



University of Pennsylvania
ScholarlyCommons

Publicly Accessible Penn Dissertations


1-1-2016

Stress Physiology and Behavior Problems

Ruiyun Chen

University of Pennsylvania, ruiyunch@sas.upenn.edu

Follow this and additional works at: <http://repository.upenn.edu/edissertations>

 Part of the [Criminology Commons](#), and the [Criminology and Criminal Justice Commons](#)

Recommended Citation

Chen, Ruiyun, "Stress Physiology and Behavior Problems" (2016). *Publicly Accessible Penn Dissertations*. 1649.
<http://repository.upenn.edu/edissertations/1649>

This paper is posted at ScholarlyCommons. <http://repository.upenn.edu/edissertations/1649>
For more information, please contact libraryrepository@pobox.upenn.edu.

Stress Physiology and Behavior Problems

Abstract

Understanding childhood externalizing problems is informative in designing interventions and reducing crime in adulthood because childhood aggression is one of the best predictors for later antisocial behavior. Childhood externalizing problems are typically studied with internalizing problems (e.g., anxiety) given their consistent correlation and seemingly opposite behavior manifestations. This dissertation examined both spectrums of behavior problems to advance our etiological understanding. Adversity and stress have been a focus in criminology research but few studies have incorporated stress physiology, the biological underpinning of how individuals deal with adversity. This dissertation comprised three papers testing the linkage of stress physiology to behavior problems. I examined how the two components of the stress system, namely the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), interact with each other, and together interact with harsh discipline in influencing externalizing and internalizing problems. Data were drawn from a community sample of 11-12 year old children (N = 446). Saliva samples were collected in the initial assessment and later assayed for cortisol (HPA) and salivary alpha-amylase (sAA; ANS). Participants and their caregivers completed questionnaires for child behavior problems initially, 3, 6, and 12 months later. Paper 1 revealed that cortisol was negatively associated with externalizing and internalizing problems but only at low levels of sAA. Paper 2 built on Paper 1 by including harsh discipline as an environmental factor and testing how the combined effect of cortisol and sAA contributed to our understanding of the heterogeneous effect of harsh discipline on behavior problems. Results showed that asymmetry in cortisol and sAA may indicate biological susceptibility to the effect of harsh discipline to develop both externalizing and internalizing problems among boys. Given the similar stress physiological patterns shown in Paper 1 and 2 for externalizing and internalizing problems, Paper 3 further explored and found that the interplay of cortisol and sAA differentiated co-occurrence of behavior problems from other developmental trajectories of behavior problems over a year. Together these findings underscore the significance of stress physiology in behavior problems. Theoretical implications in relation to differential susceptibility hypothesis and practical implications for treatment evaluation and research are discussed.

Degree Type

Dissertation

Degree Name

Doctor of Philosophy (PhD)

Graduate Group

Criminology

First Advisor

Adrian Raine

Keywords

Alpha-Amylase, Cortisol, Developmental Trajectories, Externalizing Problems, Harsh Discipline, Internalizing Problems

Subject Categories

Criminology | Criminology and Criminal Justice

STRESS PHYSIOLOGY AND BEHAVIOR PROBLEMS

Ruiyun Frances Chen

A DISSERTATION

in

Criminology

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2016

Supervisor of Dissertation

Graduate Group Chairperson

Adrian Raine, D. Phil.
Richard Perry University Professor
Departments of Criminology,
Psychology, and Psychiatry,
University of Pennsylvania

Charles Loeffler, Ph.D.
Jerry Lee Assistant Professor
Department of Criminology
University of Pennsylvania

Dissertation Committee

Adrian Raine Richard Perry University Professor, University of Pennsylvania
Sara Jaffee Professor of Psychology, University of Pennsylvania
John MacDonald Professor of Criminology and Sociology, University of Pennsylvania
Douglas Granger Professor of Psychology, Arizona State University

ACKNOWLEDGMENT

I owe my gratitude to a great many people who have contributed to the production of this dissertation and made its completion possible. Because of you, my years at Penn have been a wonderful experience.

First and foremost, I have been amazingly fortunate to have Adrian Raine as my advisor. Adrian, you gave me the freedom to explore on my own while providing me with unlimited support when I faltered. Your mentorship was paramount in giving me a well-rounded experience consistent with my long-term career goals. You encouraged me to not only immerse myself in the interdisciplinary culture of Penn to work on research projects with mentors from different disciplines, but also to seek out opportunities to serve as a course instructor and a supervisor. I don't think many graduate students are given the opportunity that I have received from you to develop their own individuality and self-sufficiency by being allowed to work with such independence; and I thank you for that!

I owe my deep gratitude to the guidance and support of my dissertation committee: Sara Jaffee, Doug Granger, and John MacDonald. Thank you all for serving on my committee and guiding me through many crises. Sara, you taught me how to formulate research questions, how to ground my research into the literature, and how to best express ideas. Your patience and support lifted me out of many episodes of self-doubt and kept me on the right track. I thank you for your constructive advice in matters of research, career development and personal life. You broaden my horizon and I always gain new perspectives talking with you. I hope one day I will become as good a

researcher and a teacher as you are. Doug, you are one of my closest collaborators. I have never met you in person but the guidance and encouragement I have received from you has been tremendous. I still remember my first email correspondence with you about our manuscripts, and your enthusiasm about research is just contagious! It has been a great pleasure to work with you, and I appreciate your insights and your sense of humor. I am very grateful that you spared so much of your time from your hectic schedule to discuss research with me, to comment on and edit my manuscripts and to connect me to many other brilliant researchers and their great research projects. John, you helped me the most to make the transition from psychology to criminology. You are my go-to person when I need advice incorporating or bridging my work with existing criminological theories. I owe a lot to you for your invaluable feedback on this dissertation.

I am fortunate to be in the Department of Criminology at UPenn to complete my doctoral training. I received enormous help throughout my time here from faculty members Emily Owens, Charles Loeffler and Sara Heller as well as department administrators Knakiya Hagens, Caroline Gonzalez Ciccone, Addie Métivier, and Janel Baselice.

Thanks to my wonderful lab mates Anna Rudo-Hutt and Jill Portnoy. Anna, you trained me to do hormone assay and skin conductance data processing, without which this dissertation could not have been completed. You inspired much of my work, and I enjoyed and learned so much when bouncing around research ideas with you. Jill, thank

you for your company and encouragement, I had a fun time going through the program with you. I am grateful to have both of you as research buddies.

I owed the most to my dear friends and family. Your love and support helped me to get to where I am today. My deepest gratitude goes to my parents, sisters and brothers, and special thanks to the most important person in my life, Dan Robbins: thank you for being there for me all these years.

Lastly, several great collaborators have contributed to the published versions of papers included in this dissertation, and I acknowledge and thank Adrian Raine, Doug Granger, Anna Rudo-Hutt, Andrea Glenn and Liana Soyfer. Data in this dissertation was drawn from the Philadelphia Healthy Brain and Behavior project, which was funded, in part, under a grant from the Pennsylvania Department of Health (SAP# 4100043366, PI: Adrian Raine). The Department specifically disclaims responsibility for any analyses, interpretations or conclusions. It was also supported by the Clinical & Translational Research Center, Perelman School of Medicine, University of Pennsylvania (grant number UL1-RR-024134, PI: Adrian Raine).

ABSTRACT

STRESS PHYSIOLOGY AND BEHAVIOR PROBLEMS

Ruiyun Frances Chen, M.S.

Adrian Raine, D. Phil.

Understanding childhood externalizing problems is informative in designing interventions and reducing crime in adulthood because childhood aggression is one of the best predictors for later antisocial behavior. Childhood externalizing problems are typically studied with internalizing problems (e.g., anxiety) given their consistent correlation and seemingly opposite behavior manifestations. This dissertation examined both spectrums of behavior problems to advance our etiological understanding. Adversity and stress have been a focus in criminology research but few studies have incorporated stress physiology, the biological underpinning of how individuals deal with adversity. This dissertation comprised three papers testing the linkage of stress physiology to behavior problems. I examined how the two components of the stress system, namely the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), interact with each other, and together interact with harsh discipline in influencing externalizing and internalizing problems. Data were drawn from a community sample of 11-12 year old children (N = 446). Saliva samples were collected in the initial assessment and later assayed for cortisol (HPA) and salivary alpha-amylase (sAA; ANS). Participants and their caregivers completed questionnaires for child behavior problems initially, 3, 6, and 12 months later. Paper 1 revealed that

cortisol was negatively associated with externalizing and internalizing problems but only at low levels of sAA. Paper 2 built on Paper 1 by including harsh discipline as an environmental factor and testing how the combined effect of cortisol and sAA contributed to our understanding of the heterogeneous effect of harsh discipline on behavior problems. Results showed that asymmetry in cortisol and sAA may indicate biological susceptibility to the effect of harsh discipline to develop both externalizing and internalizing problems among boys. Given the similar stress physiological patterns shown in Paper 1 and 2 for externalizing and internalizing problems, Paper 3 further explored and found that the interplay of cortisol and sAA differentiated co-occurrence of behavior problems from other developmental trajectories of behavior problems over a year. Together these findings underscore the significance of stress physiology in behavior problems. Theoretical implications in relation to differential susceptibility hypothesis and practical implications for treatment evaluation and research are discussed.

TABLE OF CONTENTS

ACKNOWLEDGMENT	II
ABSTRACT.....	V
LIST OF TABLES	IX
LIST OF ILLUSTRATIONS.....	X
GENERAL INTRODUCTION.....	1
Background and Objective.....	1
Data Summary	6
PAPER 1. INTERACTION OF ADRENOCORTISOL ACTIVITY AND AUTONOMIC AROUSAL ON CHILDREN’S EXTERNALIZING AND INTERNALIZING PROBLEMS.....	9
Abstract.....	9
Introduction.....	10
Methods.....	19
Results	24
Discussion.....	26
Paper 1 Tables and Figures.....	33
PAPER 2. HARSH DISCIPLINE AND BEHAVIOR PROBLEMS: THE MODERATING EFFECTS OF CORTISOL AND ALPHA-AMYLASE	40
Abstract.....	40
Introduction.....	41
Methods.....	46

Results	54
Discussion.....	57
Paper 2 Tables and Figures.....	62
PAPER 3 HYPOTHALAMIC PITUITARY ADRENAL ACTIVITY AND AUTONOMIC NERVOUS SYSTEM AROUSAL PREDICT DEVELOPMENTAL TRAJECTORIES OF CHILDREN’S COMORBID BEHAVIOR PROBLEMS.....	70
Abstract.....	70
Introduction.....	72
Methods.....	79
Results	85
Discussion.....	87
Paper 3 Tables and Figures.....	94
GENERAL DISCUSSION	101
BIBLIOGRAPHY	105

LIST OF TABLES

Table 1.1 Correlations among All Indicators	33
Table 1.2 Correlations among Predictors and Outcome Variables	34
Table 1.3 Combined Effects of Cortisol and Alpha-amylase on Levels of and Covariance between Externalizing and Internalizing problems	35
Table 2.1 Means (Standard Deviation) of All Variables in the Model.....	62
Table 2.2 Correlations among Main Variables.....	63
Table 2.3 Individual and Interactive Effects of Harsh Discipline, Latent Basal Cortisol and Latent Basal Alpha-amylase on Levels of Externalizing and Internalizing Problems in a Multi-group Structural Equation Model.....	64
Table 3.1 Descriptive Statistics for Main Variables	94
Table 3.2 Comparisons of Unconditional Latent Class Growth Models with Different Number of Classes for Co-developmental Trajectories of Externalizing and Internalizing Problems	95
Table 3.3 Descriptive Statistics by Five Classes	96
Table 3.4 Estimates from Conditional Latent Class Growth Analysis with Latent Cortisol, Alpha-amylase and Interaction as Predictors.....	97

LIST OF ILLUSTRATIONS

Figure 1.1 Combined Effect of Trait-like Cortisol and Trait-like Alpha-amylase on Behavior Problems	37
Figure 1.2 Salivary Alpha-amylase Moderates the Relation between Cortisol and Externalizing Problems (panel a) and between Cortisol and Internalizing Problems (panel b).....	38
Figure 1.3 Similar Interactive Patterns of Cortisol and Salivary Alpha-amylase on Externalizing and Internalizing Problems	39
Figure 2.1 Measurement Model and Results for Latent Variable Cortisol, Salivary Alpha-amylase and Behavior problems	65
Figure 2.2 Individual and Interactive Effects of Harsh Discipline, Latent Basal Cortisol, and Latent Basal Salivary Alpha-amylase on Externalizing and Internalizing Problems	66
Figure 2.3 Latent Basal Cortisol Regions of Significance for Slopes of Harsh Discipline on Boys' Externalizing Problems by Low (panel a) and High Levels of Latent Basal Salivary Alpha-amylase (panel b).....	67
Figure 2.4 Latent Basal Cortisol Regions of Significance for Slopes of Harsh Discipline on Boys' Internalizing Problems by Low (panel a) and High Levels of Latent Basal Salivary Alpha-amylase (panel b).....	68
Figure 2.5 Effects of Harsh Discipline on Standardized Scores of Boys' Externalizing (panel a) and Internalizing Problems (panel b) by Factorial Combination of High versus Low Latent Basal Cortisol and Salivary Alpha-amylase	69
Figure 3.1 Individual and Interactive Effects of Latent Cortisol and Latent Alpha-amylase (sAA) on Predicting Probability of Being in Different Classes that Capture the Co-development in Externalizing and Internalizing Problems	98
Figure 3.2 Five-class Solution of Cross-domain Latent Class Growth Analysis.....	99
Figure 3.3 Interaction of Cortisol and Alpha-amylase on the Probability of Being in Stable Comorbid Class (with the low class as the reference group)	100

GENERAL INTRODUCTION

Background and Objective

Childhood aggression is consistently associated with adolescent delinquency and criminality in adulthood (Coie & Dodge, 1998). A large body of research has established that childhood aggression is one of the best predictors for later aggressive, antisocial, and criminal behaviors (Farrington, 1991; Huesmann et al., 1984; Moffitt, 1990) due to the continuity and stability of antisocial behavior. Individuals who are antisocial as children have been found to impose on-going financial costs to society throughout their lives and into adulthood (Cohen, 1998; Scott, Knapp, Henderson, & Maughan, 2001). Thus, understanding childhood aggression is informative in designing interventions and reducing crime in adulthood for both safety and financial consideration.

Aggressive and rule-breaking behaviors in childhood and adolescence are widely studied in criminology, and these behaviors are commonly referred to as externalizing problems in the psychology literature. Despite different terminology, the same constructs, that is, delinquency/ aggressive behavior in criminology and externalizing problems in psychology are under investigation. Another seemingly opposite set of behavior problems, namely, internalizing problems, is investigated in psychology research along with externalizing problems because of their moderate degree of comorbidity (Kessler & Wang, 2008; Nock et al., 2007). Internalizing problems refer to problems mainly within the self, including anxiety, depression, somatic complaints and withdrawal behavior (Achenbach & Rescorla, 2001).

Theoretical propositions have been postulated to understand these two types of behavior problems based on their differential physiological correlates and behavior manifestations, such as the over- and under arousal hypotheses (Kagan, 1994). Research that examines both categories of behavior problems is needed to advance our understanding of both the commonalities and differences of their biological and psychosocial correlates, given their seemingly opposite symptoms yet positively correlated relation.

Life adversity and stress such as unemployment, family disruption and disadvantaged neighborhood have been a focus in criminology research (Farrington, 2000; McCord, 2001; Rutter & Giller, 1983; Sampson & Laub, 1993; Sampson, Raudenbush, & Earls, 1997; Wilson & Herrnstein, 1985), but very few studies in criminology have taken into account the stress physiology in their investigation of the association between life adversity and antisocial behavior. Contemporary theorists in psychology champion the role that individual differences in stress system play on children's behavior problems, given that stress system lays foundation to how we react to stress and adversity (Gunnar & Quevedo, 2007; Johnson, Riley, Granger, & Riis, 2013; Kertes, Gunnar, Madsen, & Long, 2008). This dissertation focuses on the linkage of stress physiology to behavior problems. I investigate the interplay of different components of stress systems on behavior problems, and how this physiology-behavior association is further conditional on environmental factors.

The two major components of the psychobiology of the stress response, the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS),

produce signaling molecules that may have synergistic, opposing, and redundant effects (Chrousos & Gold, 1992). The HPA axis and the ANS are grounded in related brain regions (Young et al., 2005), but the two systems function differently. For example, the HPA axis activity occurs more in situations featured emotional distress and loss of control, when subjects become immobile/ passive (Lundberg & Frankenhaeuser, 1980; Henry, 1993) whereas ANS activation taps more into a “defense reaction” (Folkow, 1985; Henry, 1993), an active, effortful response to challenges that are manageable and controllable. In addition, HPA axis activity has shown habituation after repeated exposure to stressors but ANS activation has been found generally responsive and shown uniform activation patterns with repeated exposure to stressor (Gordis et al., 2006; Schommer, Hellhammer, & Kirschbaum, 2003). Hence, the examination of the combined effect of the two systems provides unique rather than redundant information. Non-invasive measurement (in saliva) of the activity of the HPA (cortisol) and ANS (salivary alpha-amylase, sAA) opens windows of opportunity to explore this possibility in youth (Granger et al., 2012). Up till now, only a handful of published studies (El-Sheikh et al, 2008; Gordis et al., 2006; Allwood et al., 2011) have examined whether and how interactions between the HPA axis and ANS are associated with behavior problems. In Paper 1 of this dissertation I take advantage of this technology advancement and test the interactive nature of these two components of stress system to further our understanding of children’s behavior problems.

Built on Paper 1, I include harsh discipline, an environmental stressor in my inquiry of behavior problems in Paper 2. Harsh discipline encompasses a wide spectrum

of physical and verbal behavior towards offspring in face of conflict including corporal punishment, shouting, and threats (Reid, Patterson, & Snyder, 2002). Studies report that children's exposure to these negative discipline behaviors early in life is associated with later adjustment problems including higher rates of aggressive behavior (Gershoff, 2002; Gershoff, et al., 2012) and anxiety and depression (McLoyd et al., 2007; Rodriguez, 2003; Wang & Kenny, 2014). Nonetheless, these effects are not universal in that not all individuals raised in such adverse family circumstances display behavior problems later in life—adversity places some at risk, but others are resilient (e.g., Toth & Cicchetti, 2013). The impact of harsh discipline may conditional on individuals' biological wiring, particularly their stress system. Investigating how these two components interact with harsh discipline is not a new idea but only became more feasible to carry out among the youth recently with the advances of technology allowing non-invasive and easy-to-implement measures of both systems.

The first two papers adopt a variable-centered approach and cross-sectional design, and additional insight could be gained on behavior problems, particularly the co-occurrence of the externalizing and internalizing problems, through a person-centered approach with follow-up data capturing behavior development. The person-centered approach is premised on the assumption that there is unobservable heterogeneity in the development of behavior and that the heterogeneity is linked to different correlates (Muthén & Muthén, 2000). Previous studies on behavior problems typically dealt with the two spectrums of behavior problems either as independent by conducting analyses separately for each spectrum of behavior problems (Allwood et al.,

2011; El-Sheikh et al., 2008); or they include the covariance between the two behavior problems in the model at the variable level. Few studies have put the co-occurrence of behavior problems as the center of investigation and explore potential correlates that differentiate different developmental courses of behavior problems.

Investigation into the co-occurrence of behavior problems and its correlates would contribute to our theoretical construction of the physiology-behavior association. The over- and under arousal hypotheses have been proposed to account for internalizing and externalizing behavior problems (Bauer et al., 2005; Kagan, 1994; Raine et al., 2005). The over- arousal hypothesis for internalizing problems postulates that some children have over-aroused central nervous system functioning which may stem from a lower activation threshold. As a result, they are more prone to fearfulness and anxiety and compensate such hyper-arousal with withdrawal and avoidance. In contrast, the under-arousal hypothesis for externalizing problems states that some children have under-aroused central nervous system. The under-arousal state may implicate fearlessness and result in difficulty in conditioning and socialization through punishment or parenting (Raine, 2005); or indicate a relatively unpleasant state and individuals may behave aggressively to seek stimulation to increase arousal levels back to normal (Zuckerman, 1979). The over- and under-arousal hypotheses for behavior problems are intuitive yet they cannot account adequately for the co-occurrence of both internalizing and externalizing problems in one individual. Paper 3 is sought to further our understanding of behavior problems with a person-centered approach. Paper 1 adds to our knowledge of the interactive patterns of HPA axis activity and ANS arousal on

behavior problems at a variable level with a cross-sectional design adjusting for the covariance of the two spectrums of behavior problems, and Paper 3 further tests whether physiological features of the stress system can distinguish individuals who are clinically comorbid with externalizing and internalizing problems over a one-year period from those with only one spectrum of behavior problems (i.e., externalizing-only or internalizing-only).

In sum, in this dissertation I examine how HPA axis activity and ANS arousal, indexed by salivary analytes, interact to influence the levels of behavior problems (Paper 1), and how the interplay of HPA axis activity and ANS arousal further condition the association between harsh discipline and behavior problems (Paper 2). Last, I test whether the interplay of HPA axis activity and ANS arousal can predict co-occurrence of externalizing and internalizing problems from other short-term prototypical development of behavior problems such as externalizing-only and internalizing-only development (Paper 3).

Data Summary

Data in all three papers was drawn from the Philadelphia Healthy Brains and Behavior (HBB) study which was administered in 2008-2012. The project goal of the HBB study was to identify risk and protective factors for aggression and to test the effectiveness of the treatments for children with high levels of aggressive behaviors. Participants were recruited by advertisements within the city of Philadelphia and contiguous suburbs. Participants completed an initial assessment and follow-up assessments 3, 6, and 12 months later. Exclusion criteria for initial enrollment were

diagnoses of a psychotic disorder, intellectual disability, pregnant, a pervasive developmental disorder or current medication use with the potential to interfere with the measurement of salivary analytes. Participants came to university laboratories where data were collected. The initial assessment lasted 8 hours over a two-day period with the saliva samples, psychophysiological measures and questionnaires collected on the first day. After the initial assessment, high aggressive participants (defined as being diagnosed with oppositional defiant disorder (ODD) or diagnosed with conduct disorder (CD) and/or scoring at least one standard deviation above a normed population mean on the reactive or proactive components of the Reactive Proactive Aggression Questionnaire) were randomized into a control group or treatment groups (i.e., cognitive behavior therapy, nutritional supplements or the combine of the two). Participants received treatment, if assigned, between the initial assessment and the 3-month follow-up. A more comprehensive description of the HBB sample and recruitment can be found in Liu et al. (2013). Caregivers gave informed consent and youth gave assent after description of the study was given. The HBB project was approved by the University of Pennsylvania and the Philadelphia Department of Health human subjects boards.

The eligible sample from the initial assessment include 446 participants (51 % male) who self-identified as White (n = 53, or 11.88%), African American (n = 358, or 80.27%), Hispanic (n = 4, or .90%), Native American (n = 2, or .45%), Asian American (n = 2, or .45%), multiracial (n = 22, or 4.93%) and other ethnicities (n = 4, or .90%). On average they were 11.88 years of age (SD = .59), and with an average score of 3.27

(SD = .99) on Tanner's five stages of development. Parents/guardians accompanying children to the lab received an average of 13.38 years of schooling (SD = 2.25), and their monthly income was on average 1,710.46 dollars (SD = 1,692.86). Regarding their marital status, 15.92% of the parents/ guardians were either divorced or separated, 56.50% never married, and only 24.44 % were married and still lived with their spouses. Paper 1 and Paper 2 report results using data only from the initial assessment while Paper 3 utilizes data from all four waves. Fifty two of the four hundred and forty six participants enrolled too late to allow sufficient time for follow up within the time frame of the larger project. Not all participants had complete data on behavior problems throughout all four waves (7.62% missing at the 3-month follow-up; 15.02% missing at the 6-month follow-up; and 32.96% missing at the 12-month follow-up).

**PAPER 1. INTERACTION OF ADRENOCORTISOL ACTIVITY AND
AUTONOMIC AROUSAL ON CHILDREN'S EXTERNALIZING AND
INTERNALIZING PROBLEMS ¹**

Abstract

The psychobiology of stress involves two major components, the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS). Research has revealed the association between behavior problems and the psychobiology of stress, yet findings are inconsistent and few studies have addressed the moderate correlations between behavior problems. This study examines the individual and interactive effects of HPA and ANS on child behavior problems while taking into account the comorbidity of externalizing and internalizing problems. Four saliva samples were collected from each participant in a community sample (N = 429; aged 11-12 years; 50.49% male), which were assayed for cortisol (HPA) and alpha-amylase, sAA (ANS). Children's behavior problems were assessed using parent-report and self-report versions of the Child Behavior Checklist. Latent variables were constructed to represent trait-like individual differences in cortisol and sAA. Low levels of HPA axis activity were associated with higher levels of both externalizing and internalizing problems, but only among children with low ANS arousal. The association between externalizing and internalizing problems diminished to non-significant after taking into account the influence of HPA axis activity and ANS arousal, which suggests that the psychobiology

1. A version of this paper was published as Chen, F. R., Raine, A., Soyfer, L., & Granger, D. A. (2015). Interaction of adrenocortical activity and autonomic arousal on children's externalizing and internalizing problems. *Journal of Abnormal Child Psychology*, 43, 189-202.

of stress explains a fair proportion of comorbidity of behavior problems. The findings support that interaction between HPA axis and ANS functioning has potential to clarify prior mixed findings and advance our understanding of the child behavior problems.

Keywords cortisol; alpha-amylase; externalizing problems; internalizing problems; comorbidity; multisystem

Introduction

Contemporary theorists champion the role that individual differences in stress responsive and environmentally sensitive biological systems play on children's behavior problems (Gunnar & Quevedo, 2007; Johnson, Riley, Granger, & Riis, 2013; Kertes, Gunnar, Madsen, & Long, 2008). The two major components of the psychobiology of the stress response, the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS), produce signaling molecules that may have synergistic, opposing, and redundant effects (Chrousos & Gold, 1992). The complex interconnected nature of these components suggests that a multi-system approach will be conducive to advance our understanding of children's behavior problems (El-Sheikh et al., 2008; Gordis, Granger, Susman, & Trickett, 2006; Raine, 2005). This approach has potentials to clarify the mixed finding from studies examining only one system at a time. Up till now, only a handful of published studies (El-Sheikh et al., 2008; Gordis et al., 2006; Allwood et al., 2011) have examined whether and how interactions between the HPA axis and ANS are associated with behavior problems. The present study extends these findings by taking a rigorous methodological approach. We aim to examine the independent and interactive effects of HPA axis activity and ANS arousal

on the levels of externalizing and internalizing behavior problems in late childhood while taking into account the comorbidity of externalizing and internalizing problems.

Components of the Psychobiology of Stress

The psychobiology of the stress response has two major components. The first involves activation of the HPA axis and the secretion of cortisol, the main glucocorticoid in humans, into circulation. HPA stress response was sometimes termed as “defeat reaction”, a response with features of emotional distress and loss of control, when subjects become immobile/ passive (Lundberg & Frankenhaeuser, 1980; Henry, 1993). In line with this proposition, studies found that HPA axis response occur more likely when the situation is uncontrollable and generates emotional distress (Kirschbaum & Hellhammer, 1994; Lundberg & Frankenhaeuser, 1980; Peters et al., 1998).

The second involves activation of the ANS and the release of catecholamines (e.g., norepinephrine) into the blood stream (Chrousos & Gold, 1992). This component facilitates quick and diffuse reactions commonly known as “fight or flight” responses such as elevated heart rate and enhancing blood flow to muscles (Cannon, 1914). ANS activation was referred to as “defense reaction” (Folkow, 1985; Henry, 1993), an active, effortful response to challenges that are manageable and controllable. Consistent with this description, studies found that the defense reaction was enhanced for controllable stressors (Lundberg & Frankenhaeuser, 1980; Peters et al., 1998) or for individuals with tendency to exert high effort to gain control (Bauer, Quas, and Boyce, 2002;

Frankenhaeuser, Lundberg, & Forsman, 1980). In addition, studies suggested that this component of stress response was generally responsive and shows uniform activation patterns with repeated exposure to stressor (Gordis et al., 2006; Schommer, Hellhammer, & Kirschbaum, 2003).

Individual differences in the levels of HPA axis activity and ANS arousal can be assessed non-invasively in saliva (Granger et al., 2012). Examination of HPA axis activity through measuring salivary cortisol has been investigated and its validity has been extensively documented (Hellhammer, Kirschbaum, & Belkien, 1987; Kirschbaum & Hellhammer, 1994). Numerous studies and reviews have investigated salivary alpha-amylase (sAA) as a surrogate marker for ANS activity (Granger et al., 2007; Nater et al., 2005; Nater & Rohleder, 2009; Rohleder & Nater, 2009). Salivary alpha-amylase is an enzyme and its release is elicited by activation of the ANS, particularly the involvement of sympathetic nervous system. Salivary AA has been found to increase under stressful conditions known to increase other ANS markers like heart rate, plasma catecholamines and systolic blood pressure (Bosch et al., 2003; Chatterton et al., 1996; Skosnik, Chatterton, Swisher, & Park, 2000). In addition, administration of the beta-adrenergic antagonist propranolol reduced sAA (Granger et al., 2007; van Stegeren, Rohleder, Everaerd, & Wolf, 2006).

Behavior Problems and Psychobiology of Stress

Externalizing Behavior Problems and the Psychobiology of Stress

Both HPA axis activity and ANS arousal have been linked to externalizing problems (Oosterlaan, Geurts, Knol, & Sergeant, 2005; Raine, 2005). Low levels of cortisol have been found to be associated with conduct disorder (McBurnett et al., 2005; Pajer et al., 2001), disruptive behavior (McBurnett, Lahey, Rathouz, & Loeber, 2000), and clinically significant levels of externalizing problems (Cicchetti & Rogosch, 2001). The inverse association between cortisol and externalizing problems might stem from the role that the HPA axis plays in potentiating the state of fear, generating sensitivity to punishment, and inducing withdrawal behavior (Schulkin, Gold, & McEwen, 1998; van Honk et al., 2004). However, a meta-analysis based on 72 studies with 5,480 subjects (Alink et al., 2008) revealed that the relationship between the basal cortisol and externalizing behavior problems was significant with an effect size of -0.1, much smaller than what previous research had concluded (Azar et al., 2004; Hart, Gunnar, & Cicchetti, 1995; van Bokhoven, Mathys, van Goozen, & van Engeland, 2005; van Goozen, Fairchild, Snoek, & Harold, 2007; van Goozen et al., 2000). In sum, results have not been consistent regarding the inverse relation between HPA axis activity and externalizing problems.

Studies also reveal a relation between ANS arousal and externalizing problems (Raine, 2005; Sijtsema, Shoulberg, & Murray-Close, 2011). Low resting heart rate has been found to be a reliable predictor of antisocial behavior in non-institutionalized children and adolescents (Ortiz & Raine, 2004). A meta-analysis by Lorber (2004) showed that low skin conductance level was a correlate of children externalizing behavior, particularly for conduct problems. Low autonomic arousal may implicate

fearlessness, resulting in difficulty in conditioning and socialization through punishment or parenting (Raine, 2005). In line with this, impaired electrodermal fear conditioning at age 3 is associated with aggressive behavior at age 8 as well as criminal behavior 20 years later at age 23 (Gao et al., 2010a, 2010b). Alternatively, stimulation-seeking theory suggests that low arousal may be a relatively unpleasant state and individuals may behave aggressively to seek stimulation to increase arousal levels back to normal (Zuckerman, 1979). Nevertheless, the inverse relation between ANS arousal and externalizing problems has not been consistent (Lorber, 2004).

Internalizing Behavior Problems and the Psychobiology of Stress

Internalizing problems have been associated with high levels of HPA axis activity and ANS arousal, although findings are again mixed. It has been hypothesized that some children have over-aroused central nervous system functioning which may stem from a lower activation threshold (Kagan, 1994). As a result, they are more prone to fearfulness and anxiety and compensate such hyper-arousal with withdrawal and avoidance. Regarding HPA axis functioning, higher levels of cortisol have been found to be related to withdrawn temperament, inhibited characteristics, and internalizing emotion (Davis, Donzella, Krueger, & Gunnar, 1999; Kagan, Reznick, & Snidman, 1988; Schmidt et al., 1997; Smider et al., 2002). A recent meta-analysis found a tendency for depressed children to have higher basal cortisol levels than non-depressed controls (Lopez-Duran, Kovacs, & George, 2009). However, studies investigating cortisol levels with anxiety disorder were less consistent. Some observed increased cortisol activity (Vreeburg et al., 2010; Wedekind et al., 2000) while some found

normal cortisol activity (Gurguis, Mefford, & Uhde, 1991). Similarly, findings on the association between cortisol and post-traumatic stress symptoms are inconsistent (De Bellis, 2001; Cicchetti & Rogosch, 2001; Weems & Carrión, 2007). Generally, studies on behaviorally inhibited and shy children have found more consistent results than those using clinical samples (Dierckx et al., 2012).

High levels of ANS arousal have also been linked to internalizing problems. Studies on inhibited children have found level elevation in several autonomic indicators (Kagan et al., 1988). Other related research has suggested elevated autonomic sympathetic reactivity for children with high levels of anxiety (Beidel, 1991). Exaggerated ANS arousal has been found among adults with anxiety disorders (Cole et al., 1996; Harrison & Turpin, 2003), and youth with anxiety (Klimes-Dougan et al., 2001; Weems et al., 2005). There is also evidence that depressed youths showed stronger ANS arousal (Nock & Mendes, 2008; Shannon et al., 2007), yet results are not entirely consistent, with some showing an association in the opposite direction or no relation at all (Tonhajzerova et al., 2010).

Sources of inconsistent findings

The mixed findings for the relationship between behavior problems and the psychobiology of stress may derive from at least three sources. First, the consistent focus on one biological stress system at a time hinders research on links between psychobiological stress responses and behavior problems. Recently researchers have advocated examining the combined effects of multiple subsystems (Young, Abelson, &

Cameron, 2005). The HPA axis and the ANS are grounded in related brain regions (Young et al., 2005), but the two systems function differently. For example, they habituate at different rates (Schommer, Hellhammer, & Kirschbaum, 2003). In addition, some research suggests that the HPA axis is activated by negative affect associated with stress, including fear and frustration, whereas the ANS is valence non-specific (Lovallo & Thomas 2000). Given that both systems are associated with behavior problems and that results are not entirely consistent, examining the joint effects of these systems may provide additional information about the nature of their relations with behavior problems. For instance, El-Sheikh et al. (2008) found that the interaction of the basal levels of the two systems was indeed associated with behavior problems, with concurrently high HPA axis activity and ANS arousal associated with the highest risk.

Second, the methodological issue of obtaining reliable assessments of basal HPA axis activity and ANS arousal (Granger et al., 2012) may contribute to the mixed findings. Variability in either HPA axis activity or ANS arousal at any given moment can derive from different sources, including basal arousal, arousal attributable to momentary situational influences, and measurement errors (Kenny & Zautra, 2000; Shirtcliff, Granger, Booth, & Johnson, 2005; Taylor et al., 2012). For these reasons, it is hard to obtain reliable measures for basal levels unless a strict protocol is implemented to obtain such well-timed single sample (Granger et al., 2012). Alternatively, a common practice is to derive the average of multiple measures to represent the basal level (El-Sheikh et al., 2008). This approach certainly has its merit, yet the issue remains that in observed scores, the basal level of interest (i.e., the true

score) is intermixed with noise (i.e., measurement errors and other sources of variability not contributable to the “true score”). Structural equation modeling (SEM) can be used to resolve this issue by forming latent variables to represent the “true score”. SEM differentiates the variance of physiological measures into components of trait, state and error variances. Isolating the variance in salivary analyte levels attributable to stable intrinsic “trait-like” sources has been demonstrated using both cortisol (Booth, Granger, & Shirtcliff, 2008; Kirschbaum et al., 1990; Shirtcliff et al., 2005) and sAA (Out et al., 2011; Out, Granger, Sephton, & Segerstrom, 2013; Taylor et al., 2012). In the present study, we employed a multiple time point assessment strategy and SEM as a tactic to better represent individual differences in HPA axis activity and ANS arousal at the trait-like level.

Third, the comorbidity of internalizing and externalizing problems may play a part in the mixed findings. Previous studies tend to either examine one category of behavior problems or treat categories of behavior problems as orthogonal. This is surprising, because comorbidity of externalizing and internalizing problems in childhood is the norm rather than the exception (Nottelmann & Jensen, 1995). The National Comorbidity Survey Replication (NCS-R), a nationally representative household survey on the epidemiology of mental disorders in the United States, highlights the prevalence of comorbidity. For instance, seventeen other child-onset and adolescent-onset disorders (e.g., panic disorder and anxiety disorder) were found to be significantly associated with childhood attention-deficit/hyperactivity disorder (ADHD) and had an elevated bivariate odds ratio in predicting ADHD (Kessler et al., 2005).

About 92% of the respondents with lifetime oppositional defiant disorder (ODD) meet criteria for at least one other lifetime disorder, including internalizing problems such as anxiety and mood disorder (Nock, Kazdin, Hiripi, & Kessler, 2007). Impulse-control disorders (e.g., ODD, ADHD and conduct disorder) were highly comorbid with each other and with early-onset anxiety disorder (Kessler & Wang, 2008). In addition, a moderate correlation was consistently found between externalizing and internalizing behavior problems (Jaffee, Moffitt, & Caspi, 2002; Reitz, Dekovic, & Meijer, 2006; Youngstrom, Loeber, & Stouthamer-Loeber, 2000). Hence, the correlated nature of externalizing and internalizing problems should be taken into account when examining the relation of the psychobiology of stress and behavior problems.

Present Study

This study aims to test whether and how stable “trait-like” individual differences in HPA axis activity and ANS arousal contribute, independently and in combination, to children’s externalizing and internalizing problems. To address the possible sources of the mixed findings in the field, this study examined the interaction of the HPA axis and ANS, used SEM for more reliable assessments of the basal levels of the two systems, and took into account the comorbidity of externalizing and internalizing problems. In accordance to the under- and over-arousal hypothesis on externalizing and internalizing problems (Kagan, 1994; Raine, 1993), we anticipated that concurrently low levels of HPA axis activity (indexed by cortisol) and ANS arousal (indexed by sAA) would be associated with high levels of externalizing behavior, whereas concurrently high levels of both systems would be associated with high level of internalizing problems.

Methods

Overview

Participants in this study were enrolled from 2008 to 2012 in the Philadelphia Healthy Brains and Behavior (HBB) project. The HBB project aims to identify environmental and biological risk factors for aggression in childhood. Children (aged 11 to 12 years) and their caregivers were recruited by advertisements within the city of Philadelphia and contiguous suburbs. Exclusion criteria for the larger project were diagnoses of 1) a psychotic disorder, 2) mental retardation, 3) pervasive developmental disorder, 4) current psychiatric treatment or treatment, or 5) current medication use with the potential to interfere with the measurement of salivary analytes (Granger et al., 2009). A comprehensive description of the HBB sample and recruitment can be found in Liu et al. (2013). The data used here were collected as a component part of the HBB study initial assessment. Participants came to a university laboratory where saliva samples were collected and behavioral assessments were completed. Caregivers gave informed consent and youth gave assent after description of the study was given. The HBB project was approved by the University of Pennsylvania and the Philadelphia Department of Health human subjects boards.

Participants

Of the original eligible HBB study participants (N =446), sixteen children who presented as multivariate outliers were excluded (see Statistical Analysis section), yielding a valid sample of 429 children. On average they were 11.88 years of age (SD

= .59), with 210 males and 219 female children, and the majority were African-American (N = 343; 80.0%), or Caucasian (N = 52; 12.1%). Parents/guardians accompanying children to the lab received 13.39 years (SD = 2.22) of schooling, and their monthly income was 1723.33 dollars (SD = 1,704.80). About 16% of the parents/guardians were either divorced or separated, over 55% were never married, and only 25% were married and still lived with their spouses in the same household. Based on parent reports on the Child Behavior Checklist (CBCL), 29.8% and 25.8% of the participants were above the borderline clinical range on externalizing and internalizing problems. Based on the Youth Self-Report (YSR) on behavior problems, 24.7% and 34.8% were above the borderline clinical range on externalizing and internalizing problem.

Behavioral Assessments

Behavior problems were assessed with the Child Behavior Checklist (CBCL) and the Youth Self-Report (YSR) (Achenbach & Rescorla, 2001). The CBCL is a parent-report measure of children's behavior problems and the YSR is a self-report questionnaire modeled on the CBCL. There are 113 and 112 items on CBCL and YSR respectively. These items are rated on a 3-point Likert scale (0 = *not at all true*; 2 = *very true*). Both CBCL and YSR can be scored in terms of two broadband scales: one for externalizing behavior problems, including rule-breaking behavior and aggressive behavior syndrome subscales; and one for internalizing behavior problems, including anxious/depressed, withdrawn/depressed, and somatic complaints syndrome subscales. Externalizing and internalizing problems scales have excellent test-retest reliability

($r=0.91$ and 0.92 for CBCL, and $r=0.80$ and 0.89 for YSR) and internal consistency (Cronbach's $\alpha = 0.90$ and 0.94 for CBCL, 0.90 and 0.90 for YSR; Achenbach & Rescorla, 2001). The raw scores of externalizing problems and internalizing problems from CBCL and YSR were used in the current study to form latent variables of externalizing and internalizing problems.

Collection and Determination of Salivary Analytes

Across a single day, four saliva samples were collected from each participant. Participants were instructed to refrain from food and drink (except water) prior to sample donation (Granger et al., 2012). Following Granger et al.'s suggestions (2007), whole, un-stimulated saliva was collected by passive drool. Three samples were collected in the morning at 15-min intervals, and a fourth sample was collected in the afternoon before a series of lab tasks. Between sample collections, participants completed other behavior assessments for the HBB study. On average sample collection times were 9:16, 9:36, 9:58 AM and 12:40 PM. Following collection, samples were frozen at -80°C until assay.

On the day of testing, all samples were centrifuged at 3,000 rpm for 15 minutes to remove mucins. Following Granger et al. (2007), samples were assayed for sAA using a commercially available assay kit for the kinetic measurement of sAA activity (Salimetrics, State College PA). The assay utilizes a chromogenic substrate, 2-chloro-*p*-nitrophenol linked to maltotriose. The enzymatic action of sAA on this substrate yields 2-chloro-*p*-nitrophenol, which can be spectrophotometrically measured at 405 nm using

a standard laboratory plate reader. The amount of sAA activity present in the sample is directly proportional to the increase over a 2 minute period in absorbance at 405 nm. Coefficient of variation is less than 10% for both intra- and inter-assay.

Samples were assayed for salivary cortisol using a commercially available enzyme immunoassay (Salimetrics, State College, PA). The test used 25 μ l of saliva for singlet determinations and had a range of sensitivity from 0.007 to 3 μ g/dl. Samples were all assayed in duplicate and the averages of cortisol concentration were used in the current analysis. Coefficient of variation is less than 5% for intra-assay and less than 10% for inter-assay. Trait-like measures of cortisol and sAA were modeled as latent variables with four corresponding sample analytes as indicators (Out et al., 2011; Shirtcliff et al., 2005; Taylor et al., 2012).

Statistical Analyses

Univariate outliers for cortisol and sAA at each time point were defined as three standard deviations away from the mean and they were treated as missing values. Additionally, to eliminate multivariate outliers, we examined the Mahalanobis distance as suggested by Tabachnick and Fidell (2012) for all the variables in the model, with a very conservative cut-off p value (.001). This resulted in total exclusion of 16 participants (i.e., 3.6% of the incoming sample).

Two models were tested using Mplus 7 (Muthén & Muthén, 1998-2012). The model estimation method was maximum likelihood with robust standard error (MLR). Full information maximum likelihood (FIML) was employed to handle missing data.

The first model only had control variables (i.e., age, gender, ethnicity, and saliva collected time) and the outcomes. The second model included control variables, main effects, and the interaction between trait-like cortisol level and trait-like sAA level as predictors (see Figure 1.1). Trait-like cortisol and trait-like sAA were formed with the corresponding analytes assayed from the four samples as manifest indicators. The latent variables of externalizing and internalizing problems were assessed by the corresponding subscales of the CBCL and the YSR. Errors from the same reporter sources were freely correlated due to the common method. In addition, to account for the moderate correlation between externalizing and internalizing problems, the residual variances of the latent variables externalizing and internalizing problems were freely correlated as well. All predictors were centered to facilitate the interpretation.

Latent variables have arbitrary metrics, thus it is useful to present and plot interaction effects in terms of standardized latent variables (Muthén, 2012). Significant interactive terms were probed by testing the regression slope of the high (+1.5) and low (-1.5) standardized values of the moderator (Aiken & West, 1991). If the slope is significantly different from zero, it means that at this particular level of moderator there is significant association between the independent variable and outcome (Aiken & West, 1991; Jaccard, Wan & Turrisi, 1990). In the current study we did the probing twice for each significant interaction, one with trait-like sAA as the moderator and one with trait-like cortisol as the moderator.

Results

Descriptive statistics

The correlations among indicators are presented in Table 1.1. Indicators for behavior problems were significantly correlated with each other across report sources and across categories. Cortisol indicators were significantly related with each other and sAA indicators correlated significantly with each other as well, but there were no significant correlations between cortisol indicators and sAA indicators.

As seen in Table 1.2, there was a moderate correlation ($r = .52$) between the latent variables of externalizing and internalizing problems, consistent with previous studies (Youngstrom et al., 2000). Trait-like cortisol significantly correlated with trait-like sAA ($r = .46$). Trait-like cortisol was inversely related to both externalizing and internalizing problems. In addition, African American has higher externalizing and internalizing problems compared to Caucasian. There was a positive association between the first sample collected time and internalizing problems, indicating that children with more internalizing problems showed up later in the laboratory for the study.

Interaction of trait-like cortisol and trait-like alpha-amylase on behavior problems

We tested two models, and each of them converged to an admissible solution. Standardized coefficients are reported in Table 1.3. Model 1 only had control variables as predictors, and overall they explained little variance of externalizing and

internalizing problems, only 3.7% and 6.1%, respectively. After accounting for control variables, there was still significant shared variance between externalizing and internalizing problems. Model 2 included trait-like cortisol, trait-like sAA, and their interaction as predictors in addition to control variables. This model accounted for 9.4% of the total variance of externalizing problems and 22.7% of internalizing problems. As shown in Table 1.3, the interaction of trait-like cortisol and trait-like sAA was significant for both externalizing ($\beta = .16$, Wald's $z = 2.32$, $p = .02$) and internalizing problems ($\beta = .17$, Wald's $z = 2.16$, $p = .03$). The main effects of trait-like cortisol on both externalizing and internalizing problems were significant, with lower trait-like cortisol associated with higher externalizing and internalizing problems.

After modeling trait-like cortisol, trait-like sAA, and their interaction on externalizing and internalizing problems, the shared variance between externalizing and internalizing problems was no longer significant. We also tested an additional model identical to model 2 but with only the three morning indicators to construct latent variables for trait-like cortisol and trait-like sAA. Findings from this additional model were very similar to that of model 2, including the significant interactive effects, main effects of trait-like cortisol, and non-significant covariance between externalizing and internalizing problems.

To further the examination of the interaction effects of model 2, we probed and plotted them in terms of standardized latent variables, as revealed in Figure 1.2 panel a and b. High and low levels of the moderator were chosen as 1.5 and -1.5. We first probed the interaction with trait-like sAA as the moderator. For both externalizing and

internalizing problems, trait-like cortisol had a *negative* effect among children with *low* trait-like sAA level, but not among children with high trait-like sAA level. We also probed the interaction with trait-like cortisol as a moderator. There was a *negative* association between sAA and externalizing problems but only among children with *low* trait-like cortisol levels. In contrast, there was a *positive* association between sAA and internalizing problems but only among children with *high* trait-like cortisol levels. As depicted in Figure 1.3, the profiles for externalizing and internalizing problems were very similar, except that the line of high trait-like sAA shifts higher for internalizing problems compared to that for externalizing problems.

Discussion

This study examined the interaction of HPA axis activity and ANS arousal on externalizing and internalizing behavior problems. The primary findings confirm prior research at a conceptual level by underscoring the importance of a multisystem approach. Results indicated that the interaction of HPA axis activity and ANS arousal was associated with both externalizing and internalizing problems. Both externalizing and internalizing problems were *inversely* related to HPA axis activity but only among children with *low* ANS arousal. A novel observation of the current study was that after taking into account individual differences in the independent and combined influence of HPA axis activity and ANS arousal, the degree of association between externalizing and

internalizing behavior problems was significantly attenuated, which provided additional insights into the comorbidity issue.

Concurrently low levels of HPA axis activity and ANS arousal were associated with externalizing problems, which is in accordance with several previous studies (Gordis et al., 2006; de Vries-Bouw et al., 2012). This is also consistent with the physiological under-arousal hypothesis of externalizing problems (van Goozen et al., 2007). Children with under-arousal in both systems may have a higher activation threshold and appear to lack of fear (Kagan, 1994). It is possible that they have altered perceptions of stressors or threats in general which is reflected in their under-responsive physiological levels. Individuals with such physiological features of low levels across both systems are more susceptible to aggressive and antisocial behaviors compared to those with a low level in just one system. As a result, children may be less sensitive to punishment or socialization as postulated by fearlessness theory (Raine, 1993), or attempt to reach an optimal level of arousal by seeking out excitement and stimulation which in turn leads to antisocial behavior (Raine, 2002; Zuckerman, 1979).

In addition, above and beyond any interactive effect, HPA axis activity had a significant main effect on externalizing problems. As shown by a meta-analysis on 72 studies (Alink et al., 2008), there was a significant inverse association between HPA axis activity and externalizing problems but the effect size was smaller than expected. This finding could be explained by the significant interaction and significant main effect of HPA axis activity in the current study: ANS arousal moderates the inverse relation between HPA axis activity and externalizing problems, and when studies unknowingly

oversampled children with high ANS arousal, the relation between HPA axis activity and externalizing problems might be attenuated and contribute to inconsistent findings. Nevertheless, overall cortisol has a main effect, and it is this main effect that was revealed by previous meta-analysis. Furthermore, Shirtcliff et al. (2005) used the same trait-like approach for HPA axis activity and found it to be negatively related to externalizing problems. The current study confirmed Shirtcliff et al.'s (2005) findings and provides additional insight by looking into the moderating role of ANS arousal.

Whereas the findings on externalizing problems are confirmatory (Shirtcliff et al., 2005), the inverse association between HPA axis activity and internalizing problems is novel and does not follow the expectations based on the theoretical formulation that internalizing problems are associated with physiological over-arousal (El-Sheikh et al., 2008; Kagan, 1994). Instead, internalizing problems were linked to *low* HPA axis activity and this relation was moderated by ANS arousal. There were few studies that investigated the interaction of HPA axis activity and ANS arousal on internalizing problems with a trait-like variable approach. There have been a few studies focused on direct basal measures or reactivity measures for HPA axis and ANS, yet findings were mixed. For instance, Vigil et al. (2010) revealed an *inverse* association between basal cortisol and internalizing problem only among children with *high* sAA. El-Sheikh et al. (2008) found a *positive* association between cortisol and internalizing problems only among children with *high* sAA. Comparisons between studies are difficult given differences in methods, sample size and characteristics, and outcome measures. The

present study has several key methodological strengths and consequentially we suspect our findings are both valid and reliable.

Low cortisol constitutes what has been termed as “*hypocortisolism*” (Heim, Ehlert, & Hellhammer, 2000), a fairly common phenomenon associated with prolonged or chronic stressors and challenges (Hellhammer & Wade, 1993). Hypercortisolism has garnered the majority of research interest on internalizing problems (Badanes, Watamura, & Hankin, 2011). However, the past decade has witnessed increasing attempts to examine the psychopathology of hypocortisolism (Fries, Hesse, Hellhammer & Hellhammer, 2005; Gunnar & Vazquez, 2001; Heim et al., 2000). Hypocortisolism has been viewed as an indicator of maladaptation (McEwen, 1998) and reflection of allostatic load (Gunnar & Vazquez, 2001). Low basal cortisol has traditionally been postulated to be associated with externalizing problems (Alink et al., 2008; Raine, 1993), but a growing body of research is currently linking it to internalizing problems, including depression (Cicchetti, Rogosch, Gunnar, & Toth, 2010; Hart et al., 1995; Kaufman, 1991), posttraumatic stress disorder (Goenjian et al., 1996; Yehuda, Boisoneau, Mason, & Giller, 1993) and withdrawal behaviors (Alink, Cicchetti, Kim, & Rogosch, 2012). Badanes, Watamura and Hankin (2011) proposed that low cortisol may be an important marker for allostatic load for children, and for psychopathological outcomes such as depression and anxiety. The inverse link of HPA axis activity to both externalizing and internalizing problems revealed in the current study is intriguing, as it provides empirical support for the aforementioned theoretical formulation of hypocortisolism.

However, caution is warranted that hypocortisolism was not, at least in the current study, consistently associated with child behavior problems: it was moderated by ANS arousal. High ANS arousal appears to buffer individuals from behavior problems in the face of hypocortisolism. This is consistent with numerous studies that proposed the protective effect of heightened ANS arousal among vulnerable populations (Brennan et al., 1997; Lösel & Farrington, 2012; Portnoy et al., 2013; Raine, Venables, & Williams, 1995). HPA axis activity may augment the influence of ANS arousal on behavioral outcome, and when both systems are at a low level, the body fails to achieve an optimal activity level and to efficiently regulate physiological arousal (Bauer et al., 2002). Interestingly, contradictory to our prediction, concurrently high levels of HPA axis activity and ANS arousal was not associated with high risk for either dimension of behavior problems. It is possible that when individuals are under-responsive physiologically, it is a more prominent sign of maladjustment than when they are over-responsive, and children compensate such under-arousal with maladaptive behaviors, both self-direct (internalizing) and/or other-direct (externalizing) behaviors (Kagan, 1994; Zuckerman, 1979).

By forming more reliable measures at a latent level for HPA axis activity, ANS arousal, externalizing problems, and internalizing problems, we revealed relations among them that did not appear with their respective indicators (see Table 1.1 and Table 1.2). For example, we found no significant correlation between direct baseline measures for HPA axis activity and ANS arousal (see Table 1.2), consistent with previous findings (El-Sheikh et al., 2008; Vigil et al., 2010). However, trait-like level of

HPA axis activity and ANS arousal showed a *positive* correlation. It is likely that the correlation between HPA axis activity and ANS arousal were attenuated by large momentary fluctuations and situational influences when using baseline measures directly, whereas a trait-like approach models out such measurement noise. The moderate correlation between HPA axis activity (indexed by trait-like cortisol) and ANS arousal (indexed by trait-like sAA) suggests an inherent consistency of stability across stress response systems within individuals.

Lastly, the novel observation that HPA axis activity and ANS arousal attenuate the association between externalizing and internalizing problems suggested that the psychobiology of stress has the potential to account for the comorbidity of externalizing and internalizing problems. Two competitive hypotheses have been proposed to explain comorbidity (Bauer et al., 2002). One proposed that there was a physiological profile that globally classifies children as “at risk”, whereas the other postulated that comorbidity may be associated with one specific physiological pattern which substantively differs from those associated with only one category of behavior problems. Our findings, to some degree, support the first hypothesis. As revealed in the results, the unexplained covariance between externalizing and internalizing problems diminished after adding the independent and interactive effects of HPA axis and ANS, and the interactive patterns on externalizing and internalizing problems are similar. Hence, it may be this similar profiles in HPA axis and ANS of externalizing and internalizing problems rather than qualitatively different physiological profiles from pure forms of behavior problems that are associated with comorbidity,

This study has several limitations. First, it was cross-sectional and such a design precludes strong conclusions regarding causal direction. It is unclear whether physiological profiles (HPA axis activity and ANS arousal) predispose children to behavior problems or the development of behavior problems alters such physiological profiles. Second, we only had cortisol and sAA measures from a single day, which could not account for possible day-to-day variation. Future studies are needed with data from several days to form a trait-like measure of cortisol and sAA. Third, the participants in this study were in a narrow age range (i.e., age 11-12), although it provided a rather homogenous sample, the conclusion drawn from this study may not generalize to youth of other ages.

Despite these limitations, the findings suggest the possibility of a global interactive pattern of HPA axis activity and ANS arousal on externalizing and internalizing problems, and underscore the importance of a multi-system approach. Future studies may look into the interactive effects of psychobiology of stress on the stability and developmental trajectories of behavior problems, and how they may relate to treatment responsiveness so as to have practical implications. Research in this area is inevitably growing both more complex and sophisticated and future research needs to incorporate advanced analytical strategies such as growth mixture modeling to appropriately address the complexity of research design.

Paper 1 Tables and Figures

Table 1.1 Correlations among All Indicators (N = 429)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.ext_CBCL	1																
2.int_CBCL	.50**	1															
3.ext_YSR	.38**	.15**	1														
4.int_YSR	.09	.16**	.57**	1													
5.Cort1	-.08	-.05	-.06	-.06	1												
6.Cort2	-.05	-.02	-.03	-.02	.96**	1											
7.Cort3	-.07	-.05	-.07	-.07	.97**	.84**	1										
8.Cort4	-.06	-.03	-.04	-.05	.60**	.92**	.28**	1									
9.sAA1	-.01	.05	-.04	-.02	-.02	-.04	.02	-.05	1								
10.sAA2	-.04	-.01	.01	.06	-.02	-.05	.02	-.06	.68**	1							
11.sAA3	-.12*	-.10	-.04	.05	.03	.01	.05	.05	.55**	.73**	1						
12.sAA4	-.03	.02	.01	.10	-.01	.01	.00	.02	.52**	.61**	.58**	1					
13.Sex	.11*	.04	.03	-.07	.02	-.04	.05	-.05	.01	-.02	-.02	-.13**	1				
14.Age	.00	.02	.00	-.04	.04	-.02	.09	-.02	.01	-.04	-.02	.02	-.01	1			
15.Ethnicity	.10	.06	.10*	.10*	.04	.03	.03	.02	.08	.05	.07	-.01	-.05	.04	1		
16.Time1	-.03	.03	.06	.09	.03	-.12*	.10*	-.10*	.04	-.05	-.04	-.10*	.04	-.05	.07	1	
17.Time2	-.01	-.02	.01	.03	.12*	.03	.10	-.01	.00	.02	.01	.05	.01	-.05	.06	.11*	1
Means	9.58	10.74	7.28	13.48	.20	.198	.191	.146	76.16	89.35	87.63	144.13					
SD	8.97	7.76	6.70	8.36	.48	.284	.521	.181	77.16	82.74	75.69	102.28					

Note. ext_CBCL = externalizing subscales of Child Behavior Checklist; ext_YSR = externalizing subscale of Youth Self-Reports; int_CBCL = internalizing subscales of Child Behavior Checklist; int_YSR = internalizing subscale of Youth Self-Reports; Cort = cortisol; sAA = salivary alpha-amylase; Sex: 0 = girls, 1 = boys; Ethnicity: 0 = Caucasian, 1 = African American; Time1 = first saliva sample collected time; Time2 = last saliva sample collected time; * p < .05, ** p < .01

Table 1.2 Correlations among Predictors and Outcome Variables (N = 429)

	1	2	3	4	5	6	7	8	9
1.Externalizing problems	1								
2.Internalizing problems	.52**	1							
3.Trait-like cortisol	-.42**	-.39**	1						
4.Trait-like sAA	-.13**	.03	.46**	1					
5.Sex	.14**	-.13**	.05	-.01	1				
6.Age	.01	-.04	.11*	-.01	-.02	1			
7.Ethnicity	.20**	.33**	.13*	.06	-.05	.04	1		
8.Time1	.05	.27**	-.15**	-.03	.05	-.07	-.07	1	
9.Time2	.002	.02	.18**	.03	.02	-.05	.06	.11*	1

Note. sAA = salivary alpha-amylase; Sex: 0 = girls, 1 = boys; Ethnicity: 0 = Caucasian, 1 = African American; Time1 = first saliva sample collected time; Time2 = last saliva sample collected time;

* $p < .05$, ** $p < .01$

Table 1.3 Combined Effects of Cortisol and Alpha-amylase on Levels of and Covariance between Externalizing and Internalizing problems

	Model 1			Model 2				
	β^a	Wald's z^b	p	R^2	β^a	Wald's z^b	p	R^2
<i>Externalizing problems</i>				3.7%				9.4%
Sex	.12	1.78	.07		.03	1.72	.09	
Age	-.001	-.01	.99		.13	.38	.70	
Ethnicity	.15	2.56*	.01		.19	2.92**	.004	
Time1	.03	.43	.67		-.007	-.101	.920	
Time2	-.01	-.20	.84		.03	.40	.69	
Trait-like cortisol	-	-	-		-.36	-3.02**	.003	
Trait-like sAA	-	-	-		-.11	-1.29	.20	
Trait-like cortisol * trait-like sAA	-	-	-		.16	2.32*	.02	
<i>Internalizing problems</i>				6.1%				22.7%
Sex	-.07	-.70	.48		.01	-.68	.50	
Age	-.02	-.26	.80		-.07	-.070	.95	
Ethnicity	.18	1.72	.09		.19	1.86	.06	
Time1	.15	1.45	.15		-.12	-1.12	.26	
Time2	-.01	-.14	.89		.03	.30	.76	
Trait-like cortisol	-	-	-		-.36	-2.60**	.01	
Trait-like sAA	-	-	-		.01	.08	.94	
Trait-like cortisol * trait-like sAA	-	-	-		.17	2.16*	.03	
Covariance between internalizing & externalizing	7.06 ^c	2.54*	.01		4.79 ^c	1.53	.13	

Note. sAA = salivary alpha-amylase; Sex: 0 = girl, 1 = boys; Ethnicity: 0 = Caucasian, 1 = African American; Time1 = first saliva sample collected time; Time2 = last saliva sample collected time;

a. standardized coefficients; b. based on unstandardized coefficients and corresponding standard errors; ^c unstandardized coefficients;

* $p < .05$, ** $p < .01$

Figure 1.1 Combined Effect of Trait-like Cortisol and Trait-like Alpha-amylase on Behavior Problems.

Caption: Externalizing problems freely correlate with internalizing problems to take into account comorbidity. Common methods of behavior problems measures and measurement errors were adjusted. Models were controlled for age, gender, ethnicity and saliva collected time.

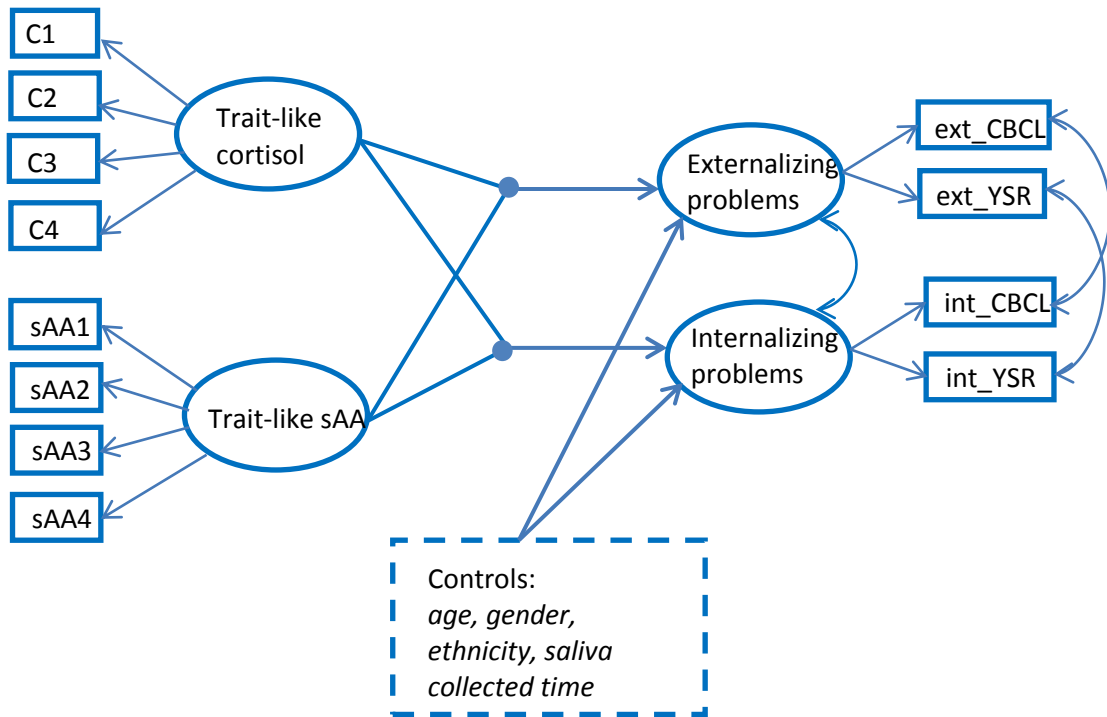


Figure 1.2 Salivary Alpha-amylase Moderates the Relation between Cortisol and Externalizing Problems (panel a) and between Cortisol and Internalizing Problems (panel b)

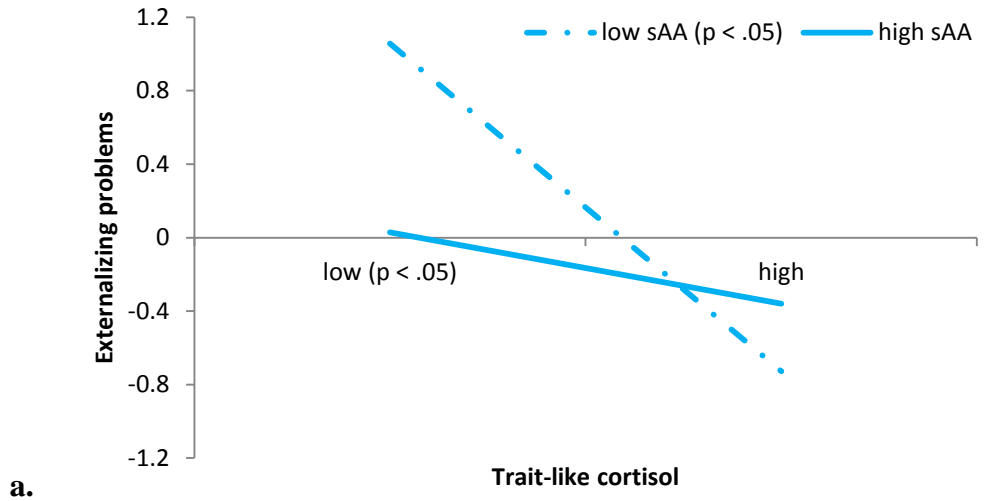
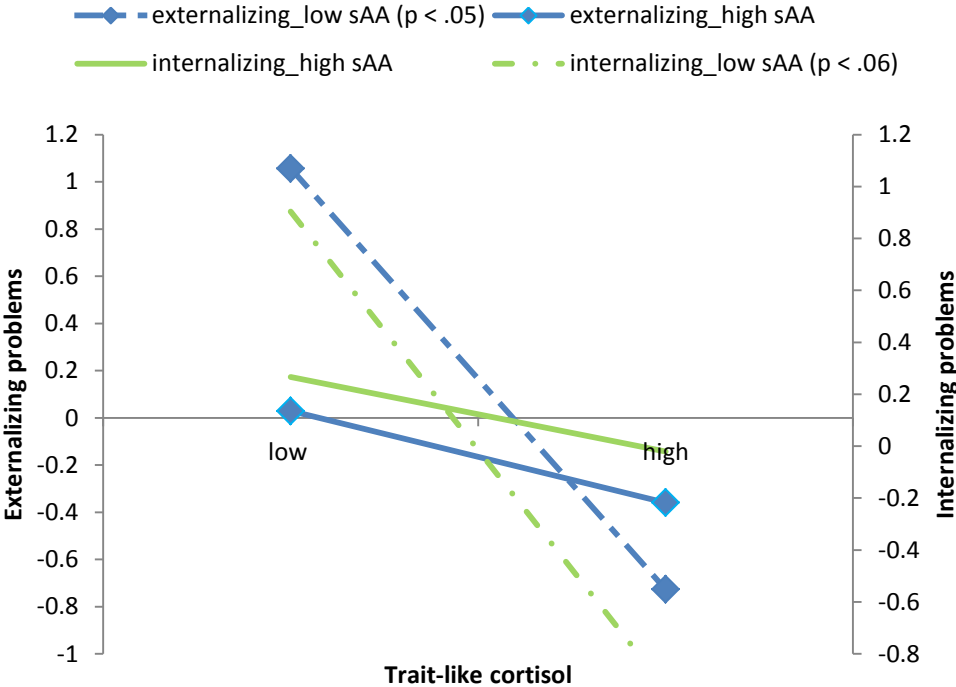


Figure 1.3 Similar Interactive Patterns of Cortisol and Salivary Alpha-amylase on Externalizing and Internalizing Problems



PAPER 2. HARSH DISCIPLINE AND BEHAVIOR PROBLEMS: THE MODERATING EFFECTS OF CORTISOL AND ALPHA-AMYLASE²

Abstract

Numerous studies link harsh discipline to adjustment problems in youth, yet not all individuals exposed to harsh discipline develop behavior problems. Contemporary theory suggests that this relationship could be moderated by individual differences in environmentally sensitive biological systems. This study investigated whether the interaction between hypothalamic-pituitary-adrenal (HPA) activity and autonomic nervous system (ANS) arousal moderated the link between harsh discipline and behavior problems. Three saliva samples were collected on a single day from 425 inner city youth (50% male, age 11-12 yrs, 80% African American) and were later assayed for cortisol (HPA) and alpha-amylase (ANS). Problem behavior was assessed by self- and parent- report using the Child Behavior Checklist. Youth also reported the level of harsh discipline that they experienced. Harsh discipline was positively associated with externalizing *and* internalizing problems only when there were asymmetrical profiles of HPA activity and ANS arousal. This pattern was evident for boys but not girls. Findings are discussed in relation to prevailing theories suggesting that biological susceptibility translates adversity into risk for behavior problems.

Keywords externalizing problems, internalizing problems, cortisol, salivary alpha-amylase, harsh discipline

² A version of this paper was published as Chen, F. R., Raine, A., Rudo-Hutt, A., Glenn, A. L., Soyfer, L., & Granger, D. A. (2015). Harsh parenting and child behavior problems: The moderating effects of cortisol and salivary alpha-amylase. *Biological Psychology*, 104, 19-27.

Introduction

Harsh discipline behavior encompasses a wide spectrum of physical and verbal behavior towards offspring including corporal punishment, shouting, and threats (Reid, Patterson, & Snyder, 2002). Studies report that children's exposure to these negative discipline behaviors early in life is associated with later adjustment problems including higher rates of aggressive behavior (Gershoff, 2002; Gershoff, et al., 2012) and anxiety and depression (McLoyd et al., 2007; Rodriguez, 2003; Wang & Kenny, 2014). It is noteworthy that these findings have been corroborated in many countries and cultures (e.g., Csorba, et al., 2001; Steely & Rohner, 2006). Nevertheless, these effects are not universal in the sense that not all individuals raised in such adverse family circumstances express behavior problems later in life—adversity places some at risk, but others are resilient (e.g., Toth & Cicchetti, 2013).

Developmental science has drawn upon notions of *diathesis-stress* and *differential susceptibility* to explain these individual differences (Belsky & Pluess, 2009; Monroe & Simons, 1991). The diathesis-stress model (or dual hazard model) postulates that biological vulnerability is exacerbated by environmental adversity (Monroe & Simons, 1991; Raine, 2005). In contrast, differential susceptibility emphasizes the role of individual differences in developmental plasticity. That is, individuals with high plasticity are assumed to be more affected by either supportive or adverse features of their social environments, whereas individuals with low plasticity show less biobehavioral reactivity to their circumstances regardless of the valence (Belsky & Pluess, 2009). Central to both models is the notion that environmentally

sensitive biological systems moderate the effects of adversity on the development of behavior problems. Most recently, researchers have operationalized individual differences in environmentally responsive biological processes as related to problem behavior with a multi-system approach and tested main and interactive effects of, hypothalamic-pituitary-adrenal (HPA) axis activity and autonomic nervous system (ANS) arousal (Chen et al., 2015a; El-Sheikh et al., 2008; Gordis et al., 2006). In the present study we attempt to extend our understanding about these complex relationships to new limits by investigating whether the interaction between HPA activity and ANS arousal moderates the link between harsh discipline and behavior problems in a large sample (n = 425) of predominantly low socioeconomic status, urban dwelling African American children (50% male, 11-12 years of age).

The psychobiology of the stress response has two major components, the HPA axis and the ANS. Activation of the HPA axis culminates in the secretion of cortisol into circulation. This response is relatively slow (in minutes) as the primary signaling molecules with this system, must be synthesized *de novo*. Studies have shown that HPA axis activation is more likely to occur when the situation is novel or unfamiliar, uncontrollable, involves social evaluative threat, and generates emotional distress (Gerra et al., 2001; Kirschbaum & Hellhammer, 1994; Peters et al., 1998; Schommer, Hellhammer, & Kirschbaum, 2003). HPA axis reactivity and regulation is considered a “defeat reaction” (Henry, 1993). By contrast, activation of the ANS occurs very rapidly (in seconds) and involves the release of catecholamines into the blood stream (e.g., Chrousos & Gold, 1992; Lundberg & Frankenhaeuser, 1980). ANS arousal facilitates

fast and diffuse reactions which are collectively known as “fight or flight” responses and include changes in physiology such as elevated heart rate and blood pressure (Cannon, 1914). ANS activation is considered a “defense reaction” (Folkow, 1985; Henry, 1993) -- an active, effortful response to environmental demands that are manageable and controllable (Lovallo & Thomas, 2000; Schommer et al., 2003). Individual differences in the levels of HPA axis activity and ANS arousal can be estimated non-invasively by assessing salivary cortisol and alpha-amylase (sAA) (Hellhammer, Kirschbaum, & Belkien, 1987; Kirschbaum & Hellhammer, 1994; Granger et al., 2007; Nater & Rohleder, 2009).

The nature of the coordination between the ANS and HPA axis reactivity and regulation in response to environmental demands is complex. At the molecular and cellular level, it is assumed that activity of the HPA axis has potential *permissive*, *stimulating*, *suppressive* and *preparative* actions on the ANS (Sapolsky, Romero & Munck, 2000). Permissive actions are typically associated with levels of cortisol whereas the other three actions are linked to acute stress-induced change in cortisol production. At the behavioral level, Bauer and colleagues (2002) proposed two working models which have been used extensively to explore the coordinated effects of the ANS and HPA on problem behavior. They hypothesized that an optimal level, the medium level, of arousal is associated with the least risk of behavior problems based on the classic theories and empirical research concerning arousal and performance. In the *additive model* it is assumed that the HPA and ANS augment each other's effect. Thus, asymmetrical profiles of HPA axis and ANS (i.e., low HPA axis activity and high ANS

arousal, or high HPA axis activity and low ANS arousal) would achieve the optimal arousal level and be associated with the lowest levels of behavior problems. In contrast, in the *interactive model* the two systems are assumed to work in a suppressive fashion, that is, one suppresses the other's effect. Thus, symmetrical profiles of HPA axis and ANS (i.e., concurrently high or concurrently low levels of HPA axis activity and ANS arousal) would achieve the optimal arousal level and be associated with the least levels of behavior problems.

Multiple studies test these theoretical models but to date findings are incomplete. Stress-related cortisol reactivity was found to be negatively associated with aggression but only among those with corresponding low sAA reactivity (Gordis et al., 2006). By contrast, cortisol levels were positively associated with both externalizing and internalizing problems but only among those with high sAA levels, and no relation was detected between cortisol and behavior problems among those with low sAA levels (El-Sheikh, et al., 2008). In the largest study (N = 429), Chen et al. (2015a) reported that cortisol levels were negatively associated with both externalizing and internalizing problems but only among those with low sAA levels.

Present Study

This study aimed to test the individual and interactive effects of harsh discipline, cortisol and sAA on externalizing and internalizing problems. We employed a multiple time point assessment strategy for salivary sAA and cortisol and used a latent variable approach as a tactic to isolate the variance in each salivary analyte attributable to stable

individual differences (Taylor et al., 2012; Booth, Granger, & Shirtcliff, 2008; Out et al., 2011; Out et al., 2013). Variability in HPA axis activity or ANS arousal at any given moment can derive from trait-like intrinsic differences, activity attributable to momentary situational influence (e.g., Adam, 2006), and measurement error (Kenny & Zautra, 2001). By minimizing the influence of variation in salivary analytes attributable to momentary situational influence (and measurement error) we expected to increase the probability of revealing the relationship between harsh discipline, stress psychobiology, and problem behavior in our models.

Given the HPA axis has the potential to exert a permissive/augmenting action on the ANS (e.g., Sapolsky et al., 2000), we anticipated an *additive* rather than *interactive* model would reflect the nature of the combined effects of HPA axis and ANS (Bauer et al., 2002). Thus, asymmetrical instead of symmetrical profiles would be associated with the optimal arousal levels. Built on this prediction, we widened the frame of reference provided by Bauer et al.'s models by incorporating a biosocial component. That is, following the logic of *differential susceptibility*, we viewed asymmetrical profiles as an index of plasticity. Correspondingly, individuals expressing asymmetrical profiles would display the highest levels of behavior problems when experiencing high levels of harsh discipline but would display the lowest levels of behavior problems when experiencing low levels of harsh discipline. In contrast, following the logic of *diathesis-stress*, symmetrical profiles were viewed as biological vulnerabilities with a resulting arousal level being too high or too low. Symmetrical profiles would be associated with the highest levels of behavior problems when individuals experienced high levels of

harsh discipline because they would possess both biological and environmental vulnerability. We hypothesized that our test of the relationship between harsh discipline, stress psychobiology, and behavior problems would reveal support for the *differential susceptibility* rather than *diathesis-stress* model.

Methods

Overview

Boys and girls aged 11 and 12 years were enrolled from 2008 to 2012 in the Philadelphia Healthy Brains and Behavior (HBB) project. HBB aimed to identify risk and protective factors for aggression and to test the effectiveness of the treatments (i.e., cognitive behavior therapy and nutrition supplements) for children with high levels of aggressive behaviors. Participants were recruited by advertisements within the city of Philadelphia and contiguous suburbs. Participants completed an initial assessment at time 1 and were followed up one year later. The initial assessment included the collection of biological, psychological, physical, demographic and social data. Exclusion criteria were diagnosis of a psychotic disorder, mental retardation, pregnancy, a pervasive developmental disorder or current medication use with the potential to interfere with the measurement of salivary analytes such as steroid based anti-inflammatory (more details see Granger et al., 2009). There were 446 children in the HBB project. For a comprehensive description of the larger project see Liu et al. (2013). The data used in the present analyses were collected during the study's initial

assessment. Participants and their caregivers came to the university laboratories where data were collected. Caregivers gave informed consent and youth gave assent after description of the study was given. The HBB project was approved by the University of Pennsylvania and the Philadelphia Department of Health human subjects boards. Parents/caregivers were compensated with gift cards for their participation.

Participants

Of the 446 available participants, 21 had missing data on key measures (i.e., harsh discipline or saliva samples) and were therefore excluded from the analysis (see Statistical Analysis). The final analytic sample comprised 425 participants (50.12% male). On average they were 11.87 years old ($SD = .60$). The sample included participants who identified themselves as African-American ($N = 341$), White ($N = 50$), or other/mixed race/ethnicity ($N = 34$). On average, the household monthly income was \$2994.56 ($SD = \3173.39). Regarding caregiver marital status, 17.65% were divorced or separated, 56.71% never married, and 24.94% were married and living with their spouses (.71% were missing marital status data).

Behavioral Assessments

Behavior Problems

Child behavior problems were assessed with the Child Behavior Checklist (CBCL; parent-report) and the Youth Self-Report (YSR) (Achenbach & Rescorla, 2001). Both CBCL and YSR can be scored in terms of two broadband scales: one for externalizing behavior problems, including rule-breaking behavior and aggressive

behavior syndrome subscales; and one for internalizing behavior problems, including anxious/depressed, withdrawn/depressed, and somatic complaints syndrome subscales. Externalizing and internalizing problem scales have excellent test-retest reliability and internal consistency (Achenbach & Rescorla, 2001).

Normalized T scores derived from national samples were used in all analyses and descriptive statistics are reported in Table 2.1. For both externalizing and internalizing problems scales, scores above 63 are within the clinical range. Based on parent-reported behavior problems, 19.4% of the participant had externalizing problems above clinical level, and 16.7% had internalizing problems above clinical level. Based on self-reported behavior problems, 13.0% of the participants had externalizing problems above clinical level, and 20.7% had internalizing problems above clinical level.

Harsh discipline

Harsh discipline was measured via the Conflict Tactics Scale (CTS; Strauss, 1979). The frequency of certain discipline behaviors by parents in the past 12 months were assessed on a 6-point scale by the children (0=*never*; 5=*most of the time*). Physically harsh discipline was measured by the CTS minor assault/corporal punishment subscale (Strauss et al., 1998). This subscale has 3 items, including “parents throwing something at you”; “pushing, grabbing or shoving you”; and “slapping or spanking you”. Following Wang and Kenny (2014), we used three items from the psychological aggression subscale of CTS to measure verbally harsh discipline,

including “parents insulting or swearing at you”; “doing or saying something to spite you”; and “threatening to hit or throw something at you”. Children’s responses to all six items were summed to form a composite score for harsh discipline (Chronbach’s $\alpha = .86$).

Collection of Saliva and Determination of Salivary Analytes

Across a single day, three saliva samples were collected from each child at the initial assessment. The children were instructed to refrain from food and drink (except water) prior to sample donation (see Granger et al., 2012). Whole, un-stimulated, saliva was collected by passive drool (Granger et al., 2007). The first sample was taken at approximately 0900, the second 15 minutes later, and the third 30 minutes later. Between sample collections, participants completed a variety of questionnaires. Immediately after collection, specimens were frozen and stored at -80°C until assay. On the day of assay, samples were centrifuged at 3,000 rpm for 15 minutes to remove mucins. Following Granger et al. (2007), samples were assayed for sAA using a commercially available assay kit for the kinetic measurement of sAA activity without modification to the manufacturers recommended protocol (Salimetrics, State College PA). The coefficient of variation is less than 10% for both intra- and inter-assay. Samples were also assayed for salivary cortisol using a commercially available enzyme immunoassay (Salimetrics, State College, PA). The assay had a range of sensitivity from 0.007 to 3.0 $\mu\text{g}/\text{dl}$. Average intra- and inter-assay coefficients of variation were less than 5% and 10%. Latent measures of cortisol and sAA were modeled as latent variables with three corresponding sample collections as indicators (Chen et al., 2015a;

Out et al., 2013; Shirtcliff et al., 2005; Taylor et al., 2012). Descriptive statistics for sAA and salivary cortisol are reported in Table 2.1.

Pubertal stage, Body Mass Index, Household Income and Ethnicity

The children self-reported the stage of their genital, pubic hair, and breast development (Morris & Udry, 1980). They were presented with drawings illustrating Tanner's five stages of pubertal development and were instructed to choose the drawing closest to their own stage of development. The stages of pubic hair and breast (girls) or genital stage (boys) were averaged to yield an overall score of puberty stage for each participant. The children's height and weight were measured and body mass index (BMI) was determined (dividing weight (kg) by the square of the height (m²)). Caregivers reported demographic information including income and ethnicity of the children. See Table 2.1 for means and standard deviations.

Statistical Analysis

All analyses were conducted in Mplus 7 (Muthén & Muthén, 1998-2012) using maximum likelihood estimation with robust standard errors. There were two parts of analysis. First, we tested the measurement model for externalizing and internalizing problems, latent cortisol level and latent sAA level (see Figure 2.1). The latent variables of externalizing and internalizing problems were assessed by the corresponding subscales of the CBCL and the YSR. Errors from the same reporter sources (e.g., errors from parent-reported externalizing and internalizing problems) were freely correlated to account for common method. Univariate outliers for cortisol and sAA at each time point

were defined as three standard deviations away from the mean (Tabachnick & Fidell, 2012). There was one outlier in each of the three cortisol samples, and five, six and four outliers in each of the sAA samples. Outliers were treated as missing values. Full information maximum likelihood (FIML) was employed to handle missing data. That is, cases with missing data would be kept in the model estimation so that other information from these cases could be utilized. Six cases had missing data on all three morning saliva samples and were excluded from analysis because no information was available from these cases to construct latent levels of cortisol and sAA. Latent levels of cortisol and sAA were formed with the corresponding analytes assayed from the three samples as manifest indicators, and these indicators were regressed on the corresponding saliva collection time to tease out the variance of the indicators that was attributable to the time of sample collection. The measurement model was evaluated with χ^2 test, the Comparative Fit Index (CFI) and the Root Mean Square Error of Approximation (RMSEA) (Hu & Bentler, 1998; Kline, 2011). The model fits the data well if the χ^2 test is not significant, the CFI has a value above .90 and the RMSEA has a value below .05.

The second part of the analysis was to test whether and how harsh discipline interacts with latent cortisol and latent sAA to predict externalizing and internalizing problems. The latent variables of externalizing and internalizing problems, the latent levels of cortisol and latent sAA were constructed in the same way as in the measurement model. Cases with missing data on harsh discipline were excluded in the analysis ($N = 15$) because the multiplicative terms for interactions with latent variables can only be produced with non-missing data. We tested a model with the main effects,

all two-way interactive effects and the three-way interactive effect of latent cortisol, latent sAA and harsh discipline as predictors and with externalizing and internalizing problems as outcomes (see Figure 2.2). To account for the moderate correlation between externalizing and internalizing problems, the residual variance of the externalizing and internalizing problems was freely correlated. The model adjusted for pubertal stage, BMI, income and ethnicity. Continuous predictors were centered to facilitate interpretation. Given the potential sex differences in the physiology-behavior association (Netherton et al., 2004), the model in Figure 2.2 were estimated with a multi-group approach (i.e., two groups: boys and girls). That is, the measurement models and the effects of covariates were all constrained to be equal across sex, whereas all the main effects, two-way interactions and three way interaction on behavior problems were freely estimated across sex. Note that all the estimations were done only in one model. In order to get a parsimonious model (i.e., reduced model), we constrained all the non-significant main effects or interactive effects in both boys and girls to be equal across sex. A Wald test was used to examine whether the parameters that were freely estimated were significantly different across sex in the reduced model. The model had interaction terms including latent variables, thus conventional model fit indices such as CFI and RMSEA were not available.

If the three-way interaction was significant for either externalizing or internalizing problems, it was probed by two steps combining the traditional pick-a-point approach (Aiken & West, 1991; Cohen, Cohen, West, & Aiken, 2003; Jaccard & Turrisi, 2003) and the Johnson-Neyman technique (J-N technique; Hayes & Mathes,

2009; Johnson & Fay, 1950; Johnson & Neyman, 1936). First, following the pick-a-point approach, we chose high (mean +1SD) and low (mean -1SD) values of the latent sAA, and for each of them, we utilized the J-N technique to examine how latent cortisol moderated the relationship between harsh discipline and externalizing (or internalizing) problems by plotting the regions of significance. Regions of significance here referred to the value range of latent cortisol in which the harsh discipline had significant effects on behavior problems (Hayes & Mathes, 2009). We could, in theory, find such significant regions of latent cortisol for *every* value of latent sAA. However, to simplify the process and illustrate the general pattern, we chose high (mean +1SD) and low (mean -1SD) values of the latent sAA for such plotting. Therefore, for the low latent sAA level, we plotted a line with the slopes of harsh discipline on behavior problems for every value of latent cortisol, testing whether each slope was significant by referring to its 95% confidence interval, and examining the trend of the slopes. The same was done for high latent sAA level. Second, to further ease the interpretation, we plotted the relationship between harsh discipline and externalizing (or internalizing) problems for four factorial combinations of high (mean +1SD) and low (mean -1SD) latent levels of cortisol and sAA. If the slope is significantly different from zero, it means that for this particular combination of moderators there is a significant association between harsh discipline and externalizing (or internalizing) problems (Aiken & West, 1991; Jaccard, Wan, & Turrisi, 1990).

Results

Descriptive Statistics and Measurement Model

Bivariate correlations among all variables are reported in Table 2.2. All indicators of cortisol were correlated at high levels and all indicators of sAA were correlated at moderate to high levels. Externalizing and internalizing problems were correlated at a moderate to high level within each report source ($r = .56$ for parent-report and $r = .60$ for self-report). Self-reported and parent-reported externalizing problems were significantly correlated at a low to moderate level ($r = .34$), and internalizing problems across report sources were significantly correlated but at a low level ($r = .17$).

The measurement model for latent cortisol, latent sAA, externalizing and internalizing problems converged to an admissible solution and fit the data well as indicated by the model fit indices ($\chi^2_{(55)} = 68.78$, $p = .10$; CFI = .99; RMSEA = .024). As seen in Figure 2.1, all factor loadings were above .3 and most of them were above .8.

Interaction of Harsh Discipline, Latent Cortisol and Latent Salivary Alpha-Amylase on Behavior Problems: a Multi-Group Model

We tested the individual and interactive effects of harsh discipline, latent cortisol, and latent sAA on externalizing and internalizing problems with a multi-group model. We further simplified the model by constraining all non-significant main effects or interactive effects in both boys and girls to be equal across sex. The reduced model converged to an admissible solution. In the reduced model, only the main effect of latent cortisol and the three-way interaction on both externalizing and internalizing

problems, and the main effect of latent sAA on externalizing problems were freely estimated across sex (see Table 2.3), all other parameters were constrained to be equal across sex. The Wald z test showed that the aforementioned five parameters were indeed significantly different across sex (Wald $z = 12.335$, $df = 5$, $p = .03$). Overall, the model explained 19.04% and 65.56% of the variance of boys' externalizing problems and internalizing problems, and explained 14.04% and 57.41% of the variance of girls' externalizing problems and internalizing problems.

The main effects of harsh discipline on both externalizing and internalizing problems were significant for boys and girls, with higher levels of harsh discipline associated with higher levels of externalizing and internalizing problems. The three-way interaction was significant for externalizing and internalizing problems among boys but not girls (see Table 2.3). Furthermore, the main effect of latent cortisol and latent sAA were both inversely associated with boys' externalizing problems but not internalizing problems. In contrast, only the main effect of latent cortisol was significantly associated with girls' internalizing problems.

To examine the three-way interaction on boys' behavior problems, we first plotted the significant regions of latent cortisol for the impact of harsh discipline on externalizing problems by high versus low latent levels of sAA (see Figure 2.3). For low latent sAA level, when latent cortisol was smaller than the value of -0.1 (the mean of latent cortisol level is zero), the slopes of harsh discipline on externalizing problems were not significant, as the 95% confidence interval enclosed the value zero; when latent cortisol was above -0.1, the slopes of harsh discipline on externalizing problems

were significant (see panel a of Figure 2.3). For high latent sAA level, harsh discipline was associated with externalizing problems when latent cortisol was below the value of .10, as the 95% confidence intervals did not enclose the value of zero (see panel b of Figure 2.3). The pattern for internalizing problems mirrored that for externalizing problems: when low latent level of sAA was coupled with latent cortisol level above the value of -.05, or when high latent level of sAA was coupled with latent cortisol level below the value of .125, harsh discipline was positively associated with internalizing problems (see panel a and b of Figure 2.4).

The impact of harsh discipline on boys' externalizing and internalizing problems by the four factorial combinations of high and low latent cortisol and sAA are plotted in Figure 2.5, harsh discipline had a positive effect on externalizing and internalizing problems but only when asymmetrical profiles of latent cortisol and latent sAA were observed, that is, low latent cortisol coupled with high latent sAA, or high latent cortisol couple with low latent sAA. Harsh discipline had a null impact on both externalizing and internalizing problems when symmetrical profiles of latent cortisol and sAA were observed, that is, concurrently high or concurrently low levels of latent cortisol and sAA, as indicated by the flat lines in Figure 2.5. Boys with asymmetrical profiles had the highest levels in both externalizing and internalizing problems when experiencing high levels of harsh discipline.

Discussion

The study is the first (to the best of our knowledge) to document significant interactive effects among harsh discipline, ANS arousal, and HPA activity, on externalizing and internalizing problems in youth. More specifically, when cortisol and sAA displayed asymmetrical profiles (i.e. low-high or high-low), harsh discipline had an impact on both externalizing and internalizing problems; but when cortisol and sAA were symmetrical (i.e., low-low or high-high), harsh discipline had a null impact on externalizing and internalizing problems. Unexpectedly, this pattern was evident for boys but not girls. That is, boys with asymmetrical HPA activity and ANS arousal had the highest levels of externalizing and internalizing problems when experiencing high levels of harsh discipline and had the lowest levels of externalizing and internalizing problems when experiencing low levels of harsh discipline. Our findings underscore the importance of the multi-system approach to index individual differences in environmentally sensitivity biological processes (e.g., Bauer et al., 2002; Gordis et al., 2006) and can be interpreted as consistent with the main tenets of the *differential susceptibility* model (Belsky & Pluess, 2009).

Children with asymmetrical profiles of ANS arousal and HPA activity differed in their levels of behavior problems if placed in drastically different caregiving circumstances. It is tempting to speculate that asymmetry in ANS arousal and HPA activity indicates an openness or plasticity to influence by social forces. Of course, a firm conclusion is premature, but such an interpretation, in general, is consistent with the assumptions of the differential susceptibility model (Belsky & Pluess, 2009). The

model postulates that individuals who are most adversely affected by various stressors in life may be the very same ones who reap the most benefit from environmental support, including the absence of adversity. Thus, in theory, individuals who express asymmetrical profiles of ANS arousal and HPA activity may be the most at risk, but also following the logic of the differential susceptibility model, they may be most likely to benefit from intervention efforts aimed to change their adverse circumstances. If the observations noted here proved reliable, their implications could be far reaching as a potential mechanism for explaining why not all individuals raised in such adverse family circumstances express behavior problems later in life. Future studies aimed to repeat these findings and test this possibility seem well worthwhile.

It is equally interesting that the combined effects of harsh discipline and asymmetry in ANS arousal and HPA activity were observed on total problem behavior (*both* externalizing and internalizing) than either externalizing or internalizing problem behavior specifically. As noted above, children's exposure to these negative discipline behaviors early in life is associated with later adjustment problems including higher rates of aggressive behavior (Gershoff, 2002) but also anxiety and depression (McLoyd et al., 2007; Rodriguez, 2003; Wang & Kenny, 2014). The profound impact of comorbidity of externalizing and internalizing problems in children's lives is well documented (Kessler & Wang, 2008). Children with comorbid behavior problems have an earlier-onset and more serious and chronic problems (Oland & Shaw, 2005; Youngstrom, Findling, & Calabrese, 2003), worse developmental outcomes such as risky behavior, substance abuse and adult criminality (Fanti & Henrich, 2010; Keiley et

al., 2003; Nottelmann & Jense, 1995) and high usage rates of psychiatric services (Costello et al., 1996; Kovacs et al., 1988). In the present study, as in many previous reports, externalizing and internalizing problems were associated modestly or strongly. If asymmetry in ANS arousal and HPA activity could be construed as translating environmental adversity into differential risk for comorbidity this possibility could have important research implications.

Unlike what we observed for boys, the impact of harsh discipline on behavior problems appeared to exert an impact on girls' behavior problems relatively independent of their ANS arousal or HPA activity. No interactive effects were revealed for either externalizing or internalizing behavior problems. It is possible that harsh discipline has a more direct impact on girls' behavior problems. Alternatively, the impact of harsh discipline on girls' behavior problems could be moderated by other biological or social mechanisms that were not examined here. Indeed, contemporary theory suggests that females' responses to adversity may be more marked by a pattern of biological responses related to "tending-and-befriending" (Taylor et al., 2000) rather than the classic physiological responses associated with the males' "fight-or-flight" response. Using an evolutionary perspective, Taylor et al. (2000) argued that females' quieting and caring for offspring (i.e., tending) and affiliating with social networks (i.e., befriending) may be more effective for addressing threats. Correspondingly, biobehavioral mechanisms implicated in caregiving-attachment, such as oxytocin, opioids and dopaminergic pathways are more likely to be underpinning this "tending-and-befriending" pattern (Taylor, 2006). Thus, activity of the ANS and HPA may not

necessarily be the important biobehavioral mechanisms involved in moderating the association between harsh discipline and girls' behavior problems. At a minimum, our findings highlight the importance of considering the possibility of sex differences in the mechanisms thought to underlie individual differences in biological susceptibility.

This study has several advantages but also some limitations. First, we only assessed cortisol and sAA levels on a single day. The stability of the latent variables constructed from multiple samples within one day may be different than if multiple day measurements were employed. Second, the participants in this study were drawn from a very narrow age span ranging only from age 11 to 12, and most were of African-American descent. Thus, the conclusions drawn may not readily generalize to youth in different developmental stages and of other ethnic/racial backgrounds. Last, the differential susceptibility model is typically tested with environment features ranging from positive to negative. The absence of harsh discipline behavior does not equate to socially appropriate discipline behavior and discipline. Future research on this topic would be well served to study the spectrum of both positive-socially appropriate and negative-harsh discipline behavior. These relative limitations should be balanced with the strengths and significance of the study. We used a sophisticated measurement model to get less biased assessments for our predictors and outcomes. For example, externalizing and internalizing problems were constructed with measures from both self-reported and parent-reported sources which allowed us to model the common method bias; cortisol and sAA were modeled with a latent variable approach in order to tease out the variance attributable to momentary change and saliva collection time.

Furthermore, the relatively large sample size affords more statistical power than most studies to date and enabled us to satisfactorily test and interpret this complex three-way interaction.

Concluding Comment

This study highlights a potentially important role of the coordinated effects of the ANS and HPA as a mechanism in the linkage of environmental adversity and children's behavior problems. Findings, if replicated and robust, have potentials to advance our understanding of risk and resilience in face of adversity to new limits. They also suggest that in future studies more theoretical and empirical attention should be given to potential sex differences in our models of differential susceptibility.

Paper 2 Tables and Figures

Table 2.1 Means (Standard Deviation) of All Variables in the Model

		Boys (N = 213)	Girls (N = 212)	Overall (N = 425)
Externalizing problems (T score)	CBCL	54.50 (10.56)	52.66 (11.25)	53.58 (10.94)
	YSR	51.73 (10.71)	50.91 (10.55)	51.32 (10.63)
Internalizing problems (T score)	CBCL	53.34 (10.74)	51.86 (10.78)	52.60 (10.77)
	YSR	56.71 (9.93)	53.28 (10.47)	54.99 (10.33)
Harsh discipline		4.21 (5.60)	4.97 (5.69)	4.59 (5.65)
Cortisol ($\mu\text{g/dl}$)	Sample 1	.17 (.20)	.20 (.19)	.18 (.19)
	Sample 2	.19 (.16)	.21 (.21)	.20 (.18)
	Sample 3	.17 (.13)	.19 (.22)	.18 (.18)
salivary alpha-amylase (U/ml)	Sample 1	72.24 (59.71)	75.63 (57.61)	73.93 (58.63)
	Sample 2	84.23 (71.49)	88.79 (65.87)	86.51 (68.69)
	Sample 3	90.26 (79.43)	86.96 (67.30)	88.59 (73.48)
Saliva collection time (hour)	Sample 1	9.31 (.32)	9.29 (.37)	9.30 (.34)
	Sample 2	9.57 (.32)	9.55 (.37)	9.56 (.35)
	Sample 3	9.82 (.32)	9.81 (.38)	9.82 (.35)
Pubertal stage		3.06 (1.01)	3.47 (.93)	3.27 (.99)
BMI		20.97 (5.15)	22.75 (6.27)	21.86 (5.80)
Income		3271.37 (3550.91)	2724.14 (2734.12)	2994.56 (3173.39)
ethnicity	African- American	79.81%	80.66%	80.24%

Note. CBCL = Child Behavior Checklist; YSR = Youth Self-Report; BMI = Body mass index

Table 2.2 Correlations among Main Variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.EXT_CBCL	1														
2.EXT_YSR	.34**	1													
3.INT_CBCL	.56**	.17**	1												
4.INT_YSR	.13*	.60**	.17**	1											
5.cortisol 1	-.08	-.00	-.04	.04	1										
6.cortisol 2	-.08	.02	.01	.08	.81**	1									
7.cortisol 3	-.04	.03	.04	.13*	.54**	.78**	1								
8.sAA 1	-.08	-.07	-.00	-.01	.03	.04	.10*	1							
9.sAA 2	-.05	-.06	-.00	.01	.04	.03	.06	.67**	1						
10.sAA 3	-.04	-.07	-.04	.01	.02	.03	.09	.57**	.68**	1					
11.harsh discipline	.13**	.35**	.12	.29**	.03	.01	.03	-.04	-.07	-.07	1				
12.pubertal stage	-.06	.07	-.05	-.03	.08	.08	.07	.15**	.08	-.01	.07	1			
13.BMI	.04	.08	.12*	.05	-.00	-.01	.02	.04	-.03	-.13**	.03	.23**	1		
14.income	-.20**	-.22**	-.16**	-.19**	-.07	-.11*	-.08	-.02	-.01	-.02	.00	-.13*	-.11	1	
15.ethnicity	.07	.09	.11*	.08	-.01	-.01	-.02	.06	.02	.12*	.01	.11*	.07	-.26**	1

Note. EXT_CBCL = externalizing problems from parent-reported Child Behavior Checklist (CBCL); EXT_YSR = externalizing problems from Youth Self-Report (YSR); INT_CBCL = internalizing problems from parent-reported Child Behavior Checklist (CBCL); INT_YSR = internalizing problems from Youth Self-Report (YSR); BMI = body mass index; Ethnicity: 1 = African-American, 0 = non-African-American

* $p < .05$, ** $p < .01$

Table 2.3 Individual and Interactive Effects of Harsh Discipline, Latent Basal Cortisol and Latent Basal Alpha-amylase on Levels of Externalizing and Internalizing Problems in a Multi-group Structural Equation Model

	B (SE)		p	
Outcome variable: latent externalizing problems				
<i>Effects constrained to be equal across</i>				
<i>sex</i>				
Ethnicity	-.02 (1.40)		.89	
Pubertal stage	-.06 (.59)		.92	
BMI	.11 (.09)		.23	
Income	-.79** (.16)		.00	
Harsh discipline	.64** (.09)		.00	
Latent basal cortisol * latent basal sAA	-6.34 (8.53)		.46	
Harsh discipline *latent basal sAA	.17 (.19)		.37	
Harsh discipline *latent basal cortisol	-.43 (.85)		.61	
<i>Effects freely estimated for boys and girls</i>				
	<i>Girls</i>	<i>Boys</i>	<i>Girls</i>	<i>Boys</i>
Latent basal cortisol	2.68 (4.46)	-8.12* (4.17)	.55	.05
Latent basal sAA	-.15 (1.27)	-2.28* (.99)	.91	.02
Harsh discipline*latent basal sAA*latent basal cortisol	3.00 (2.69)	-5.57** (2.14)	.27	.009
Outcome variable: latent internalizing problems				
<i>Effects constrained to be equal across</i>				
<i>sex</i>				
Ethnicity	.65 (1.36)		.63	
Pubertal stage	-1.06* (.49)		.03	
BMI	.18* (.08)		.02	
Income	-.69** (.17)		.00	
Harsh discipline	.54** (.08)		.00	
Latent basal sAA	.27 (.94)		.77	
Latent basal cortisol * latent basal sAA	.22 (6.24)		.97	
Harsh discipline *latent basal sAA	.21 (.19)		.26	
Harsh discipline *latent basal cortisol	-.24 (.48)		.61	
<i>Effects freely estimated for boys and girls</i>				
	<i>Girls</i>	<i>Boys</i>	<i>Girls</i>	<i>Boys</i>
Latent basal cortisol	7.53* (2.7)	-.01 (3.69)	.006	.997
Harsh discipline*latent basal sAA*latent basal cortisol	1.37 (1.89)	-5.11** (1.64)	.47	.002

Note. B = unstandardized coefficients; SE = standard error; Ethnicity: 1 = African-American, 0 = non-African-American; * p < 0.05, ** p < 0.01

Figure 2.1 Measurement Model and Results for Latent Variable Cortisol, Salivary Alpha-amylase and Behavior problems

Caption: Circles represent latent variables and rectangles represent observed variables; EXT_CBCL = externalizing problems from parent-reported Child Behavior Checklist (CBCL); EXT_YSR = externalizing problems from Youth Self-Report (YSR); INT_CBCL = internalizing problems from parent-reported Child Behavior Checklist (CBCL); INT_YSR = internalizing problems from Youth Self-Report (YSR); sAA = saliva alpha-amylase; standardized coefficients are reported; * $p < .05$, ** $p < .01$

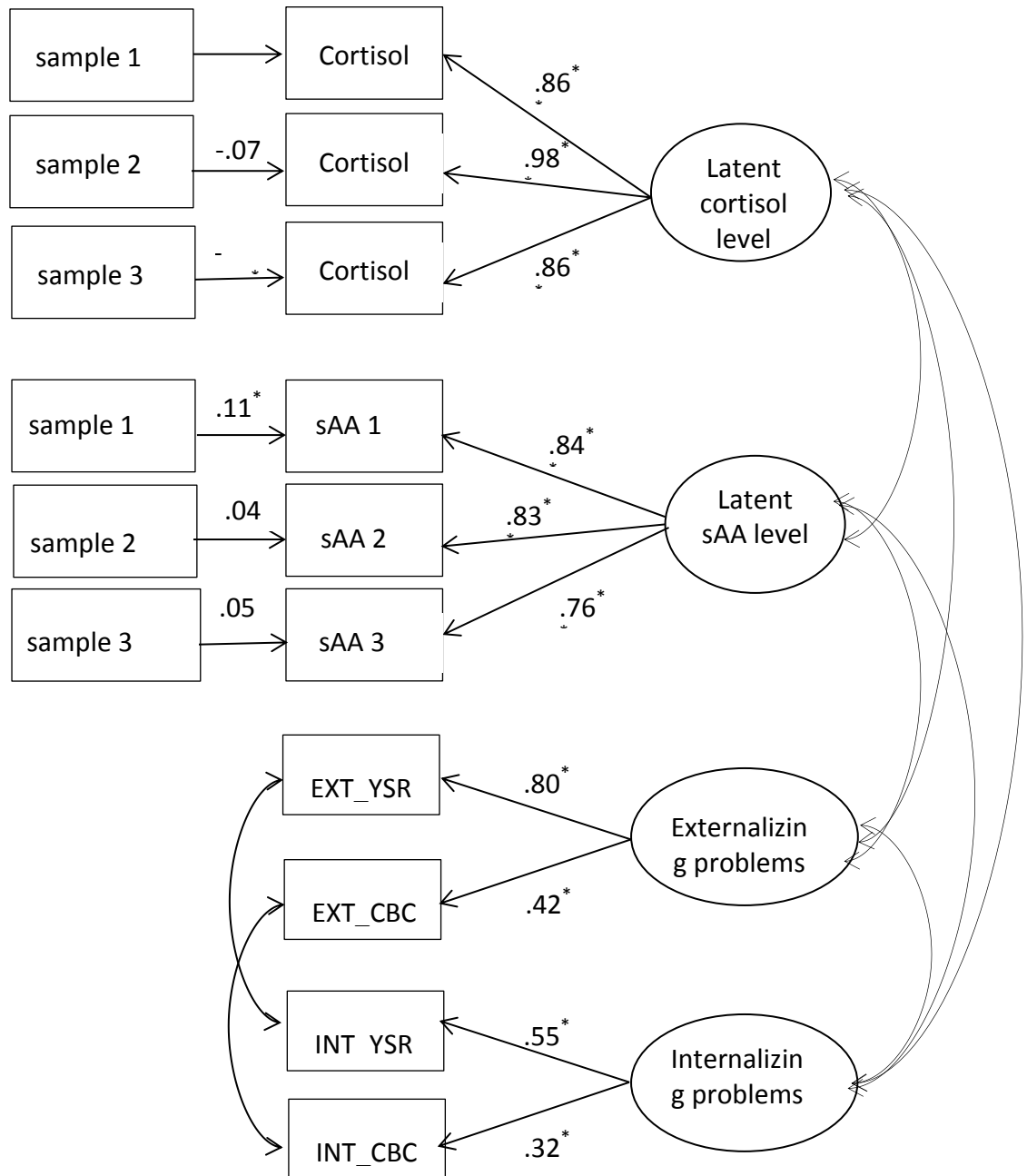


Figure 2.2 Individual and Interactive Effects of Harsh Discipline, Latent Basal Cortisol, and Latent Basal Salivary Alpha-amylase on Externalizing and Internalizing Problems

Caption: Model adjusted for income, BMI, puberty stage and ethnicity. Circles represent latent variables, rectangles represent observed variables and dots represent interaction involving latent variables. Indicators that were used to construct the latent variables are omitted in this figure. Green lines and dot represent the three-way interaction on externalizing and internalizing problems, the interest of the current study.

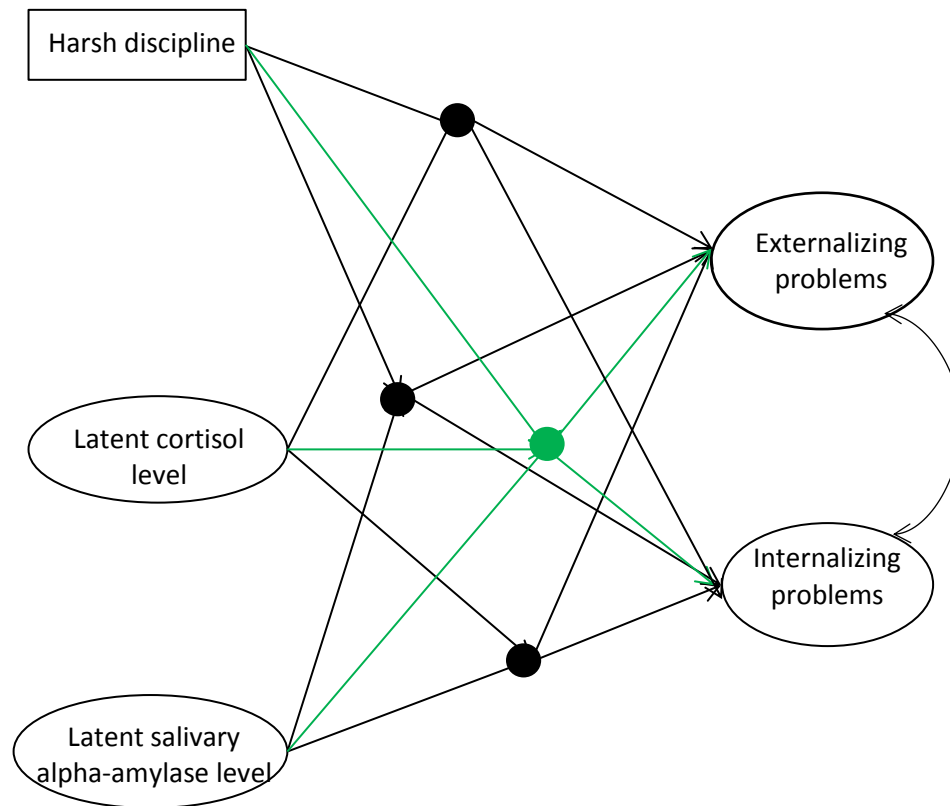


Figure 2.3 Latent Basal Cortisol Regions of Significance for Slopes of Harsh Discipline on Boys' Externalizing Problems by Low (panel a) and High Levels of Latent Basal Salivary Alpha-amylase (panel b)

Caption: When low latent basal sAA is coupled with latent basal cortisol above the value of $-.01$ (mean $=0$), the slopes of harsh discipline on externalizing problems are significant as the confidence intervals do not enclose the value of zero; when high latent basal sAA is coupled with latent basal cortisol below the value of $.10$ (mean $=0$), the slopes of harsh discipline on externalizing problems are significant as the confidence intervals do not enclose the value of zero. Regions of significance are marked by shadow.

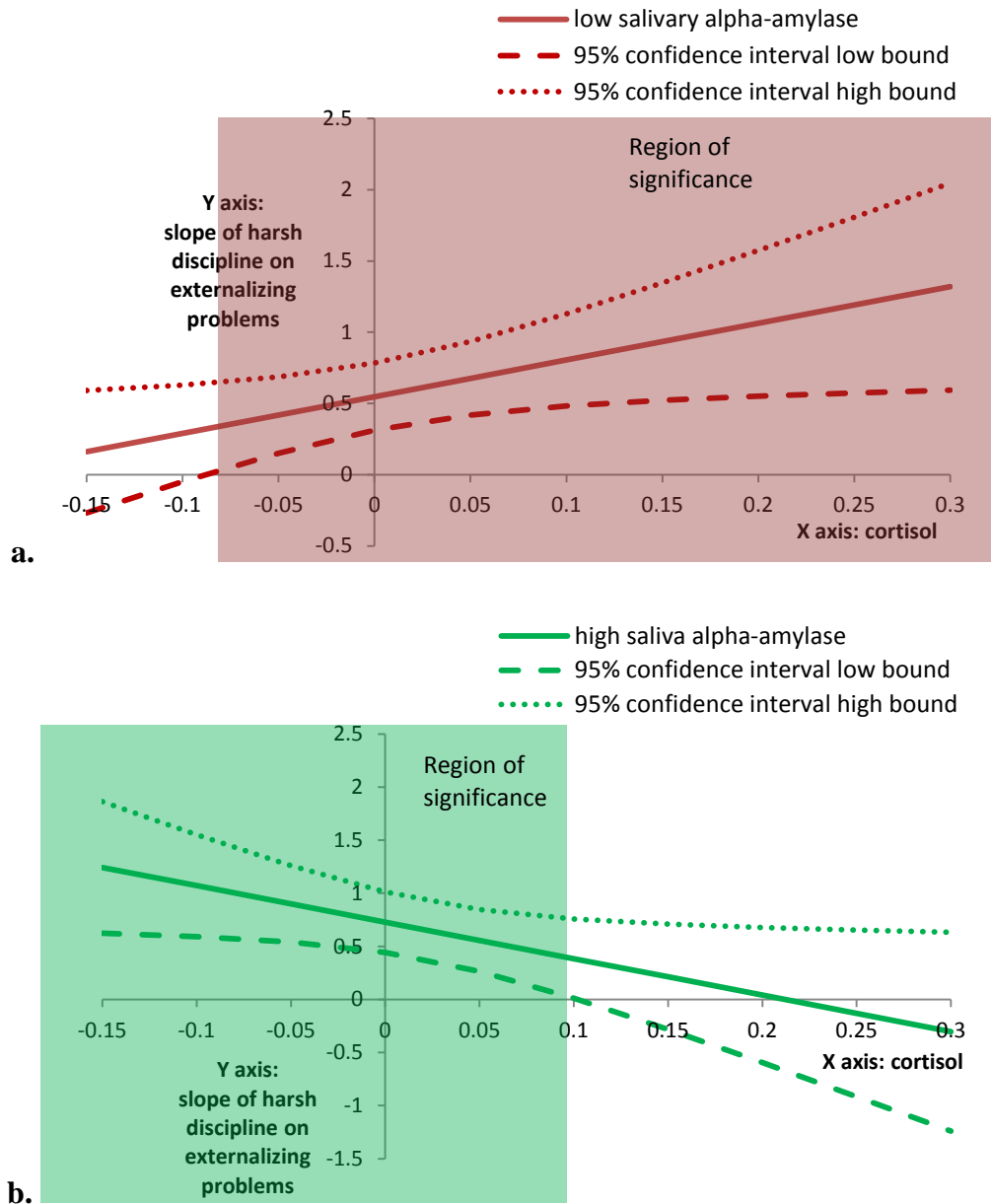


Figure 2.4 Latent Basal Cortisol Regions of Significance for Slopes of Harsh Discipline on Boys' Internalizing Problems by Low (panel a) and High Levels of Latent Basal Salivary Alpha-amylase (panel b)

Caption: When low latent basal sAA is coupled with latent basal cortisol level above the value of -.05 (mean = 0), the slopes of harsh discipline on externalizing problems are significant as the confidence intervals do not enclose the value of zero; when high latent basal sAA is coupled with latent basal cortisol below the value of .125 (mean =0), the slopes of harsh discipline on externalizing problems are significant as the confidence intervals do not enclose the value of zero. Regions of significance are marked by the shadow.

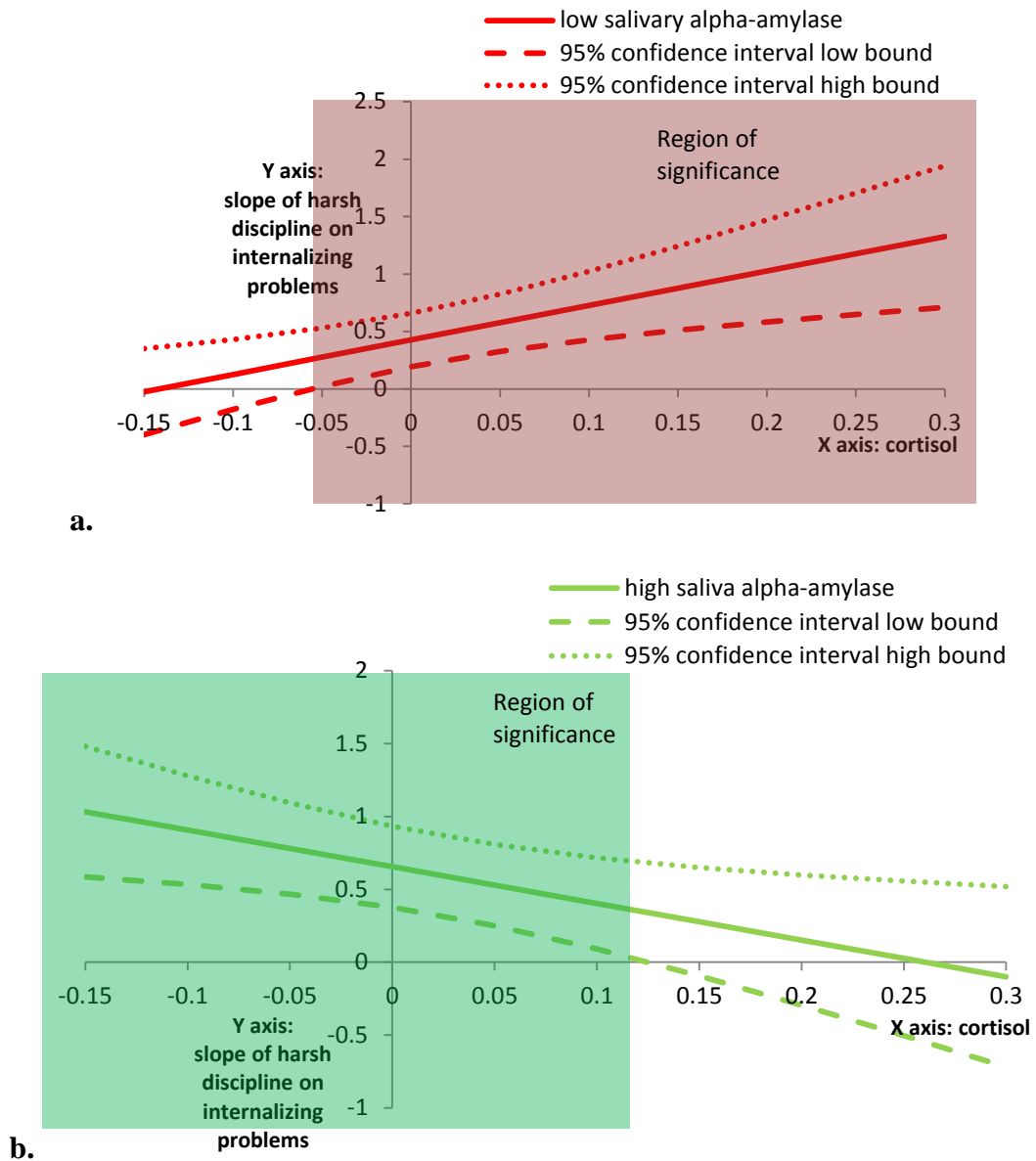
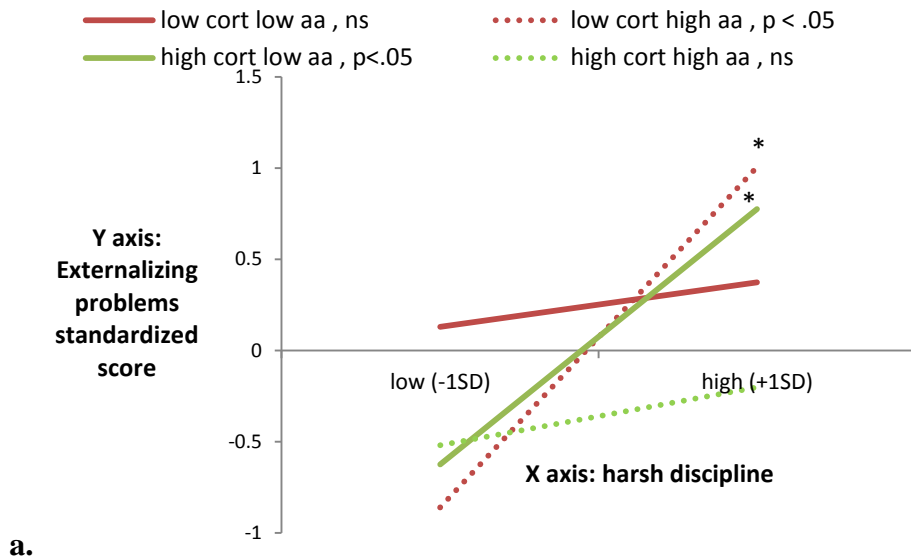
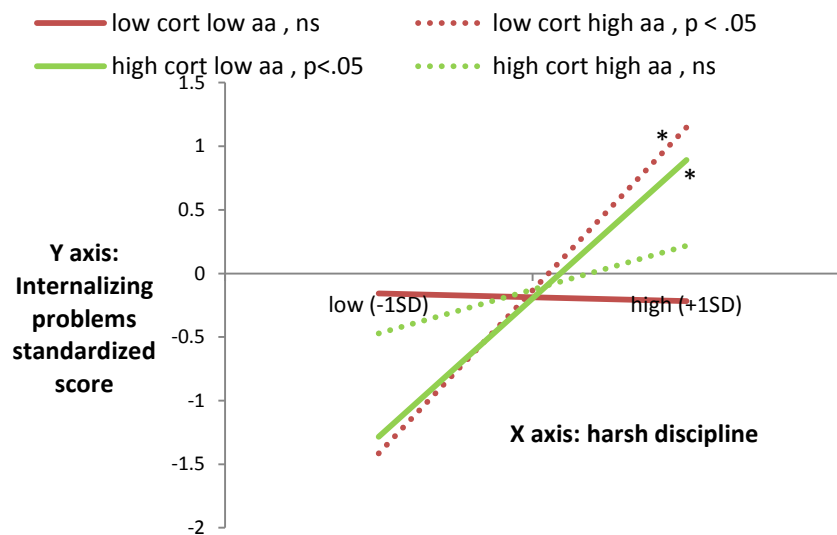


Figure 2.5 Effects of Harsh Discipline on Standardized Scores of Boys' Externalizing (panel a) and Internalizing Problems (panel b) by Factorial Combination of High versus Low Latent Basal Cortisol and Salivary Alpha-amylase

Caption: Harsh discipline has significant impacts on externalizing and internalizing problems for asymmetrical profiles of latent basal cortisol and sAA (marked by asterisk), that is, when low latent basal sAA is coupled with high latent basal cortisol (green line) or when high latent basal sAA is coupled with low latent basal cortisol (red dashed line).



a.



b.

**PAPER 3 HYPOTHALAMIC PITUITARY ADRENAL ACTIVITY AND
AUTONOMIC NERVOUS SYSTEM AROUSAL PREDICT
DEVELOPMENTAL TRAJECTORIES OF CHILDREN'S COMORBID
BEHAVIOR PROBLEMS³**

Abstract

The combined effects of hypothalamic-pituitary-adrenal (HPA) axis activity and autonomic nervous system (ANS) arousal were examined on developmental trajectories of children's comorbid internalizing and externalizing problems. Participants were 394 urban dwelling, primarily African American, youth (50% male, age 11-12 years). Parent-reported child behavior problems were obtained initially, 3, 6, and 12 months later. Saliva samples (collected at the initial assessment) were assayed for cortisol (HPA) and alpha-amylase (ANS). Cross-domain latent class growth analysis identified a stable comorbid trajectory and four other distinct short-term developmental trajectories of internalizing and externalizing behavior problems. ANS arousal was negatively associated with the probability of stable comorbidity, but only among youth who also had *high* levels of HPA axis activity. Findings underscore the predictive value of the interaction of HPA axis activity and ANS arousal in differentiating children with stable comorbidity and have important implications for etiological theories and treatment outcome research.

³ A version of this paper was published as Chen, F. R., Raine, A., Glenn, A. L., & Granger, D. A. (in press). Hypothalamic pituitary adrenal activity and autonomic nervous system arousal predict developmental trajectories of children's comorbid behavior problems. *Developmental Psychobiology*. doi: 10.1002/dev.21379, available at <http://onlinelibrary.wiley.com/doi/10.1002/dev.21379/epdf>

Keywords cortisol; salivary alpha-amylase; externalizing problems; internalizing problems; comorbidity development

Introduction

The National Comorbidity Survey Replication in the United States underscores that comorbidity of internalizing and externalizing problem behavior is commonplace (Nock, Kazdin, Hiripi, & Kessler, 2007) and that children with comorbidity have serious and chronic problems (Youngstrom, Findling, & Calabrese, 2003). As adults, children with comorbidity are at high risk for criminality and substance abuse (Capaldi, 1992), have high rates of divorce and unemployment, low educational attainment, and disproportionately use psychiatric and social services (Costello et al., 1996). Not surprisingly, understanding the determinants of comorbidity in childhood is a central component of the national scientific agenda on mental health (Kessler & Wang, 2008). Numerous studies underscore the role of stress and adversity in predisposing children to risk for externalizing and internalizing problems (Keiley et al., 2003; Kim et al., 2003). Collectively, the findings raise the possibility that the study of individual differences in environmentally sensitive biological systems may advance our understanding of comorbidity to new limits.

The central tenet of contemporary theory is that the psychobiology of the stress response actively and adaptively calibrates to the environment (Chrousos & Gold, 1992; McEwen & Wingfield, 2003; Weiner, 1992). The working parameters including baseline activation and its responsivity to external events, are strikingly different across individuals (Del Giudice, Ellis, & Shirtcliff, 2011), and there are widespread allelic variations in many genes that can affect the functioning of the stress response systems (Alexander et al., 2009; Ouellet-Morin et al., 2008). Most importantly,

several leading theorists speculate that individual differences in the psychobiology of the stress response influence the translation of adverse early experience into problematic developmental trajectories (McEwen & Stellar, 1993; McEwen & Wingfield, 2003). The two spectrums of behavior problems refer to externalizing and internalizing problems. Although comorbidity of behavior problems can refer to the co-occurring problems within each spectrum, such as co-occurring depression and anxiety, we focus primarily on co-occurring problems across two spectrums in the current study. We address the possibility that individual differences in the psychobiology of the stress response may have the potential to differentiate children who develop co-occurring externalizing and internalizing problems from those who have only pure forms of problems.

The psychobiology of the stress response has two main components, the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) (Chrousos & Gold, 1992). The HPA axis response to stress is considered to reflect a “defeat reaction” – a response in situations featured emotional distress and loss of control, where individuals become immobile/passive (Lundberg & Frankenhaeuser, 1980; Henry, 1993). Studies report that cortisol (the primary product of the HPA axis) reactivity is more likely when the situation is uncontrollable and generates emotional distress (Kirschbaum & Hellhammer, 1994; Lundberg & Frankenhaeuser, 1980; Peters et al., 1998). In contrast, ANS activation is quick and diffuse and is considered a “defense reaction” (Folkow, 1985; Henry, 1993) – an active, effortful response to

challenges that are manageable and controllable (Lundberg & Frankenhaeuser, 1980; Peters et al., 1998).

Both HPA axis activity and ANS arousal have been linked to externalizing problems (Oosterlaan, Geurts, Knol, & Sergeant, 2005; Raine, 2005). Low levels of cortisol have been found to be associated with conduct disorder (McBurnett et al., 2005; Pajer et al., 2001), disruptive behavior (McBurnett, Lahey, Rathouz, & Loeber, 2000), and clinically significant levels of externalizing problems (Cicchetti & Rogosch, 2001), but the effect was recently showed to be much smaller than previous assumed in a meta-analysis based on 72 studies with 5,480 subjects (Alink et al., 2008). Studies also reveal a relation between ANS arousal and externalizing problems (Raine, 2005; Sijtsema, Shoulberg, & Murray-Close, 2011). Most of the prior studies used heart rate (or skin conductance) as an index of ANS functioning. Low resting heart rate has been found to be a reliable predictor of antisocial behavior in non-institutionalized children and adolescents (Ortiz & Raine, 2004). A meta-analysis by Lorber (2004) showed that low skin conductance level was a correlate of children externalizing behavior, particularly for conduct problems, although the inverse relation between ANS arousal and externalizing problems has not been consistent.

It has been hypothesized that some children have over-aroused central nervous system functioning which may stem from a lower activation threshold (Kagan, 1996). As a result, they are more prone to fearfulness and anxiety and compensate such hyper-arousal with withdrawal and avoidance, but findings are again mixed. Regarding HPA axis functioning, higher levels of cortisol have been found to be

related to withdrawn temperament, inhibited characteristics, and internalizing emotion (Davis, Donzella, Krueger, & Gunnar, 1999; Kagan, Reznick, & Snidman, 1988; Schmidt et al., 1997; Smider et al., 2002). A recent meta-analysis found a tendency for depressed children to have higher basal cortisol levels than non-depressed controls (Lopez-Duran, Kovacs, & George, 2009). Yet with respect to cortisol levels and anxiety disorder, some studies have observed increased cortisol activity (Vreeburg et al., 2010; Wedekind et al., 2000) while others have found normal cortisol activity (Gurguis, Mefford, & Uhde, 1991). Similarly, researchers have argued for elevated autonomic sympathetic activation in individuals with high levels of anxiety (Harrison & Turpin 2003; Weems et al., 2005). There is also evidence that depressed youths showed stronger ANS arousal (Nock & Mendes, 2008; Shannon et al., 2007), yet results are not entirely consistent, with some showing an association in the opposite direction or no relation at all (Tonhajzerova et al., 2010).

Recently, researchers have advocated examining the combined effects of multiple subsystems (Young, Abelson, & Cameron, 2005). The HPA axis and the ANS are grounded in related brain regions (Young et al., 2005), but the two systems function differently. For example, the HPA axis activity occurs more in situations featured emotional distress and loss of control, when subjects become immobile/passive (Lundberg & Frankenhaeuser, 1980; Henry, 1993) whereas ANS activation taps more into a “defense reaction” (Folkow, 1985; Henry, 1993), an active, effortful response to challenges that are manageable and controllable. In addition, HPA axis activity has shown habituation after repeated exposure to stressors but ANS activation

has been found generally responsive and shown uniform activation patterns with repeated exposure to stressor (Gordis et al., 2006; Schommer, Hellhammer, & Kirschbaum, 2003). Hence, the examination of the combined effect of the two systems provides unique rather than redundant information. Non-invasive measurement (in saliva) of the activity of the HPA (cortisol) and ANS (salivary alpha-amylase, sAA) opens windows of opportunity to explore this possibility in youth (Granger et al., 2012).

Our understanding of the combined effects of the HPA axis activity and ANS arousal on behavior problems, particularly on the comorbidity of behavior problems, is in its infancy. Previous studies in this field thus far have utilized cross-sectional designs (Allwood et al., 2011; Chen, Raine, Soyfer, & Granger, 2015a; El-Sheikh et al., 2008; Gordis et al., 2006). These studies have revealed inconsistent findings. For example, El-Sheikh et al. (2008) found that HPA axis activity was positively associated with externalizing and internalizing problems but only when ANS arousal was low, whereas Chen et al. (2015a) revealed that HPA axis activity was negatively associated with externalizing and internalizing problems when ANS arousal was low. In addition, the comorbidity of externalizing and internalizing problems has rarely been put in the center of research investigations examining the association between stress systems and behavior problems. That is, externalizing and internalizing problems have been either treated as independent by conducting analyses separately for each type of behavior problems (Allwood et al., 2011; El-Sheikh et al., 2008); or the comorbidity of externalizing and internalizing problems has been viewed as a

potential source for inconsistent findings and subsequently controlled for in the model (Chen et al., 2015a). For example, Chen et al. (2015a) addressed the confounding effect of comorbidity by allowing the residual variance of externalizing and internalizing problems to correlate, but their variable-centered approach cannot directly investigate whether HPA axis activity and ANS arousal distinguish individuals with comorbidity from those with pure form of behavior problems.

The current study built upon prior work (Chen et al., 2015a) by employing a longitudinal design and analytical approach to model heterogeneity in developmental trajectory. Trajectory analysis is premised on the assumption that there are unobserved and distinct groups in the population that follow different developmental trajectories and that latent classes can be identified which capture distinct patterns in trajectory (Muthén & Muthén, 2000). Although trajectory analysis studies on one category of behavior problems (either internalizing or externalizing problems) are commonplace (McCrae, 2009; Woodruff & Lee, 2011), few studies have addressed trajectories involving the correlated nature of externalizing and internalizing problems. To address the heterogeneity of correlated behavior problem development, one solution is to employ cross-domain latent class growth analysis (LCGA) where class memberships are conditional simultaneously on the trajectory of both externalizing and internalizing problems. Few studies have used cross-domain LCGA to simultaneously model the development of both forms of behavior problems and to test potential factors that may differentiate classes. In this study we addressed this knowledge gap. We tested individual and interactive effects of HPA axis activity and ANS arousal on odds of

being stable comorbid in a one-year period using cross-domain LCGA. Variability in either HPA axis activity or ANS arousal at any given moment can derive from basal arousal, arousal attributable to momentary situational influence, and measurement errors. Hence, we employed a latent variable approach as a tactic to isolate the variance in salivary analyte levels attributable to stable individual difference. This latent variable approach has been demonstrated using both cortisol (e.g., Shirtcliff et al., 2005) and sAA (e.g., Taylor et al., 2012). With respect to the heterogeneity in the co-development of behavior problems, we expected that at least four prototypic classes would emerge: a comorbid class, an externalizing-dominant class, an internalizing-dominant class and a normative class. We anticipated that the interaction between the ANS arousal and HPA axis activity would differentiate the comorbid class from the remaining classes. Specifically, we expected that for those with low versus high levels of HPA axis activity, there would be different association patterns between ANS arousal and the probability of being stable comorbid. Consistent with the rich literature linking hypocortisolism to psychopathological outcomes in children (Badanes, Watamura, & Hankin, 2011; McBurnett, Lahey, Rathouz, & Loeber, 2000), we anticipated when individuals have low levels of HPA axis activity, the probability of being stable comorbid would be high regardless of their ANS arousal. In contrast, we anticipated that when individuals had high levels of HPA axis activity, the probability of being stable comorbid would be associated with ANS arousal, but the specific direction of such association remained unknown at this point due to the lack of consistency regarding which end of ANS arousal would be associated with the comorbidity of externalizing and internalizing problems.

Methods

Participants aged 11 and 12 were enrolled from 2008 to 2012 in the Philadelphia Healthy Brains and Behavior (HBB) project which aimed to identify risk and protective factors for aggression and to test the effectiveness of the treatments for children with high levels of aggressive behaviors. Participants were recruited by advertisements within the city of Philadelphia and contiguous suburbs. Participants completed an initial assessment and were followed at 3, 6, 12 months later. Exclusion criteria for initial enrollment were diagnoses of a psychotic disorder, intellectual disability, pregnant, a pervasive developmental disorder or current medication use with the potential to interfere with the measurement of salivary analytes. Participants came to university laboratories where data were collected. After the initial assessment, high aggressive participants (defined as being diagnosed with oppositional defiant disorder (ODD) or diagnosed with conduct disorder (CD) and/or scoring at least one standard deviation above a normed population mean on the reactive or proactive components of the Reactive Proactive Aggression Questionnaire) were randomized into a control group or treatment groups (e.g., cognitive behavior therapy or nutritional supplements). Participants received treatment, if assigned, between the initial assessment and the 3-month follow-up. For a more comprehensive description of the HBB sample and recruitment see Liu et al. (2013). Caregivers gave informed consent and youth gave assent after description of the study was given. The HBB project was approved by the University of Pennsylvania and the Philadelphia Department of Health human subjects boards.

There were 446 participants enrolled in the initial study, of which 52 enrolled too late to allow sufficient time for follow up within the time frame of the larger project. Thus, the final analytic sample in the current study comprised 394 participants (52% male). Not all participants had complete data on behavior problems throughout all four waves (8.6% missing at the 3-month follow-up; 17% missing at the 6-month follow-up; and 37.3% missing at the 12-month follow-up), but we retained all 394 cases in the analysis and used full information maximum likelihood to handle missing data. The final sample included participants who self-identified as White ($n = 43$, or 10.91%), African American ($n = 323$, or 81.98%), Hispanic ($n = 3$, or 76%), Native American ($n = 2$, or .51%), Asian American ($n = 1$, or .25%), multiracial ($n = 17$, or 4.31%) and other ethnicities ($n = 4$, or 1.02%). On average they were 11.87 years of age ($SD = .59$), and with an average score of 3.27 on Tanner's five stages of development. Parents/guardians accompanying children to the lab received an average of 13.33 years of schooling ($SD = 2.14$), and their monthly income was, on average, 1,717.61 dollars ($SD = 1,710.01$; annual income was \$20611.32 on average). Regarding their marital status, 15.2% of the parents/ guardians were either divorced or separated, 57.4% never married, and only 24.1 % were married and still lived with their spouses. There were fewer boys in the excluded sample (37% boys among excluded participants; $\chi^2 = 4.71$, $p = .03$). Nevertheless, participants excluded from the final analysis ($n = 52$) and those retained ($n = 394$) were not different in many other demographic characteristics. Specifically, the excluded participants did not differ from the retained participants on age ($M = 11.94$, $SD = .60$ for excluded participants; $t = .75$, $p = .45$), caregiver's monthly income ($M = 1,655.45$, $SD = 1,569.93$ for excluded

participants; $t = -.24, p = .81$), mother's years of schooling ($M = 13.36, SD = 2.26$ for excluded participants; $t = .75, p = .45$), biological parent's marital status ($\chi^2 = 4.49, p = .34$), ethnicity ($\chi^2 = 10.99, p = .14$) or pubertal development ($M = 3.31, SD = .95$ for excluded participants; $t = .24, p = .81$).

Behavioral Assessments

Behavior problems were measured with the parent-report Child Behavior Checklist (CBCL) (Achenbach & Rescorla, 2001) at initial assessment and follow-ups (3, 6, 12 months later). The CBCL can be scored in terms of externalizing problems (rule-breaking behavior and aggressive behavior syndrome subscales) and internalizing problems (anxious/depressed, withdrawn/depressed, and somatic complaints syndrome subscales). Externalizing and internalizing scales have excellent test-retest reliability ($r = 0.91$ and 0.92) and internal consistency (Cronbach's $\alpha = 0.90$ and 0.94 ; Achenbach & Rescorla, 2001). In the current study, both externalizing and internalizing scales showed excellent internal consistency (Cronbach's $\alpha = 0.98, 0.89, 0.91$ and 0.98 for externalizing problems from wave 1 to wave 4; and Cronbach's $\alpha = 0.98, 0.88, 0.88$, and 0.98 for internalizing problems from wave 1 to wave 4) Normalized T scores derived from national samples were used in this study (Achenbach & Rescorla, 2001). For both externalizing and internalizing problems scales, scores from 60 to 63 (84th through 90th percentile) are in borderline clinical range, and those above 63 are within the clinical range. Descriptive statistics are reported Table 2.1.

Analytes from Saliva Samples

Across a single day, three saliva samples were collected from each child at the initial assessment. The children were instructed to refrain from food and drink (except water) prior to sample donation (see Granger et al., 2012). Whole, un-stimulated, saliva was collected by passive drool (Granger et al., 2007). The first sample was taken in the laboratory in the university at approximately 0919 (SD = 20mins), the second 15 minutes later, and the third 30 minutes later. Between sample collections, participants completed a variety of questionnaires. Immediately after collection, specimens were frozen and stored at -80°C until assay. On the day of assay, samples were centrifuged at 3,000 rpm for 15 minutes to remove mucins. Following Granger et al. (2007), samples were assayed for sAA using a commercially available assay kit for the kinetic measurement of sAA activity without modification to the manufacturers recommended protocol (Salimetrics, State College PA). The coefficient of variation is less than 10% for both intra- and inter-assay. Samples were also assayed for salivary cortisol using a commercially available enzyme immunoassay (Salimetrics, State College, PA). The assay had a range of sensitivity from 0.007 to 3.0 µg/dl. Average intra- and inter-assay coefficients of variation were less than 5% and 10%. Latent measures of cortisol and sAA were modeled as latent variables with three corresponding sample collections as indicators. Descriptive statistics are reported Table 2.1.

Covariate: Puberty stage

Participants reported their stage of puberty in terms of their pubic hair development and genital or breast development (Morris & Udry, 1980). Participants

were presented with drawings illustrating Tanner's five stages of development of breasts and pubic hair, or male genitalia and pubic hair. They were instructed to choose the drawing closest to their own stage of development. The stages were averaged to yield an overall score of puberty stage.

Statistical Analysis

We first model co-developmental trajectories of externalizing and internalizing problems (see Model 1 in Figure 3.1), then we tested individual and interactive effects of latent cortisol and latent sAA on predicting the probability of being in different developmental trajectories of behavior problems, with a prime focus on comorbidity development. All models were estimated using maximum likelihood with robust standard error, and missing data were handled by full information maximum likelihood using Mplus 7 (Muthén & Muthén, 1998-2012).

We ran a series of unconditional cross-domain LCGA. LCGA estimates a different mean growth curve for each class. Within-class variations in growth factors are constrained to be zero. Cross-domain LCGA simultaneously models the influence of internalizing and externalizing problems on class membership. Thus, the classes were derived from six latent factors: three representing development in externalizing problems (initial level, linear and quadratic changes in externalizing problems as a function of time) and three representing development in internalizing problems (initial level, linear and quadratic changes in internalizing problems as a function of time) (see Model 1 in Figure 3.1). Bayesian information criterion (BIC), Lo-Mendell-Rubin

likelihood ratio tests (LMR-LRT) and the meaningfulness of class were all taken into account to decide the optimal class number (Nylund, Asparouhov, & Muthén, 2007). The model with the optimal number of classes should have the smallest BIC, a significant LMR-LRT and meaningful classes.

Note that homogeneity of trajectory group members is built upon an assumption that events (except for extreme treatment or medication) do not fundamentally alter behavior. That is, change is incremental and not dramatic; events cause deviation from the mean behavior of most individuals of the same trajectory but not so large as to bear no similarity to the original trajectory (Nagin, 2005). The short-term psychotherapy effect would be incremental and was unlikely to alter individuals' class membership. Nevertheless, the treatment may introduce effects on the shape of the trajectory when modeling the heterogeneity in the behavior problems and we need to control for it even in the unconditional model. The treatment component of the HBB were implemented only between initial assessment and 3-month follow-up and was targeted on aggression, but we included the treatment effect on the 3-, 6-, and 12-month scores of both externalizing and internalizing problems in case of spill-over effects across time and across category of behavior problems (see Model 1 in Figure 3.1). With the 3-, 6-, and 12-month behavior scores regressed on treatment, the shapes of the trajectory (the linear and quadratic terms of time) were a better reflection of the mean trajectory.

Next, we examined Model 2 with cortisol, sAA and their interaction as focal predictors (see Figure 3.1). In order to test the unique contribution of the focal

variables, we need to control for other potential confounds, including age, sex, ethnicity, puberty stage, and treatment assignment. Among these variables, those that differed significantly across classes would be included as control variables in Model 2. Latent cortisol and sAA were formed from three corresponding analytes from saliva samples. Any significant interaction was probed by testing regression slopes of the high (mean +1SD) and low (mean -1SD) values of the moderator (Aiken & West, 1991). An LCGA model with predictors is essentially a multi-nominal logistic regression. The linear relation lies in the predictors and the *log odd* of being in a certain class. We plotted the interaction in terms of the *probability* of being in a certain class to ease interpretation.

Results

Modeling co-developmental trajectories of behavior problems: Unconditional Models

A 5-class solution had the best model: the BIC continued to decline from 1-class to 5-class solution but increased for the 6-class solution, hence the 5-class solution had the smallest BIC; The LMT-LRT for 5 versus 4 classes was significant ($p = .04$), but the LMT-LRT for 6 versus 5 classes was not significant ($p = .55$), suggesting that the 5-class solution substantively improved the model but that the 6-class solution did not. Furthermore, the 5-class solution revealed meaningful classes in

line with our prediction. Details of model fit indices see Table 3.2. The class features are reported in Table 3.3 and plotted in Figure 3.2.

The *low class* and the *average declining class* both represented individuals with normative growth, but the former had none or very few problems whereas the latter had average levels of problems (50th percentile) initially and continued to decline. These two classes together comprised half of the sample. Individuals in the “*internalizing slow declining*” class had high levels of internalizing problems (81st percentile) which then gradually decreased, along with an average level of externalizing problems. The “*clinical externalizing relapse*” class included individuals whose externalizing problems were very high (95th percentile) and above the clinical level, reduced substantively, but then climbed back into clinical range. This class also exhibited high levels of internalizing problems (80th percentile). We identified a small class characterized as “stable comorbid” (8% of the sample, n = 31) who displayed very high levels of both externalizing and internalizing problems, 96th percentile and 99th percentile respectively, and remained unchanged over time.

Interaction of latent cortisol and latent alpha-amylase on the co-development of behavior problems: Conditional Model with predictors

No significant differences were found in all covariates but percentages in treatment groups across classes (see Table 3.1). This was not surprising given that participants with high aggression were randomized into treatment and control groups. The clinical externalizing relapse and stable comorbid groups are bound to have a

higher percentage of participants who had been assigned into treatments. Thus, treatment assignment was included as the only control variable for Model 2 so that we could assess the predictive effect of latent cortisol and latent sAA for class memberships over and above the treatment assignment.

The stable comorbid class was served as the reference and results are reported in Table 3.4. The interaction of cortisol and sAA predicted the odds of being stable comorbid as opposed to being in each of the other four classes. There was a marginally significant effect of latent cortisol on the odds of being stable comorbid (see coefficients in the second column of Table 3.4). We altered the reference but cortisol and sAA did not differentiate between other four classes.

We probed the interaction. Latent sAA was negatively associated with the odds of being in the stable comorbid class but only for individuals with high latent cortisol. In addition, for those with low latent cortisol, their probability of being in the stable comorbidity class was high regardless of their latent AA level. We plotted the probability of being in the stable comorbid class in Figure 3.3 with the low class as the reference class. Similar patterns of probability of being stable comorbid class were found when comparing stable comorbid class with the other three classes.

Discussion

Interaction of HPA axis activity and ANS arousal differentiated comorbidity from other developmental trajectory of behavior problems. ANS arousal was

negatively associated with the probability of being in the stable comorbid class but only among youth with high levels of HPA axis activity. In addition, there was a marginal main effect of HPA axis activity: *low* levels of HPA axis activity were associated with a high probability of being stable comorbid. This finding was robust in a sense that they differentiated children with persistent comorbidity from children from the other classes. Findings are among the first to document the interaction of two major stress systems on the odds of developing comorbid behavior problems in youth. Our results lend support to the hypothesis that comorbidity of internalizing and externalizing behavior in youth is associated with distinctive patterns of physiological activity and arousal (Bauer et al., 2002; Rutter, 1997).

We identified a group of children who had clinical levels of both externalizing and internalizing problems. Despite the seemingly small number (i.e., 8%), this is a significant group of children given that the 90th percentile is the clinical cut-off point when the norm was constructed (Achenbach & Rescorla, 2001). The clinical cut-off point has been found to be the most efficient cut point in terms of minimizing false negatives and false positives in classifying individuals in samples of referred and non-referred youth (Achenbach & Rescorla, 2001). Thus, having 8% of our sample with both externalizing and internalizing problems continuously above clinical cut off points is not negligible.

There were three physiological profiles linked to stable comorbidity, namely, the two asymmetrical profiles and one of the symmetrical profiles of HPA axis activity and ANS arousal. It has been postulated that the asymmetry in HPA axis and ANS

may be associated with the optimal level of arousal (Bauer et al., 2002), and in turn to be most responsive to environment and carry high level of plasticity (Chen, Raine, Rudo-Hutt, Glenn, Soyfer, & Granger, 2015b). Individuals with high plasticity are assumed to be more affected by either supportive or adverse features of their social environments, whereas individuals with low plasticity show less biobehavioral reactivity to their circumstances regardless of the valence (Belsky & Pluess, 2009). Thus the highest level of behavior problems would be observed among individuals with high plasticity when they are in an adverse environment. It is noteworthy that participants in the current study were predominantly from lower class, from disadvantageous neighborhoods and reside in a single-parent household. Finding that individuals with comorbidity were more likely to have asymmetry in HPA axis and ANS profile is in line with this.

Stable comorbidity was also found to be associated with one of the symmetrical profile. Concurrently low levels of HPA axis activity and ANS arousal increased the probability of being stable comorbid. Previous studies have found this profile to be closely linked to externalizing problems (Gordis et al., 2006). This symmetry is consistent with the under-arousal hypothesis of externalizing problems (van Goozen et al., 2007): individuals with under-arousal in both systems may have a higher activation threshold and appear fearless and hyperactive. Consequently, individuals may be less sensitive to punishment or socialization as postulated by fearlessness theory, or attempt to reach an optimal level of arousal by seeking out excitement and stimulation, which, in turn, leads to antisocial behavior (Raine, 2002).

In this case, internalizing problems might be the subsequent result of externalizing problems as suggested by some researchers (Patterson & Stoolmiller, 1991; Oland & Shaw, 2005). Externalizing problems have been found to be associated with academic and interpersonal failure (van Lier & Koot, 2013) which, in turn, may fuel internalizing problems (Parker et al., 2006). It is possible that concurrently low levels of HPA axis activity and ANS arousal not only predispose children to externalizing problems, but also predispose them to develop subsequent internalizing problems, whereas youth displaying externalizing problems without such physiological characteristics may not be susceptible to later internalizing problems.

We postulate that even within this stable comorbid class there is heterogeneity of symptom expression (i.e., concurrent versus subsequent), and that this heterogeneity is linked to different characteristics of psychobiology of stress as discussed above. However, participants in our sample were in their late childhood to early adolescence, and the stable comorbid class already had clinical levels of externalizing and internalizing problems at the initial stage of the study. We had little information regarding the onset of their problems, that is, whether externalizing and internalizing problems start to become expressed at the same time (i.e., concurrent expression) or in a sequential order (i.e., subsequent expression such as externalizing problems followed by internalizing problems). The interpretations given here must therefore be regarded as tentative until formally tested and replicated, although, at the same time, they offer new avenues for future research investigating physiology-comorbidity associations.

We also revealed a marginal inverse relation between cortisol and the odds of comorbidity, as seen from Figure 3.3 with different marginal mean odds of being comorbid at low versus high latent cortisol. This is consistent with the hypothesized detrimental effects of hypocortisolism (Heim, Ehlert, & Hellhammer, 2000). Hypocortisolism is proposed to relate to prolonged stress (Hellhammer & Wade, 1993). An increasing body of research has linked hypocortisolism not only to externalizing problems (Alink et al., 2008; McBurnett et al., 2000) but also to internalizing problems (Cicchetti, Rogosch, Gunnar, & Toth, 2010). Badanes, Watamura and Hankin (2011) proposed that low cortisol may be an important marker for allostatic load for children, and for psychopathological outcomes. Nevertheless, effect of HPA axis is not absolute and is conditional on the level of ANS arousal. Admittedly, from our findings, youth with low levels of cortisol seem to have a higher chance of being in the stable comorbid class regardless of their ANS arousal, but those with high levels of cortisol may also develop comorbid behavior problems when coupled with low ANS arousal. Future research exploring effect of hypocortisolism is advised to take into account ANS arousal in order to get a complete picture.

There are several limitations. First, the causal direction is undetermined even though we ensured the temporal order of the events. It is unclear whether patterns of HPA axis activity and ANS arousal are the underlying causes of comorbidity or the results of such comorbidity. However, these physiological characteristics did consistently differentiate the comorbid class from the remaining four classes in our study. Second, the time span here is only one year from late childhood to early

adolescence and we were unable to verify the onset of behavior problems, which prevents us from testing the differential links of physiological patterns with concurrent expression or subsequent expression of problems. Third, because we used the same behavior measures at four time points over a one year period, there could be practice effect in responding to the questionnaire. However, it is difficult to develop an alternative solution to solve this problem because in order to model the change of the same construct we have to repeatedly measure the same construct over time. Fourth, we did not have information on waking time and it would strengthen the findings if we could have incorporated this information in the analysis, although it has been suggested that time since waking may not make a big difference when modeling latent cortisol level (Doane et al., 2015). Fifth, the participants were primarily African-Americans and came from a relatively high-risk community sample given their demographic features (e.g., low income). We caution therefore that the conclusions drawn from this study may not readily generalize to other ethnic groups or other socioeconomic classes. Last, some individuals received treatments between the initial assessment and the 3-month follow-up. Although we modeled the impact of treatment, replication of our findings from studies with no treatment components are needed.

Despite these limitations, this study has theoretical and intervention implications. Built upon the rich literature that associated stress and behavior problems, our study highlights the unique connection between biological stress systems and comorbidity of externalizing and internalizing problems. Comorbidity may be qualitatively different from pure forms of behavior problems, and more

research with respect to its distinctive correlates will add to our knowledge of why individuals are susceptible to one but not both forms of behavior problems and vice versa. Future research should further explore the interplay between the HPA axis and ANS and why they appeared to be closely linked to comorbidity. Inevitably, saliva samples will never replace baseline clinical assessments when making diagnostic and treatment decisions. Nevertheless, with further replication and extension there exists the potential for HPA axis activity and ANS arousal indicators (and their interaction) to be assessed at baseline to provide added value in predicting the likelihood of comorbidity or treatment responsiveness. In doing so, a more focused evaluation on treatment outcomes for youth with externalizing-only or internalizing-only problems can potentially be made, and alternative treatment for youth with comorbidity can be devised to better take into account their unique physiological characteristics.

Paper 3 Tables and Figures

Table 3.1 Descriptive Statistics for Main Variables

	0 th month	Repeated measures		
		3 rd month	6 th month	12 th month
Externalizing problem				
T score	54.49 (11.11)	51.33 (10.67)	51.00 (10.47)	52.45 (10.83)
Range	33-80	33-78	33-74	34-80
Normal range ^a (%)	67.13	74.9	78.31	73.10
Borderline range ^b (%)	10.4	12.35	8.04	10.66
Clinical range ^c (%)	22.47	12.75	13.65	16.24
Internalizing problem				
T score	53.23 (10.92)	50.04 (10.50)	48.26 (10.48)	49.46 (10.81)
Range	33-85	33-85	33-80	33-77
Normal range (%)	71.91	84.46	84.74	81.22
Borderline range (%)	10.96	6.78	4.82	7.1
Clinical range (%)	17.13	8.76	10.44	11.68
		0 th month		
	Sample 1	Sample 2	Sample 3	
Cortisol (µg/dl)	.17 (.15)	.19 (.15)	.16 (.12)	
sAA (U/ml)	74.57 (56.79)	83.92 (63.18)	82.88 (63.37)	

Note. sAA = salivary alpha-amylase; ^a T score below 60 (below 84th); ^b T score from 60 to 63 (84th to 90th); ^c T score above 63 (above 90th)

Table 3.2 Comparisons of Unconditional Latent Class Growth Models with Different Number of Classes for Co-developmental Trajectories of Externalizing and Internalizing Problems

Class number	Log likelihood	BIC	entropy	LMT-LRT p value
1	-7865.05	16055.64	1	-
2	-7653.77	15468.89	.81	< .001
3	-7542.08	15287.36	.82	< .001
4	-7472.99	15191.02	.79	.002
5	-7421.67	15130.21	.80	.04
6	-7400.88	15130.46	.79	.55

Note. BIC, Bayesian information criterion; LMR-LRT, Lo-Mendell-Rubin likelihood ratio test for k versus k-1 classes

Table 3.3 Descriptive Statistics by Five Classes

		Low	Average declining	Internalizing slow declining	Clinical externalizing relapse	Stable comorbid	Comparison (F)
Class makeup		16.87%	33.54%	19.13%	22.52%	7.94%	
Externalizing problems development ^a	Intercept	40.54** (1.33)	53.84** (1.12)	50.13** (1.29)	66.13** (1.18)	66.86** (1.73)	2086.42**
	Slope	-3.20* (1.33)	-3.71** (.97)	-3.55** (1.23)	-4.91** (1.48)	-1.83 (1.71)	965.80**
	Quadratic	.69* (.30)	.74* (.22)	.55 (.29)	1.06** (.40)	-.05 (.52)	889.86**
Internalizing Problems development ^a	Intercept	42.60** (1.32)	47.81** (1.15)	58.73** (1.17)	58.37** (-1.07)	72.28** (2.03)	1710.69**
	Slope	-3.06* (1.66)	-3.84** (1.07)	-2.80* (1.12)	-4.41** (1.67)	-1.83 (1.71)	873.63**
	Quadratic	.68 (.38)	.65** (.26)	.43 (.27)	.85 (.47)	-.05 (.52)	930.13**
Age ^b		11.98 (.63)	11.85 (.57)	11.85 (.62)	11.86 (.59)	11.80 (.52)	.72
Puberty stage ^b		3.40 (1.07)	3.33 (.93)	3.06 (1.04)	3.30 (.97)	3.11 (1.06)	1.35
Sex	Male	46.97%	52.67%	54.67%	55.91%	48.28%	.40
Ethnicity	African-American	84.13%	86.61%	84.85%	94.05%	96.15%	1.60
Treatment		45.45%	48.85%	45.33%	64.52%	68.97%	3.08*

Note. ^a Standard errors in parenthesis; ^b Standard deviation in parenthesis; * p < .05, ** p < .01

Table 3.4 Estimates from Conditional Latent Class Growth Analysis with Latent Cortisol, Alpha-amylase and Interaction as Predictors

	cortisol		sAA		cortisol by sAA	
	B ^a	z	B ^a	z	B ^a	z
Reference class: stable comorbid						
1 Low	1.53 [*]	2.03	.26	1.93 ⁺	.25 [*]	2.09
2 Average declining	1.45 ⁺	1.94	.22	1.67	.24 [*]	2.06
3 Internalizing slow declining	1.48 ⁺	1.93	.20	1.49	.32 [*]	2.45
4 Clinical externalizing relapse	1.45 ⁺	1.92	.21	1.59	.27 [*]	2.31

Note. sAA = salivary alpha-amylase; a. unstandardized coefficients; CI, confidence interval;

* p < .05, +p < .06

Figure 3.1 Individual and Interactive Effects of Latent Cortisol and Latent Alpha-amylase (sAA) on Predicting Probability of Being in Different Classes that Capture the Co-development in Externalizing and Internalizing Problems

Note. ext = externalizing problems; int = internalizing problems

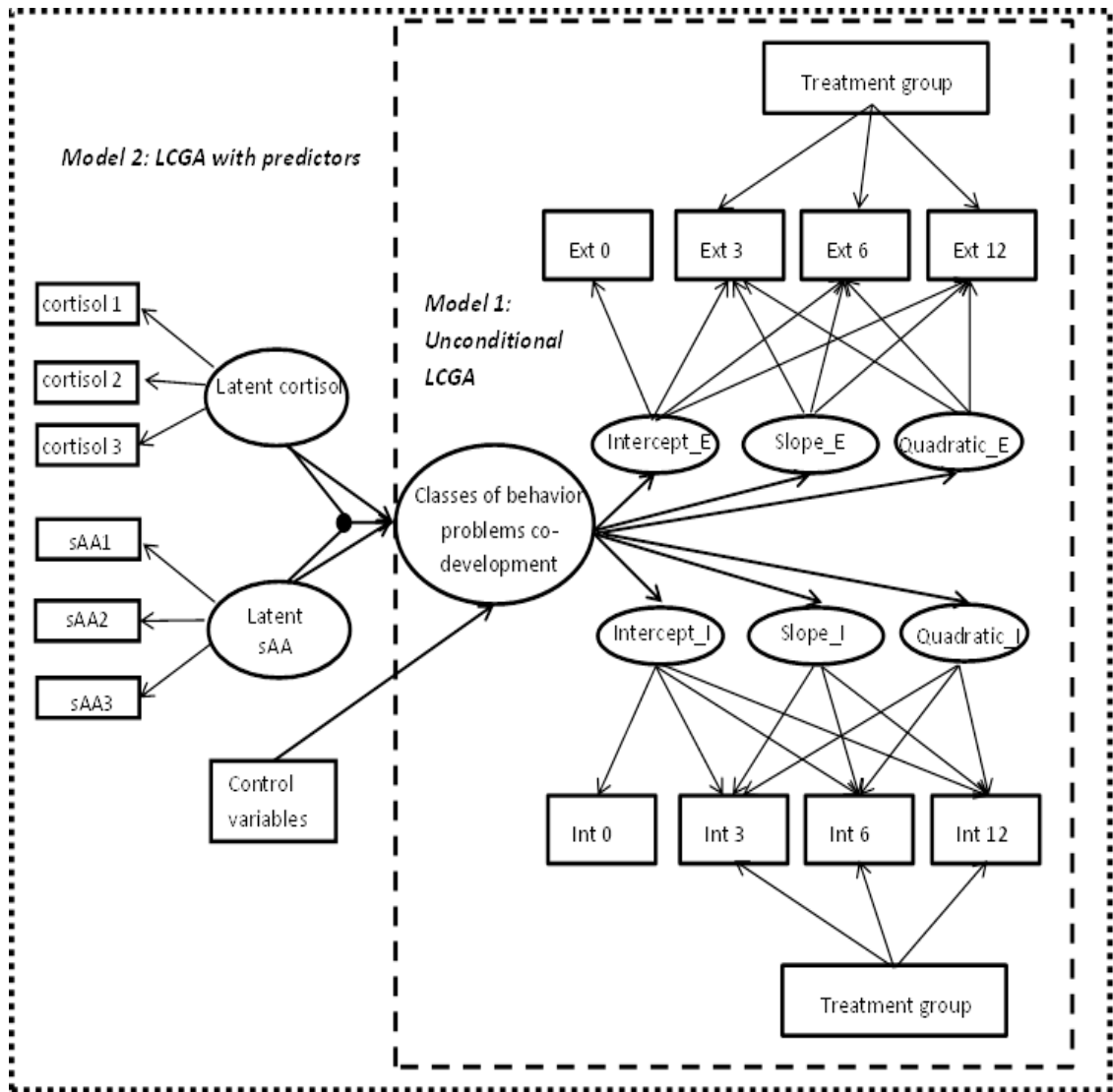
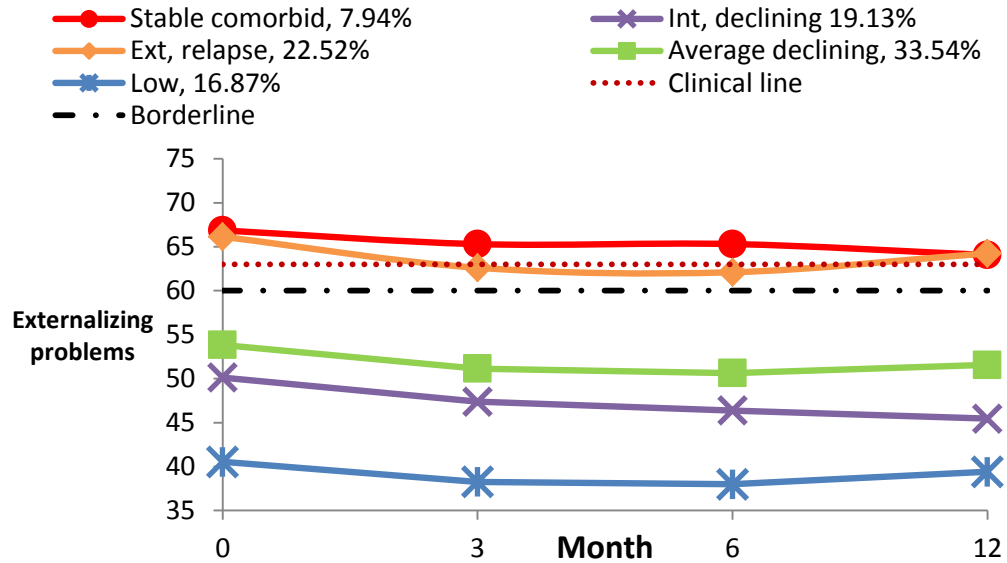


Figure 3.2 Five-class Solution of Cross-domain Latent Class Growth Analysis

a. Estimated Means for Externalizing Problems for Each Class

b. Estimated Means for Internalizing Problems for Each Class

a.



b.

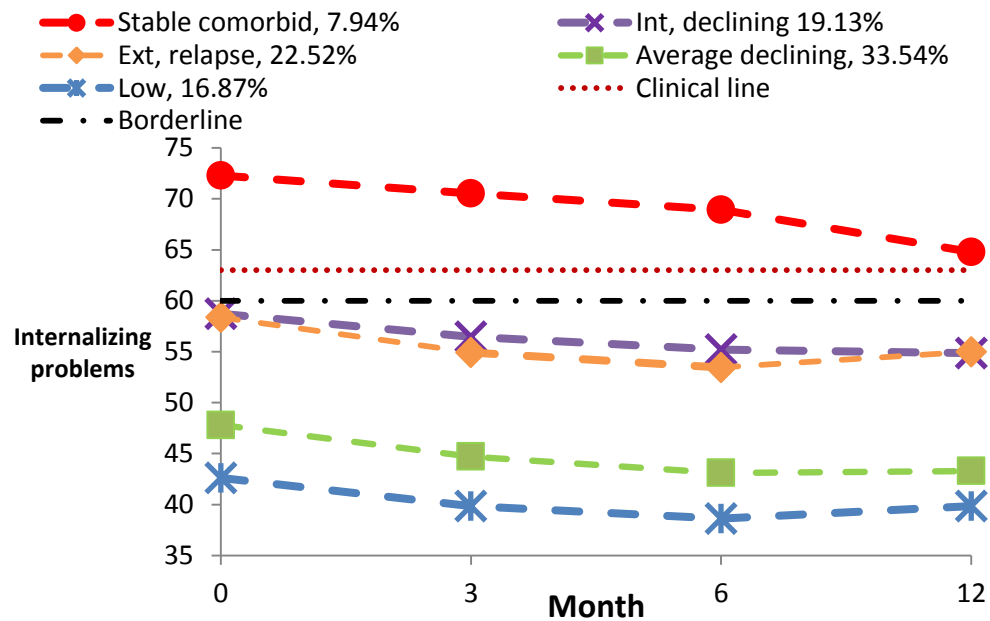
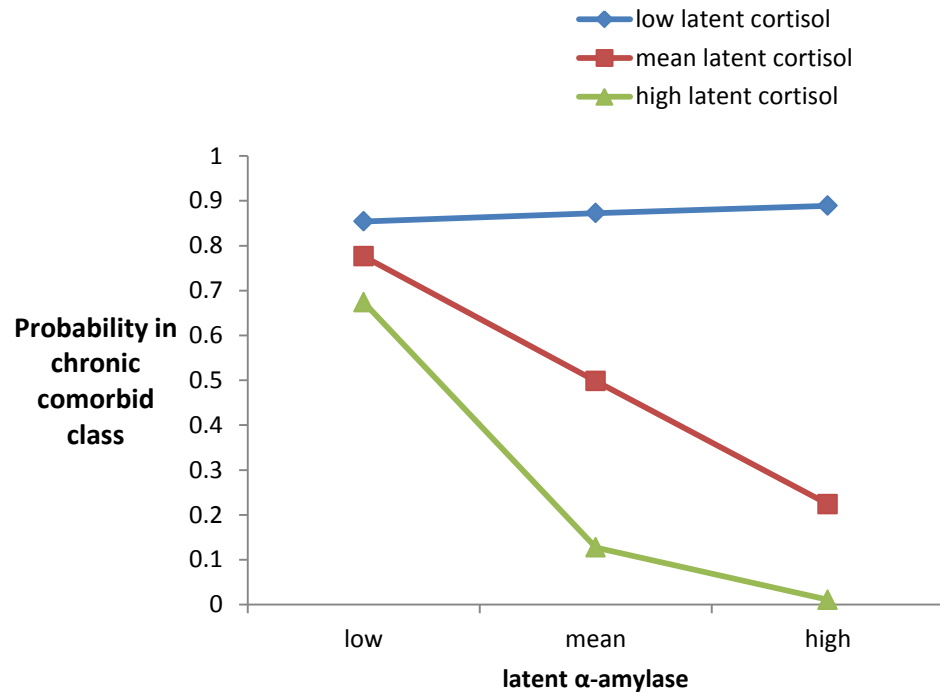


Figure 3.3 Interaction of Cortisol and Alpha-amylase on the Probability of Being in Stable Comorbid Class (with the low class as the reference group)



GENERAL DISCUSSION

These three papers together showed that investigation into stress physiology, especially the interplay of the two components of stress physiology, is of great significance in our understanding of externalizing problems, internalizing problems and the co-occurrence of them. The two components of stress system are associated with behavior problem in a non-linear fashion. More importantly, this dissertation demonstrates that a biosocial interactive perspective provides us with a more complete picture of behavior problems. Stress physiology is not standing alone and it exerts its influence on behavior problems in the context of environmental factors.

Findings warrant several limitations in addition to what have been discussed in each paper. First, the measures for HPA axis and ANS arousal are the end product of the stress system, but stress physiology involves a multi-level complicated system with negative feedback regulation. Nonetheless, there are merits to study the end product of the stress system because it is parsimonious and it provides us initial insight into a complicated regulating system. Second, causal inferences cannot be drawn from any of the three studies due to the cross-sectional design. We cannot conclude from the current study that stress physiology contributes to behavior problems, because it is equally possible that the observed stress physiology characteristics are the result of long-term behavior problems. Nevertheless, the current study provides an initial description of the relationship between stress physiology and behavior problems which would guide future studies to further explore the nature and etiology of behavior problems. Third, the follow-up period in the current project was relatively

short and late childhood is not the ideal age to observe the initiation of the behavior problems, which limits our interpretation of the co-occurrence of behavior problems. The longitudinal piece in this dissertation informs us more of the stability of behavior problems than the development process that encompasses initiation, maintenance and desistance.

The limitations should be balanced by the strength and the contributions. The current findings have both theoretical and practical implications. At a theoretical level, this dissertation is in support of the multi-system approach of studying association between stress physiology and behavior problems, and findings from the biosocial interaction of stress physiology and harsh discipline are most consistent with the basic tenets of differential susceptibility hypothesis. It appears that asymmetry of HPA axis activity and ANS arousal (i.e., high HPA axis activity coupled with low ANS arousal, or low HPA axis activity coupled with high ANS arousal) may indicate high susceptibility to environmental influences in that it is associated with the most behavior problems at high levels of harsh discipline and with the least behavior problems at low levels of harsh discipline. Furthermore, stress physiology adds to our knowledge of the comorbidity of behavior problems, and the interaction of HPA axis activity and ANS arousal differentiates co-occurrence of externalizing and internalizing problems from other developmental trajectories of behavior problems over a year, suggesting that co-occurrence of behavior problems may be qualitatively different from single forms of behavior problems.

The findings also have policy implications, particularly for treatment evaluation research. First, as shown in the findings, individuals have different susceptibility in their stress physiology to environmental influences. Understanding what biological features moderate the intervention outcome is beneficial because we can better evaluate the efficacy of interventions and design interventions catered to subgroups of individuals with distinctive biological characteristics. There are some great attempts in recent years of investigating treatment moderators. For example, Albert and colleagues (2015) identified sources of genomic variations in the effects of the Fast Track Randomized Control Intervention Trial. The Fast Track Intervention is a ten-year long randomized controlled prevention program designed in part to improve emotion regulation and self-control in the context of social stress with the ultimate goal to reducing persistent externalizing problems. Albert and colleagues found that variants of the gene NRC31, a gene that encodes glucocorticoid receptor protein for inhibitory signaling in the HPA axis, moderate the treatment outcomes of Fast Track Intervention. This finding highlights the key role that HPA axis plays in influencing individuals' response to environment.

Second, the incorporation of biological indices like stress physiology would further our understanding of treatment mechanism. Incorporation of biological indices in research and treatment do not necessarily mean manipulating biology directly. Instead, the incorporation opens window for researchers to elucidate treatment mechanism by understanding how interventions may act on biology which in turn elicits behavioral changes. A great case in point is O'Neal and colleagues' efficacy

study on a family intervention for preschoolers (2010). O'Neal and colleagues (2010) included cortisol measures before and after the randomized controlled intervention trials targeted to improve parenting behavior and parent-child interaction. Their findings revealed that cortisol changes in stress response mediated the effect of intervention on child aggressive behavior. That is, family intervention may normalize individuals' stress response physiology to result in reduction in aggressive behaviors. Research in this line would largely benefit intervention practices by highlighting effective components of treatment. Last, inclusion of salivary samples in research would provide abundant biological information in a relatively economical way because analytes from various biological systems/axes such as stress system (cortisol, alpha-amylase), immune system (C-reactive protein) and reproductive system (testosterone) can be assayed from salivary samples. For example, if researchers are interested in victimization and its consequences, they can potentially investigate objective biological measures like cortisol, C-reactive protein to tap into how their stress system and immune system may be altered by their victimization experience. Future research should examine biological indices such as stress physiology together with social factors with a perspective longitudinal design to better derive causal inference. This type of research would be critical in advancing our knowledge antisocial behavior.

BIBLIOGRAPHY

- Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA school-age forms & profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.
- Adam, E. K. (2006). Transactions among adolescent trait and state emotion and diurnal and momentary cortisol activity in naturalistic settings. *Psychoneuroendocrinology*, *31*(5), 664-679.
- Aiken, L. S., & West, S. G. (1991). *Multiple regression: Testing and interpreting interactions*. Thousand Oaks, CA: Sage.
- Alert, D., Belsky, D. W., Crowley, D. M., Latendresse, S. J., Aliev, F., Riley, B., ... & Dodge, K. (2015). Can genetics predict response to complex behavioral interventions? Evidence from a genetic analysis of the Fast Track Randomized Control Trial. *Journal of Policy Analysis and Management*, *34* (3), 497-518.
- Alexander, N., Kuepper, Y., Schmitz, A., Osinsky, R., Kozyra, E., & Hennig, J. (2009). Gene-environment interactions predict cortisol responses after acute stress: implications for the etiology of depression. *Psychoneuroendocrinology*, *34*, 1294-1303.
- Alink, L. R. A., Cicchetti, D., Kim, J., & Rogosch, F. A. (2012). Longitudinal associations among child maltreatment, social functioning, and cortisol regulation. *Developmental Psychology*, *48*, 224-236.

- Alink, L. R. A., van IJzendoorn, M. H., Bakerman-Kranenburg, M. J., Mesman, J., Juffer, F., & Koot, H. M. (2008). 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. *Developmental Psychobiology*, 50, 427-450.
- Allwood, M. A., Handwerger, K., Kivlighan, K. T., Granger, D. A., & Stroud, L. R. (2011). Direct and moderating links of salivary alpha-amylase and cortisol stress-reactivity to youth behavioral and emotional adjustment. *Biological Psychology*, 88, 57-64.
- Azar, R., Zoccolillo, M., Paquette, D., Quiros, E., Baltzer, F., & Tremblay, R. E. (2004). Cortisol levels and conduct disorder in adolescent mothers. *Journal of the American Academy of Child and Adolescent Psychiatry*, 43, 461-468.
- Badanes, L. S., Watamura, S. E., & Hankin, B. L. (2011). Hypocortisolism as a potential marker of allostatic load in children: associations with family risk and internalizing disorders. *Development and Psychopathology*, 23, 881-896.
- Bauer, A. M., Quas, J. A., & Boyce, W. T. (2002). Associations between physiological reactivity and children's behavior: Advantages of a multisystem approach. *Journal of Developmental and Behavioral Pediatrics*, 23(2), 102-113.
- Beidel, D. C. (1991). Determining the reliability of psycho-physiological assessment in childhood anxiety. *Journal of Anxiety Disorders*, 5, 139-150.

- Belsky, J., & Pluess, M. (2009). Beyond diathesis stress: differential susceptibility to environmental influences. *Psychological Bulletin*, 135(6), 885-908.
- Booth, A., Granger, D. A., & Shirtcliff, E. A. (2008). Gender- and age- related differences in the association between social relationship quality and trait-levels of salivary cortisol. *Journal of Research on Adolescence*, 18, 239-260.
- Bosch, J. A., de Geus, E. J., Veerman, E. C., Hoogstraten, J., & Nieuw-Amerongen, A.V. (2003). Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. *Psychosomatic Medicine*, 65, 245-258.
- Brennan, P. A., Raine, A., Schulsinger, F., Kirkegaard-Sorenson, L., Knop, J., Hutchings, B., ... & Mednick, S. A. (1997). Psychophysiological protective factors for male subjects at high risk for criminal behavior. *American Journal of Psychiatry*, 154, 853-855.
- Cannon, W. B. (1914). The interrelations of emotions as suggested by recent physiological researches. *American Journal of Psychology*, 25, 256-282.
- Capaldi, D. (1992). Co-occurrence of conduct problems and depressive symptoms in early adolescent boys: II. A 2-year follow-up at Grade 8. *Development and Psychopathology*, 4, 125-144.

- Chatterton Jr., R.T., Vogelsong, K.M., Lu, Y.C., Ellman, A.B., & Hudgens, G.A. (1996). Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clinical Physiology*, 16, 433-448.
- Chen, F. R., Raine, A., Soyfer, L., & Granger, D. A. (2015a). Interaction of adrenocortical activity and autonomic arousal on children's externalizing and internalizing problems. *Journal of Abnormal Child Psychology*, 43, 189-202.
- Chen, F. R., Raine, A., Rudo-Hutt, A., Glenn, A. L., Soyfer, L., & Granger, D. A. (2015b). Harsh parenting and child behavior problems: the moderating effects of cortisol and salivary alpha-amylase. *Biological Psychology*, 104, 19-27.
- Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress system disorders: overview of behavioral and physical homeostasis. *JAMA*, 267, 1244-1252.
- Cicchetti, D., & Rogosch, F. A. (2001). The impact of child maltreatment and psychopathology on neuroendocrine functioning. *Development and Psychopathology*, 13, 783-804.
- Cicchetti, D., Rogosch, F. A., Gunnar, M. R., & Toth, S. L. (2010). The differential impacts of early physical and sexual abuse and internalizing problems on daytime cortisol rhythm in school-aged children. *Child Development*, 81, 252-269.
- Cohen, M. A. (1998). The monetary value of saving a high-risk youth. *Journal of Quantitative Criminology*, 14, 5-33.

- Cohen, J., Cohen, P., West, S. G., & Aiken, L. S. (2003). *Applied multiple regression/correlation analysis for the behavioral sciences* (3rd Ed). Mahwah, NJ: Erlbaum.
- Coie, J. D., & Dodge, K. A. (1998). Aggression and antisocial behavior. In W. Damon (Eds.), *Handbook of child psychology* (5th Ed.), vol. 3. New York: Wiley.
- Cole, P., Zahn-Waxler, C., Fox, N., Usher, B., & Welsh, J. (1996). Individual differences in emotion regulation and behavior problems in preschool children. *Journal of Abnormal Psychology*, 105, 518-529.
- Costello, E., Angold, A., Burns, B., Stangl, D., Tweed, D., Erkanli, A., & Worthman, C. (1996). The Great Smoky Mountains Study of youth: Goals, design, methods, and the prevalence of DSM III-R disorders. *Archives of General Psychiatry*, 53(12), 1129–1136.
- Csorba, J., Rozsa, S., Vetro, A., Gadoros, J., Makra, J., Somogyi, E., Kaczvinszky, & Kapornay, K. (2001). Family and school-related stresses in depressed Hungarian children. *European Psychiatry*, 16(1), 18-26.
- Davis, E. P., Donzella, B., Krueger, W. K., & Gunnar, M. R. (1999). The start of a new school year: individual differences in salivary cortisol response in relation to child temperament. *Developmental Psychobiology*, 35, 188-196.

- de Bellis, M.D. (2001). Developmental traumatology: The psychobiological development of maltreated children and its implications for research, treatment, and policy. *Development and Psychopathology*, 13, 539-564.
- de Vries-Bouw, M., Jansen, L., Vermeiren, R., Doreleijeers, T., van de Ven, P., & Popma, A. (2012). Concurrent attenuated reactivity of alpha-amylase and cortisol is related to disruptive behavior in male adolescents. *Hormones and Behavior*, 62, 77-85.
- Del Giudice, M., Ellis, B. J., & Shirtcliff, E. A. (2011). The adaptive calibration models of stress responsivity. *Neuroscience and Biobehavioral Reviews*, 35, 1562-1592.
- Dierckx, B., Dieleman, G., Tulen, J. H. M., Treffers, P. D. A., Utens, E. M. W. J., Verhulst, F. C., & Tiemeier, H. (2012). Persistence of anxiety disorders and concomitant changes in cortisol. *Journal of Anxiety Disorder*, 26, 635-641.
- Doane, L. D., Chen, F. R., Sladek, M. R., van Lenten, S. A., & Granger, D. A. (2015). Latent trait cortisol (LTC) levels: Reliability, validity, and stability. *Psychoneuroendocrinology*, 55, 21-35.
- El-Sheikh, M., Erath, S. A., Buckhalt, J. A., Granger, D. A., & Mize, J. (2008). Cortisol and children's adjustment: the moderating role of sympathetic nervous system activity. *Journal of Abnormal psychology*, 36, 601-611.

- Farrington, D. P. (1991). Childhood aggression and adult violence: early precursors and later-life outcomes. In D.J. Pepler, & K.H. Rubin, (Eds.), *The development and treatment of childhood aggression* (pp. 5-29). Hillsdale, NJ: Lawrence Erlbaum.
- Farrington, D. P. (2000). Psychosocial predictors of adult antisocial personality and adult convictions. *Behavior Sciences and the Law*, 18, 605-622.
- Fanti, K. A., & Henrich, C. C. (2010). Trajectories of pure and co-occurring internalizing and externalizing problems from age 2 to age 12: findings from the National Institute of Child Health and Human Development Study of Early Child Care. *Developmental Psychology*, 46(5), 1159-1175.
- Folkow, B. (1985). Stress and blood pressure in adrenergic blood pressure regulation. In Birkenhager, W. H., Folkow, B., & Struyker, H. A. J (Eds.), *Current Clinical Practice Series* (pp.87-93). Amsterdam: Excerpta Medica.
- Frankenhaeuser, M., Lundberg, U., & Forsman, L. (1980). Dissociation between sympathetic-adrenal and pituitary-adrenal responses to an achievement situation characterized by high controllability: Comparison between Type A and Type B males and females. *Biological Psychology*, 10, 79-91.
- Fries, E., Hesse, J., Hellhammer, J., & Hellhammer, D. H. (2005). A new view on hypocortisolism. *Psychoneuroendocrinology*, 30, 1010-1016.

- Gao, Y., Raine, A., Venables, P. H., Dawson, M. E., & Mednick, S. A. (2010a). Association of poor childhood fear conditioning and adult crime. *American Journal of Psychiatry*, *167*, 56-60.
- Gao, Y., Raine, A., Venables, P. H., Dawson, M. E., & Mednick, S. A. (2010b). Reduced electrodermal fear conditioning from ages 3 to 8 years is associated with aggressive behavior at age 8 years. *Journal of Child Psychology and Psychiatry*, *51*, 550-558.
- Gerra, G., Zaimovic, A., Mascetti, G.G., Gardini, S., Zambelli, U., Timpano, M., Raggi, M. A., & Brambilla, F. (2001). Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. *Psychoneuroendocrinology*, *26*, 91-107.
- Gershoff, E. T. (2002). Corporal punishment by parents and associated child behaviors and experiences: A meta-analytic and theoretical review. *Psychological Bulletin*, *128*, 539-579.
- Gershoff, E. T., Lansford, J. E., Sexton, H. R., Davis-Kean, P. E., & Sameroff, A. J. (2012). Longitudinal links between spanking and children's externalizing behaviors in a national sample of White, Black, Hispanic, and Asian American families. *Child Development*, *83*, 838-843.
- Goenjian, A. A., Yehuda, R., Pynoos, R. S., Steinberg, A. M., Tashjian, M., Yang, R. K., Najarian, L. M., & Fairbanks, L. A. (1996). Basal cortisol, dexamethasone

- suppression of cortisol, and MHPG in adolescents after the 1988 earthquake in Armenia. *American Journal of Psychiatry*, 153, 929-934.
- Gordis, E. B., Granger, D. A., Susman, E. J., & Trickett, P. K. (2006). Asymmetry between salivary cortisol and α -amylase reactivity to stress: Relation to aggressive behavior in adolescents. *Psychoneuroendocrinology*, 31, 976-987.
- Granger, D. A., Fortunato, C. K., Beltzer, E. K., Virag, M., Bright, M. A., & Out, D. (2012). Focus on methodology: salivary bioscience and research on adolescence: an integrated perspective. *Journal of Adolescence*, 35, 1081-1095.
- Granger, D. A., Hibel, L. C., & Fortunato, C. K., & Kapelewski, C. H. (2009). Medication effects on salivary cortisol: Tactics and strategy to minimize impact in behavioral and developmental science. *Psychoneuroendocrinology*, 34, 1437-1448.
- Granger, D. A., Kivlighan, K.T., El-Sheikh, M., Gordis, E.B., & Stroud, L.R. (2007). Salivary alpha-amylase in biobehavioral research: recent developments and applications. *Annals of the New York Academy of Sciences*, 1098, 122-144.
- Gurguis, G. N., Mefford, I. N., & Uhde, T. W. (1991). Hypothalamic-pituitary-adrenocortical activity in panic disorder: relationship to plasma catecholamine metabolites. *Biological Psychiatry*, 30(5), 502-506.

- Harrison, L., & Turpin, G. (2003). Implicit memory bias and trait-like anxiety: A psychophysiological analysis. *Biological Psychology*, 62, 97-114.
- Hart, J., Gunnar, M., & Cicchetti, D. (1995). Salivary cortisol in maltreated children: evidence of relations between neuroendocrine activity and social competence. *Development and Psychopathology*, 7, 11-26.
- Hayes, A. F., & Matthes, J. (2009). Computational procedures for probing interactions in OLS and logistic regression: SPSS and SAS implementations. *Behavior Research Methods*, 41, 924-936.
- Heim, U., Ehlert, D. H., & Hellhammer, D. H. (2000). The potential role of hypocortisolism in pathophysiology of stress related bodily disorders. *Psychoneuroendocrinology*, 25, 1-35.
- Hellhammer, D. H., Kirschbaum, C., & Belkien, L. (1987). Measurement of salivary cortisol under psychological stimulation. In Hingtgen, J. N., Hellhammer, D. H., & Huppmann, G. (Eds.), *Advanced Methods in Psychobiology* (pp. 281-289). Hogrefe: Toronto.
- Hellhammer, D., & Wade, S. (1993). Endocrine correlates of stress vulnerability. *Psychotherapy and Psychosomatics*, 60, 8-17.
- Henry, J. P. (1993). Biological basis of the stress response. *Physiology*, 8, 69-73.

- Hu, L., & Bentler, P. M. (1998). Fit indices in covariance structure modeling: Sensitivity to underparameterized model misspecification. *Psychological Methods*, 3, 424-53.
- Huesmann, L. R., Eron, L. D., Lefkowitz, M. M., & Walder, L. O. (1984). Stability of aggression over time and generations. *Developmental Psychology*, 20, 1120-1134.
- Jaccard, J., & Turrisi, R. (2003). *Interaction effects in multiple regression* (2nd Ed.). Thousand Oaks, CA: Sage.
- Jaccard, J., Wan, C. K., & Turrisi, R. (1990). The detection and interpretation of interaction effects between continuous variables in multiple regression. *Multivariate Behavioral Research*, 25, 467-478.
- Jaffee, S. R., Moffitt, T. E., & Caspi, A. (2002). Influence of adult domestic violence on children's externalizing and internalizing problems: an environmentally informative twin study. *Journal of the American Academy of Child and Adolescent Psychiatry*, 42(9), 1095-1103.
- Johnson, P. O., & Fay, L. C. (1950). *The Johnson–Neyman technique, its theory and application*. *Psychometrika*, 15, 349-367.
- Johnson, P. O., & Neyman, J. (1936). Tests of certain linear hypotheses and their application to some educational problems. *Statistical Research Memoirs*, 1, 57-93.

- Johnson, S. B., Riley, A. W., Granger, D. A., & Riis, J. (2013). The science of early life toxic stress for pediatric practice and advocacy. *Pediatrics*, 131, 319-27.
- Kagan, J. (1994). *Galen's prophecy: temperament in human nature*. New York: Basic Books.
- Kagan, J., Reznick, J. S., & Snidman, N. (1988). Biological bases of childhood shyness. *Science*, 240, 167-171.
- Kaufman, J. (1991). Depressive disorders in maltreated children. *Journal of the American Academy of Child and Adolescent Psychiatry*, 30, 257-265.
- Keiley, M. K., Lofthouse, N., Bates, J. E., Dodge, K. A., & Pettit, G.S. (2003). Differential risks of covarying and pure components in mother and teacher reports of externalizing and internalizing behavior across ages 5 to 14. *Journal of Abnormal Child Psychology*, 31, 267-283.
- Kenny, D. A., & Zautra, A. (2001). Trait-state models for longitudinal data. In L.M. Collins & A. G. Sayer (Eds.), *New methods for the analysis of change* (pp. 243-263). Washington, DC: American Psychological Association.
- Kertes, D. A., Gunnar, M. R., Madsen, N. J., & Long, J. D. (2008). Early deprivation and home basal cortisol levels: a study of internationally adopted children. *Development and Psychopathology*, 20(2), 473-491.
- Kessler, R. C., Adler, L. A., Barkley, R., Biederman, J., Conners, C. K., Faraone, S. V., & Zaslavsky, A. M. (2005). Patterns and predictors of attention-

deficit/hyperactivity disorder persistence into adulthood: results from the national comorbidity survey replication. *Biological Psychiatry*, 57(11), 1442-1451.

Kessler, R. C., & Wang, P. S. (2008). The descriptive epidemiology of commonly occurring mental disorders in the United States. *Annual Review of Public Health*, 29, 115-129.

Kim, I., Ge, X., Brody, G., Conger, R., Gibbons, F., & Simons, R. (2003). Parenting behaviors and the occurrence and co-occurrence of depressive symptoms and conduct problems among African American children. *Journal of Family Psychology*, 17(4), 571–583.

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*, 19, 313-333.

Kirschbaum, C., Steyer, R., Eid, M., Patalla, U., Schwenkmezger, P, & Hellhammer, D. (1990). Cortisol and behavior: 2. Application of a latent state-trait-like model to salivary cortisol. *Psychoneuroendocrinology*, 15, 297-307.

Klimes-Dougan, B., Hastings, P. D., Granger, D. A., Usher, B. A., & Zahn-Waxler, C. (2001). Adrenocortical activity in at risk and normally developing adolescents: Individual differences in salivary cortisol basal levels, diurnal variation, and

responses to social challenges. *Development and Psychopathology*, 13, 695-719.

Kline, R. B. (2011). *Principles and practice of structural equation modeling* (3rd Ed.). New York: Guilford Press.

Kovacs, M., Paulauskas, S., Gatsonis, C., & Richards, C. (1988). Depressive disorders in childhood: III. A longitudinal study of comorbidity with and risk for conduct disorders. *Journal of Affective Disorders*, 15, 205–217.

Liu, J., Richmond, T. S., Raine, A., Cheney, R., Brodtkin, E. S., Gur, R. C., & Gur, R. E. (2013). The Healthy Brains and Behavior Study: Objectives, Design, Recruitment, and Population Coverage. *International Journal of Methods in Psychiatric Research*, 22(3), 204-216.

Lopez-Duran, N. L., Kovacs, M., & George, C. J. (2009). Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. *Psychoneuroendocrinology*, 34, 1272-1283.

Lorber, M. F. (2004). Psychophysiology of aggression, psychopathy, and conduct problems: A meta-analysis. *Psychological Bulletin*, 130, 531-552.

Lösel, F., & Farrington, D. P. (2012). Direct protective and buffering protective factors in the development of youth violence. *American Journal of Preventive Medicine*, 43, S8-S23.

- Lovallo, W. R., & Thomas, T. L. (2000). Stress hormones in psychophysiological research: emotional, behavioral, and cognitive implications. In J. T. Cacioppo, L. G. Tassinary, & G.G. Bernston. (Eds.), *Handbook of psychophysiology* (2nd Ed, pp. 342-367). Cambridge University Press, New York.
- Lundberg, U., & Frankenhaeuser, M. (1980). Pituitary-adrenal and sympathetic-adrenal correlates of distress and effort. *Journal of psychosomatic research*, 24, 125-130.
- McBurnett, K., Lahey, B. B., Rathouz, P. J., & Loeber, R. (2000). Low salivary cortisol and persistent aggression in boys referred for disruptive behavior. *Archives of General Psychiatry*, 57, 38-43.
- McBurnett, K., Raine, A., Stouthamer-Loeber, M., Loeber, R., Kumar, A. M., Kumar, M., & Lahey, B. B. (2005). Mood and hormone responses to psychological challenge in adolescent males with conduct problems. *Biological Psychiatry*, 57(10), 1109-1116.
- McCrae, J. S. (2009). Emotional and behavioral problems reported in child welfare over 3 years. *Journal of Emotional and Behavioral Disorders*, 17, 17-28.
- McCord, J. (2001). Psychosocial contributions to psychopathy and violence. In A. Raine & J. Sanmartin (Eds.), *Violence and psychopathy* (pp. 141-170). New York: Kluwer Academic/ Plenum.

- McEwen, B. S. (1998). Stress adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, 840, 33-44.
- McEwen, B. S., & Stellar, E. (1993). Stress and the individual: Mechanisms leading to disease. *Archives of internal medicine*, 153(18), 2093–2101.
- McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, 43(1), 2-15.
- McLoyd, V. C., Kaplan, R., Hardaway, C. R., & Wood, D. (2007). Does endorsement of physical discipline matter? Assessing moderating influences on the maternal and child psychological correlates of physical discipline in African American Families. *Journal of Family Psychology*, 21(2), 165-175.
- Moffitt, T. E. (1990). Juvenile delinquency and attention deficit disorder: boy's developmental trajectories from age 3 to age 15. *Child Development*, 61, 893-910.
- Monroe, S. M., & Simons, A. D. (1991). Diathesis-stress theories in the context of life stress research: Implications for the depressive disorders. *Psychological Bulletin*, 110, 406–425.
- Morris, N. M., & Udry, J. R. (1980). Validation of a self-administrated instrument to assess stage of adolescent development. *Journal of Youth and Adolescence*, 9(3), 271-280.

- Muthén, B. (2012). Latent variable interactions. *Statmodel.com*. Retrieved July 19, 2013, from <http://www.statmodel.com/download/LV%20Interaction.pdf>.
- Muthén, B. O., & Muthén, L. K. (2000). Integrating person-centered and variable-centered analysis analyses: growth mixture modeling with latent trajectory classes. *Alcoholism: Clinical and Experimental Research*, 24(6), 882-891.
- Muthén, L.K., & Muthén, B.O. (1998-2012). *Mplus User's Guide*. (7th Edition). Los Angeles, CA: Muthén & Muthén.
- Nagin, D. S. (2005). *Group-based modeling of development*. Cambridge, MA: Harvard University Press.
- Nater, U.M., & Rohleder, N. (2009). Salivary alpha-amylase as a noninvasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*, 34, (4), 486-496.
- Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., & Ehlert, U. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology*, 55, 333-342.
- Netherton, C., Goodyer, I., Tamplin, A., & Herbert, J., (2004). Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology* 29, 125-140.
- Nock, M. K., Kazdin, A. E., Hiripi, E., & Kessler, R. C. (2007). Lifetime prevalence, correlates, and persistence of oppositional defiant disorder: results from the

- national comorbidity survey replication. *Journal of Child Psychology and Psychiatry*, 48(7), 703-713.
- Nock, M. K., & Mendes, W. B. (2008). Physiological arousal, distress tolerance, and social problem-solving deficits among adolescent self-injurers. *Journal of Consulting and Clinical Psychology*, 76, 28–38.
- Nottelmann, E., & Jensen, P. (1995). Comorbidity of disorders in children and adolescents: Developmental perspectives. *Advances in Clinical Child Psychology*, 17,109-155.
- Nylund, K. L., Asparouhov, T., & Muthén, B. O. (2007). Deciding on the number of classes in latent class analysis and growth mixture modeling: a Monte Carlo Simulation study. *Structural Equation Modeling: 14*(4), 535-569.
- Oland, A. A., & Shaw, D. S. (2005). Pure versus co-occurring externalizing and internalizing symptoms in children: the potential role of socio-developmental milestones. *Clinical Child and Family Psychology Review*, 8(4), 247-270.
- O'Neal, C. R., Brotman, L. M., Huang, K., Gouley, K. K., Kamboukos, D., Calzada, E. J., & Pine, D. S. (2010). Understanding Relations Among Early Family Environment, Cortisol Response, and Child Aggression via a Prevention Experiment. *Child Development*, 81(1), 290-305.

- Oosterlaan, J., Geurts, H. M., Knol, D. L., & Sergeant, J. A. (2005). Low basal salivary cortisol is associated with teacher-reported symptoms of conduct disorder. *Psychiatry Research*, 134, 1-10.
- Ortiz, J., & Raine, A. (2004). Heart rate level and antisocial behavior in children and adolescents: a meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry*, 43(2), 154-162.
- Ouellet-Morin, I., Boivin, M., Dionne, G., Lupien, S. J., Arseneault, L., Barr, R. G., Pérusse, D., & Tremblay, R. E. (2008). Variations in heritability of cortisol reactivity to stress as a function of early familial adversity among 19-month-old twins. *Archives of General Psychiatry*, 65, 211-218.
- Out, D., Bakermans-Kranenburg, M., Granger, D. A., Cobbaert, C. M., & van IJzendoorn, M. H. (2011). State and trait variance in salivary alpha-amylase: a behavior genetic study. *Biological Psychology*, 88, 147-154.
- Out, D., Granger, D. A., Sephton, S. E., & Segerstrom, S. C. (2013). Disentangling sources of individual differences in diurnal salivary alpha-amylase: reliability, stability and sensitivity to context. *Psychoneuroendocrinology*, 38(3), 367.
- Pajer, K., Gardner, W., Rubin, R. T., Perel, J., & Neal, S. (2001). Decreased cortisol levels in adolescent girls with conduct disorder. *Archives of General Psychiatry*, 58, 297-302.

- Parker, J., Rubin, K. H., Erath, S., Wojslawowicz, J. C., & Buskirk, A. A. (2006). Peer relationships and developmental psychopathology. In D. Cicchetti & D. Cohen (Eds.), *Developmental psychopathology: Risk, disorder, and adaptation* (Vol. 2, 2nd Ed., pp. 419-493). Hoboken, NJ: Wiley.
- Patterson, G., & Stoolmiller, M. (1991). Replications of a dual failure model for boys' depressed mood. *Journal of Consulting and Clinical Psychology, 59*(4), 491-498.
- Peters, M. L., Godaert, G. L., Ballieux, R. E., van Vliet, M., Willemsen, J. J., Sweep, F. C. G. J., & Heijnen, C. J. (1998). Cardiovascular and endocrine responses to experimental stress: Effects of mental effort and controllability. *Psychoneuroendocrinology, 23*, 1-17.
- Portnoy, J., Chen, F. R., & Raine, A. (2013). Biological protective factors for antisocial and criminal behavior. *Journal of Criminal Justice, 41*(5), 292-299.
- Raine, A. (1993). *The psychopathology of crime: Criminal behavior as a clinical disorder*. San Diego, California: Academic Press.
- Raine, A. (2002). Biosocial studies of antisocial and violent behavior in children and adults: A review. *Journal of Abnormal Child Psychology, 30*, 311-326.
- Raine, A. (2005). The interaction of biological and social measures in the explanation of antisocial and violent behavior. In D. Stoff & E. Susman. (Eds),

Developmental Psychobiology of Aggression (pp. 13-42). Cambridge University Press, New York.

Raine, A., Venables, P. H., & Williams, M. (1995). High autonomic arousal and electrodermal orienting at age 15 years as protective factors against criminal behavior at age 29 years. *American Journal of Psychiatry*, 152, 1595-1600.

Reitz, E., Dekovic, M., & Meijer, A. M. (2006). Relations between parenting and externalizing and internalizing behavior problems in early adolescence: child behavior as moderator and predictor. *Journal of Adolescence*, 29(3), 419-436.

Reid, J. B., Patterson, G. R., & Snyder, J. (2002). *Antisocial behavior in children and adolescents: A developmental analysis and the Oregon model for intervention*. Washington, DC: American Psychological Association.

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary alphaamylase in humans and methodological considerations. *Psychoneuroendocrinology*, 34(4), 469-485.

Rodriguez, C. M. (2003). Parental discipline and abuse potential affects on child depression, anxiety, and attributionS. *Journal of Marriage and Family*, 65(4), 809-817.

Rutter, M. (1997). Comorbidity: concepts, claims and choices. *Criminal Behavior and Mental Health*, 7, 265-285.

- Rutter, M. & Giller, H. (1983). *Juvenile delinquency: trends and perspectives*. New York: Guilford Press.
- Sampson, R. J., & Laub, J. H. (1993). *Crime in the making: pathways and turning points through life*. Cambridge: Harvard University Press.
- Sampson, R. J., Raudenbush, S. W., & Earls, F. (1997). Neighborhoods and violent crime: a multilevel study of collective efficacy. *Science*, 227, 918-924.
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress response? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21(1), 55-89.
- Schommer, N. C., Hellhammer, D. H., & Kirschbaum, C. (2003). Dissociation between reactivity of the hypothalamus-pituitary-adrenal and the sympathetic-adrenal-medullary system to repeated psychosocial stress. *Psychosomatic Medicine*, 65, 450-460.
- Schmidt, L. A., Fox, N. A., Rubin, K. H., Sternberg, E. M., Gold, P. W., Smith, C. C., & Schulkin, J. (1997). Behavioral and neuroendocrine responses in shy children. *Developmental psychobiology*, 30, 127-140.
- Schulkin, J., Gold, P. W., & McEwen, B. S. (1998). Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology*, 23, 219-243.

- Scott, S., Knapp, M., Henderson, J., & Maughan, B. (2001). Financial cost of social exclusion: Follow up study of antisocial children into adulthood. *British Medical Journal*, 323, 1-5.
- Shannon, K. E., Beauchaine, T. P., Brenner, S. L., Neuhaus, E., & Gatzke-Kopp, L. (2007). Familial and temperamental predictors of resilience in children at risk for conduct disorder and depression. *Development and Psychopathology*, 19, 701-727.
- Shirtcliff, E. A., Granger, D. A., Booth, A., & Johnson, D. (2005). Low salivary cortisol levels and externalizing behavior problems in youth. *Development and psychopathology*, 17, 167-184.
- Sijtsema, J. J., Shoulberg, E. K., & Murray-Close, D. (2011). Physiological reactivity and different forms of aggression in girls: Moderating roles of rejection sensitivity and peer rejection. *Biological Psychology*, 86, 181-192.
- Skosnik, P. D., Chatterton, R. T., Swisher, T., & Park, S. (2000). Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. *International Journal of Psychophysiology*, 36, 59-68.
- Smider, N. A., Essex, M. J., Kalin, N. H., Buss, K. A., Klein, M. H., Davidson, R. J., & Goldsmith, H. H. (2002). Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: a perspective study. *Child Development*, 73, 75-92.

- Steely, A. C., & Rohner, R. P. (2006). Relations among corporal punishment, perceived parental acceptance, and psychological adjustment in Jamaican youths. *Cross-Cultural Research, 40*(3), 268-286.
- Strauss, M. A. (1979). Measuring intrafamily conflict and violence: The Conflict Tactics Scales. *Journal of Marriage and the Family, 41*, 75-88.
- Strauss, M. A., Hamby, S. L., Finkelhor, D., Moore, D. W., & Runyan, D. (1998). Identification of child maltreatment with the parent-child Conflict Tactics Scales: development and psychometric data for a national sample of American parents. *Child Abuse and Neglect, 22*(4), 249-270.
- Tabachnick, B. G., & Fidell, L. S. (2012). *Using multivariate statistics* (6th Edition). Boston: Pearson Education, Inc.
- Taylor, S. E. (2006). Tend and befriend: biobehavioral bases of affiliation under stress. *Current Directions in Psychological Science, 15*(6), 273-277.
- Taylor, S. E., Klein L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A. R., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychological Review, 107*(3), 411-429.
- Taylor, Z. E., Spinrad, T. L., VanSchyndel, S. K., Eisenberg, N., Huynh, J., Sulik, M. J., & Granger, D. A. (2012). Sociodemographic risk, parenting, and effortful control: relations to salivary alpha-amylase and cortisol in early childhood. *Developmental Psychobiology, 55*(8), 869-880.

- Tonhajzerova, I., Ondrejka, I., Javorka, K., Turianikova, Z, Farsky, I., & Javorka, M. (2010). Cardiac autonomic regulation is impaired in girls with major depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 34, 613-618.
- Toth, S. L., & Cicchetti, D. (2013). A developmental psychopathology perspective on child harsh discipline. *Child Harsh discipline*, 18(3), 135-139.
- van Bokhoven, I., Matthys, W., van Goozen, S. H. M., & van Engeland, H. (2005). Prediction of adolescent outcome in children with disruptive behavior disorders: A study of neurobiological, psychological and family factors. *European Child and Adolescent Psychiatry*, 14, 153-163.
- van Honk, J., Schutter, D. J. L. G., Hermans, E. J., Putman, P., Tuiten, A., & Koppeschaar, H. (2004). Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. *Psychoneuroendocrinology*, 29, 937-943.
- van Lier, P. A. C & Koot, H. M. (2013). Developmental cascades of peer relations and symptoms of externalizing and internalizing problems from kindergarten to fourth-grade elementary school. *Development and Psychopathology*, 22, 569-582.

- van Goozen, S. H. M., Fairchild, G., Snoek, H., & Harold, G.T. (2007). The evidence for a neurobiological model of childhood antisocial behavior. *Psychological Bulletin*, 133, 149-182.
- van Goozen, S. H. M., Matthys, W., Cohen-Kettenis, P. T., Buitelaar, J. K., & van Engeland, H. (2000). Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. *Journal of the American Academy of Child and Adolescent Psychiatry*, 39, 1438-1445.
- van Stegeren, A., Rohleder, N., Everaerd, W., & Wolf, O.T. (2006). Salivary alpha-amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology*, 31, 137-141.
- Vigil, J. M., Geary, D. C., Granger, D. A., & Flinn, M. V. (2010). Sex differences in salivary cortisol, alpha-amylase, and psychological functioning following hurricane Katrina. *Child Development*, 81(4), 1228-1240.
- Vreeburg, S. A., Zitman, F. G., van Pelt, J., Derijk, R. H., Verhagen, J. C., van Dyck, R., Hoogendijk, W. J., Smit, J. H., & Penninx, B. X. (2010). Salivary cortisol levels in persons with and without different anxiety disorders. *Psychosomatic Medicine*, 72(4), 340-347.
- Wang, M., & Kenny, S. (2014). Longitudinal links between fathers' and mothers' harsh verbal discipline and adolescents' conduct problems and depressive symptoms. *Child Development*, 85(3), 908-923.

- Wedekind, D., Bandelow, B., Broocks, A., Hajak, G., & Ruther, E. (2000). Salivary, total plasma and plasma free cortisol in panic disorder. *Journal of Neural Transmission, 107*(7), 831-837.
- Weems, C. F., & Carrión, V. G. (2007). The association between PTSD symptoms and salivary cortisol in youth: The role of the time since the trauma. *Journal of Traumatic Stress, 20*, 903-907.
- Weems, C. F., Zakem, A. H., Costa, N. M., Cannon, M. F., & Watts, S. E. (2005). Physiological response and childhood anxiety: Association with symptoms of anxiety disorders and cognitive bias. *Journal of Clinical Child & Adolescent Psychology, 34*, 712–723.
- Weiner, B. (1992). *Human Motivation: Metaphors, Theories, and Research*. Thousand Oaks, CA: Sage.
- Wilson, J. Q., & Herrnstein, R. (1985). *Crime and human nature*. New York: Simon and Schuster.
- Woodruff, K., & Lee, B. (2011). Identifying and predicting problem behavior trajectories among pre-school children investigated for child abuse and neglect. *Child Abuse and Neglect, 35* (7), 491-503.
- Yehuda, R., Boisoneau, D., Mason, J. W., & Giller, E. L. (1993). Glucocorticoid receptor number and cortisol excretion in mood, anxiety, and psychotic disorders. *Society of Biological Psychiatry, 34*, 18-25.

- Young, E. A., Abelson, J., & Cameron, O. G. (2005). Interaction of brain noradrenergic system and the hypothalamic-pituitary-adrenal (HPA) axis in man. *Psychoneuroendocrinology*, 30, 807-814.
- Youngstrom, E., Findling, R., & Calabrese, J. (2003). Who are the comorbid adolescents? Agreement between psychiatric diagnosis, youth, parent, and teacher report. *Journal of Abnormal Child Psychology*, 31(3), 231–245.
- Youngstrom, E., Loeber, R., & Stouthamer-Loeber, M. (2000). Patterns and correlations of agreement between parent, teacher, and male adolescent ratings of externalizing and internalizing problems. *Journal of Consulting and Clinical Psychology*, 68(6), 1038-1050.
- Zuckerman, M. (1979). *Sensation seeking: Beyond the optimum level of arousal*. Hillsdale, NJ: Erlbaum.