO.

10. Daoust, A. R. *Fossil taphonomy in the early Permian Richards Spur locality, Oklahoma, USA.* Poster presentation: Ontario Biology Day, 2014 Mar 22 – 23; Mississauga, ON.

ACADEMIC HONOURS AND ACHIEVEMENTS

2018 – 2019	QOL Graduate Research Fellowship Children's Health Research Institute
2017 – 2018	QOL Graduate Research Fellowship Children's Health Research Institute
2017 – 2018	Ralph S. Devereux Award in Psychology University of Western Ontario
2017 – 2018	Ontario Graduate Scholarship (OGS) University of Western Ontario

2016 -2017	QOL Graduate Research Fellowship Children's Health Research Institute
2016 – 2017	Ontario Graduate Scholarship (OGS) University of Western Ontario
2016	Best Student Presentation Toronto Forensic Research Exchange
2011-2015	Dean's List (3.5+ GPA) University of Toronto
2011	Entrance Scholarship University of Toronto
2011	Academic Achievement Scholarship Rotary Club of Oakville

RESEARCH EXPERIENCE

2016 - Present	Graduate Student under Dr. Elizabeth Hayden Personality and Emotion Development Lab University of Western Ontario
2015 – 2016	Research Intern under Dr. Judith Andersen HART ("Health Adaptation Research on Trauma" Lab) University of Toronto at Mississauga
2014	Research Assistant under Christine Nguyen Dr. Janet Polivy, Polivy "Market Research" Laboratory University of Toronto at Mississauga
2014 – 2015	Independent Research BIO318 Animal Behaviour Research Project University of Toronto at Mississauga
2013 – 2014	Research Opportunity Project under Dr. Robert Reisz Reisz Paleontology Laboratory University of Toronto at Mississauga

CLINICAL EXPERIENCE

Jan. – Apr. 2018	Initial Assessment Practicum, Child & Adolescent Psychoeducational Assessment Child and Parent Resource Institute
Jan. – Apr. 2018	Initial Assessment Practicum, Adult

	Neuropsychology University Hospital
Apr. – Aug. 2017	Initial Intervention Practicum, Adult Student Development Center University of Western Ontario

OTHER WORK AND TRAINING

Sep. 2017- Apr. 2018	Undergraduate Thesis Co-Supervisor, Andrea Sandstrom
Apr. 2017	Growth Modelling with MPlus Enablytics Workshop
Jan. – Apr. 2017	Teaching Assistant University of Western Ontario Abnormal Child Psychology (Undergraduate)
2014	SafeTALK Crisis Management Workshop
2014	Distress Line Volunteer Training, Oakville Distress Centre

VOLUNTEER EXPERIENCE

2015 – 2016	Phone Line Operator, Oakville Distress Center