GENETIC, HORMONE, AND FAMILY ENVIRONMENTAL INFLUENCES ON THE
DEVELOPMENT OF ADOLESCENT INTERNALIZING AND EXTERNALIZING
BEHAVIOR

A Dissertation in
Psychology
by
Kristine Marceau

© 2013 Kristine Marceau

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2013
The dissertation of Kristine P. Marceau was reviewed and approved* by the following:

Jenae M. Neiderhiser  
Liberal Arts Research Professor of Psychology  
Dissertation Advisor  
Chair of Committee

Elizabeth Susman  
Jean Phillips Shibley Professor of Biobehavioral Health

Alysia Blandon  
Assistant Professor of Psychology

Ginger Moore  
Associate Professor of Psychology

Melvin M. Mark  
Professor of Psychology  
Head of the Department of Psychology

*Signatures are on file in the Graduate School.
Abstract

Our understanding of the development of internalizing and externalizing problems can be greatly strengthened through interdisciplinary research using a family-based approach combining the strengths of behavioral genetics and behavioral endocrinology. This dissertation thesis presents a rationale and conceptual framework for testing the complex transactional associations between genetic, prenatal, endocrine, and family environmental influences hypothesized by existing literature. Empirical support for the conceptual framework is presented in the form of three studies designed to test important facets of the proposed conceptual model. The first study probed the gene-environment interplay underlying the association between parental negativity and externalizing problems in adolescence and shows that parental negativity is a response to adolescents’ genetically informed externalizing problems - not an environmental influence causing adolescents’ externalizing problems. The second study tested the associations between reactivity of several hormones to mother-child conflict, parental negativity and internalizing and externalizing problems during adolescence, and suggests that parental conflict and negativity can moderate hormone-behavior associations. Finally, the third study tested the associations and interactions among select genetic, prenatal, endocrine, and postnatal environmental influences on the development of internalizing and externalizing problems across infancy to middle childhood, and demonstrates that prenatal and endocrine influences can mediate genetic and environmental influences on internalizing, but not externalizing problems during childhood. Together, these studies generally support the proposed conceptual framework for the integration of genetic, prenatal, endocrine, and family environmental influences on the development of adolescent externalizing problems.
# TABLE OF CONTENTS

List of Tables ........................................................................................................... v
List of Figures ............................................................................................................. vi
Acknowledgements .................................................................................................... vii

Chapter 1. INTRODUCTION......................................................................................... 1
Chapter 2. BEHAVIOR GENETICS AND BEHAVIORAL ENDOCRINOLOGY ................... 6
Chapter 3. DEVELOPMENT OF INTERNALIZING AND EXTERNALIZING BEHAVIOR ...... 17
Chapter 4. PARENTING AND THE FAMILY CONTEXT.................................................. 27
Chapter 5. GENE-ENVIRONMENT CORRELATION UNDERLYING THE ASSOCIATION BETWEEN PARENTAL NEGATIVITY AND ADOLESCENT EXTERNALIZING PROBLEMS................................................................. 38
Chapter 6. NEUROENDOCRINE RESPONSES TO CONFLICT AND BEHAVIOR PROBLEMS: THE MODERATING ROLE OF PARENT-ADOLESCENT CONFLICT FOR GIRLS’ AND BOYS’ ADJUSTMENT......................................................... 60
Chapter 7. COMBINED INFLUENCE OF GENES, PRENATAL ENVIRONMENT, CORTISOL, AND PARENTING ON THE DEVELOPMENT OF CHILDREN’S INTERNALIZING VS. EXTERNALIZING PROBLEMS........................................ 91
Chapter 8. SUMMARY AND FUTURE DIRECTIONS......................................................... 112

References .................................................................................................................. 116
List of Tables

Table 5.1. Intra-Class Correlations for Parental Negativity and Child Externalizing in Each Sample .................................................................52

Table 6.1. Sex Specific Descriptive Statistics for Study Variables........................................72
Table 6.2. Correlations Among Study Variables.................................................................76
Table 6.3. Parameter Estimates for Models Including Proximal, Observed Maternal Negativity and Hormone Responses........................................78
Table 6.4. Parameter Estimates for Models Including Global Mother-Adolescent and Father-Adolescent Conflict Intensity and Hormone Responses..........................82

Table 7.1. Sample Descriptive Statistics............................................................................98
Table 7.2. Means, Standard Deviations, and Correlations among Study Variables............101
Table 7.3. Parameter Estimates.......................................................................................107
List of Figures

Figure 1.1. Conceptual Framework for the Development of Internalizing and Externalizing Problems

Figure 5.1. Extended Children of Twins Model

Figure 5.2. Results for Maternal Negativity ECOT Model

Figure 5.3. Results for Paternal Negativity ECOT Model

Figure 6.1. Cortisol Responsivity Moderates Maternal Negativity-Externalizing Association for Girls

Figure 6.2. Father-Son Conflict Moderates Cortisol Response-Externalizing Association for Boys

Figure 6.3. Father-Son Conflict Moderates DHEA Response-Externalizing Association for Boys

Figure 6.4. DHEA Response Moderates Mother-Son Conflict Intensity-Externalizing Association

Figure 7.1. Model Fitting Results
Acknowledgements

I would like to acknowledge my first mentors, Carolyn Zahn-Waxler and Birdie Shirtcliff, for sparking my interest in developmental psychology and continuing our collaboration after my undergraduate career, and more recent mentors Jenae Neiderhiser, Liz Susman, and Nilam Ram, for guiding and challenging me through my graduate career, and for their flexibility and assistance as my research interests to have taken shape. I would also like to acknowledge my many collaborators on work presented in and related to this dissertation, Briana Horwitz, Paula Ruttle, Lorah Dorn, Jody Ganiban, Erica Spotts, David Reiss, Leslie Leve, Jurgita Narusyte, Paul Lichtenstein, Misaki Natsuaki, Heidemarie Laurent, Nastassia Hajal, Phil Fisher, Danny Shaw, Paul Hastings, Bonnie Klimes-Dougan, Marilyn Essex, Mikhila Humbad, Alex Burt, Kelly Klump, Renate Houts, Kevin Grimm, Kim Kendziora, and Linda Mayes, without whom I would not have been able to accomplish the work presented here. I would like to acknowledge Jurgita Narusyte for her integral assistance in understanding the statistical model presented in the first empirical paper included in this dissertation, Birdie Shirtcliff and Liz Susman for their prominent role in my understanding of behavioral endocrinology, and Jenae Neiderhiser for her prominent role in my understanding of behavioral genetics. I would especially like to thank Jenae Neiderhiser, David Reiss, and Paul Lichtenstein, for access to data used in the first empirical paper; Carolyn Zahn-Waxler and Bonnie Klimes-Dougan for access to data used in the second empirical paper, and Jenae Neiderhiser, Leslie Leve, and David Reiss for access to data used in the final empirical paper, as well as the research teams and participants involved in data collection for all of the studies used in the present dissertation. Funding for this dissertation was provided by the National Institute on Drug Abuse (F31 DA033737). I would also like to thank my dissertation committee for providing guidance throughout the process of crafting this dissertation. Last but not least, I thank my family and friends for supporting me during graduate school and providing constructive criticism and encouragement when needed.
CHAPTER 1: INTRODUCTION

This dissertation focuses on biological and environmental influences on the development of externalizing and internalizing behavior across childhood and adolescence. Children with externalizing and internalizing behavior are more likely to go on to engage in more deviant and criminal behavior and develop substance use problems than children who do not display these behaviors (e.g., Disney, Elkins, McGue, & Iacono, 1999; Helstrom, Bryan, Hutchison, Riggs, & Blechman, 2004; King, Iacono, & McGue, 2004; Loeber, Stouthamer-Loerber, & Raskin White, 1999). Therefore, understanding the development of internalizing and externalizing behavior and particularly identifying which youth are likely to develop internalizing and/or externalizing problems is important for discerning which youth are likely to exhibit more severe, lifelong problems, including later substance use problems.

Three comprehensive, broad developmental approaches have been described for the development of emotional and behavioral problems in children and adolescents: additive, interactionist, and transactional (e.g. Dodge & Petit, 2003; Kimonis & Frick, 2010). In additive models, different developmental influences work together in an aggregate way, each producing independent effects to influence trajectories of development. In interactionist models, distinct developmental influences produce a joint effect on development of the phenotype through moderation, modifying or amplifying the influence of other developmental influences. In transactional models, developmental factors influence both each other and the phenotype of interest across development. The proposed dissertation is conceptualized primarily in the transactional framework because of the complicated associations between genetic, prenatal, hormonal, and family environmental influences in developmental trajectories of internalizing and externalizing problems (reviewed below).

The conceptual model for the current dissertation falls under the broad scope of biosocial theory (e.g. Booth, Johnson, Granger, Crouer, & McHale, 2003; Cairns, Gariepy, & Hood, 1990; Gottlieb, 1992), and uses an interdisciplinary approach. Recent work in the field of psychology has favored the use of interdisciplinary approaches to gain a more comprehensive understanding of the development of emotional and behavioral problems. Biosocial theories are inherently interdisciplinary because emphasis is given to the confluence of genetic, physiological, and social factors for the development of behavior (e.g. Dodge & Petit, 2003), particularly the role of genetics and hormones in the negotiation and renegotiation of social relationships (e.g. Booth et al., 2003).

Through the use of interdisciplinary biosocial approaches, researchers have discovered that there are diverse developmental pathways to and among different types of mood and behavior problems. The vast majority of this work on the development of internalizing and externalizing
problems focuses either on the extent to which early behavioral problems predict later and more severe problems including school dropout, incarceration, early parenthood, inter-partner violence, and substance use (e.g. Broidy, Nagin, Tremblay, Bates, Brame, Dodge et al., 2003; Campbell, Shaw, & Gilliom, 2000; Feldman, 1997; Keenan, Loeber, & Green, 1999; King et al., 2004; Leve & Chamberlain, 2004; Reid, Patterson, & Snyder, 2002) or on the precursors of internalizing and externalizing behavior, including parent psychopathology, exhibiting emotional or behavioral problems before kindergarten, and environmental adversity (Moffitt, 1993; see Zahn-Waxler, Shirtcliff, & Marceau, 2008 for review). Much of this literature takes a biosocial approach, simultaneously studying biological and social risk factors for psychopathology (e.g. Broidy et al., 2003; Campbell et al., 2000), while some theories of the behavioral development focus on different levels of environmental factors including family, peer, and societal influences (e.g. Reid et al., 2002). Together, this broad body of research illustrates the variety of developmental pathways from biological, family environmental and earlier behaviors to later, more serious types of problems.

Drawing together transactional and interdisciplinary approaches for testing the tenants of biosocial theory, this dissertation aims to advance our understanding of the development of internalizing and externalizing behavior by examining the transactions among genetic, neuroendocrine, and prenatal and postnatal environmental factors that may convey risk for these behaviors, and more serious problems later in life. This dissertation extends existing work using a biosocial approach by bridging across behavioral genetic and behavioral endocrinology approaches and examining these transactions specifically in the family context. Through work on this dissertation, I have developed a conceptual framework designed to delineate multiple mechanisms (explained below) for the development of internalizing and externalizing behavior while building on existing developmental research in the related, but yet relatively disparate fields of family studies, behavioral genetics, and behavioral endocrinology.

**Conceptual Framework**

The conceptual framework is presented in Figure 1.1. In this section, each component of the conceptual model I’ve constructed through my work thus far and the work of others will be described briefly. Detailed supporting evidence from various disciplines will be provided in subsequent sections. As described above, potentially the best predictor of adolescent internalizing and externalizing are earlier internalizing and externalizing behaviors (e.g., Moffit, 1993; Zahn-Waxler et al., 2008), depicted by the directional arrow along the x-axis, since emotions, behavior, and emotional and behavioral problems develop over time. Another very strong predictor of internalizing and externalizing behavior is the family context, or parenting (e.g., Baumrind, 1991; Stice & Barrera, 1995; Shaw & Bell, 1993). Evidence from family systems theory (Minuchin, 1985) suggests that
parenting demands and practices change over time, and there is evidence that the family context continually changes with the age of the child (Steinberg, Dahl, Keating, Kupfer, Masten, & Pine, 2004; Steinberg & Morris, 2001). Therefore, parental negativity is also depicted by a directional arrow (center). Specifically, family systems theory espouses bidirectional, transactional relations between parenting and child behavior over time (Deater-Deckard & Dodge, 1997; Dishion, Patterson, Stoolmiller, & Skinner, 1991; Patterson & Fisher, 2002; Pettit & Arsiwalla, 2008; Zadeh, Jenkins, & Peplar, 2010), which are depicted with curved arrows linking the block arrows (labeled a).

Figure 1.1. Conceptual Framework for the Development of Internalizing and Externalizing Problems. The Figure is laid out from left to right, with the earliest influences arranged on the left hand side. Block arrows contain developmental influences that change over time, whereas time invariant influences are depicted in circles. Outcomes, adolescent internalizing and externalizing problems, are depicted in the bottom right. Curved block arrows represent bidirectional influences, whereas line arrows represent a hypothesized directional effect.

A special challenge of studying child behavior in the family context is the confounding of genetic and environmental influences within parent-child relationships, since parents and offspring share both genes and the rearing environment. Genetically informed designs can help to disentangle family influences into genetic and environmental components (Horwitz, Marceau, & Neiderhiser, 2011; Marceau & Neiderhiser, in press). Thus, genetic influences are explicit in the conceptual model. Specifically, there are genetic influences on internalizing and externalizing behavior across infancy through adulthood (e.g., Burt & Neiderhiser, 2009; Rhee & Waldman, 2002; Rice & Thapar, 2009; Kendler, Prescott, Myers, & Neale, 2003; represented by path b). Further, there are genetic influences on aspects of the family context, including parenting (e.g., Neiderhiser, Marceau, & Reiss, 2013). Genetic influences on family context are often conceptualized in terms of gene-environment
correlation (described below), therefore gene-environment correlation is represented via a curved arrow (labeled c). Finally, genetic influences may also play a role in the transactional associations between the family context and behavior over time (Burt, McGue, Krueger, & Iacono, 2005; Larsson, Viding, Rijsdijk, & Plomin, 2008; Neiderhiser Reiss, Hetherington, & Plomin, 1999; represented with path d).

A major limitation of quantitative genetic approaches is that latent genetic factors do not inform researchers of how (or which) genes can exert influence on emotions, behavior, and relationships across development. Genes can only modify behavior via biological pathways. Hormones are a likely candidate for genetic influences, as hormones are one of the types of messengers in the body which can relay genetic information to keep the body running, affect moods and behavior, and can also in turn affect gene expression (Griffin & Ojeda, 1996; depicted with a curved arrow, e). Indeed, there is a large literature on hormone-behavior associations across childhood and adolescence (path f), such that levels and changes in several hormones are associated with internalizing and externalizing behavior (see Marceau, Ruttle, Shirtcliff, Essex, & Susman, accepted). In some cases, especially for externalizing problems, there is evidence that behavior problems precede hormone dysregulation, which can subsequently influence behavioral development (Ruttle et al., 2011), thus the hormone-behavior relationship (path f) is presented as a transactional influence. The endocrine system also develops, and the levels and patterns of hormone releases change over time (Marceau, Ruttle, et al., accepted), and so endocrine development is depicted by a block arrow (top). The prenatal environment is particularly important for endocrine development (Phillips & Jones, 2006, path g), and the prenatal environment also influences emotional and behavioral development during childhood (e.g., Allen, Lewinsohn, & Seeley, 1998; McNeil, 1995; Williams & Ross, 2007, path h). Further, there is evidence that genetic and prenatal influences are correlated (Marceau, Hajal et al., in press, path i), and therefore the prenatal environment may mediate genetic influences on hormone functioning and internalizing and externalizing behavior.

The literature on hormone-behavior associations is mixed, potentially indicating the presence of a moderator of hormone-behavior associations, especially during adolescence. Given the small but growing body of literature suggesting a transactional association between family context and endocrine development across childhood and adolescence (e.g., Marceau, Dorn, & Susman, 2012, curved paths j), and the emerging developmental evolutionary theories (e.g., Adaptive Calibration Model, Del Giudice, Ellis, & Shirtcliff, 2011; Biological Sensitivity to Context, Ellis & Boyce, 2008; Differential Susceptibility, Belsky, 2005), I consider genetic (path k), prenatal (path l), and parenting influences (depicted by path m traveling through family context) as potential moderators of hormone-behavior associations.
To summarize, there are several hypothesized ways in which genetic and environmental influences may affect behavioral development. First, prenatal and endocrine influences may mediate genetic and parenting influences on the development of internalizing and externalizing behavior. Second, parenting influences may moderate genetic and hormone influences on the development of internalizing and externalizing behaviors. Third, genetic influences may moderate later hormone and parenting influences on the development of internalizing and externalizing behavior. Endocrine development and changes in parenting and the family context over time are explicitly accounted for in this conceptual framework. By explicitly separating genetic, prenatal, hormone and family environment influences, transactions among these influences can be tested and incorporated into models of the development of behavior problems. Thus, in this framework, multiple developmental mechanisms may operate simultaneously, and different mechanisms may lead to internalizing, externalizing, and comorbid behavior.

The remainder of this dissertation is organized as follows: Chapter 2 is an introductory chapter providing an overview of the key concepts involved in behavioral genetics and behavioral endocrinology. Chapters 3 and 4 are also introductory chapters, briefly reviewing extant literature on the development of internalizing and externalizing behavior (Chapter 3), and parenting influences on internalizing and externalizing behavior (Chapter 4). Chapters 5-7 are empirical papers testing portions of the conceptual framework presented above. The first paper (Chapter 5) uses a behavioral genetic approach to probe the mechanisms underlying the association between parental negativity and adolescent externalizing behavior, conceptually testing parts of paths a through d of the conceptual model. The second paper (Chapter 6) examines whether family context can moderate associations between hormone responses to parent-adolescent conflict and internalizing and externalizing problems during adolescence, conceptually testing components of paths a, j, and m. The third paper (Chapter 7) combines behavioral genetic and behavioral endocrinology approaches to examine whether prenatal influences and cortisol functioning transmit genetic and or environmental influences on internalizing and externalizing behavior during childhood, conceptually linked to paths a through j. Finally, Chapter 8 summarizes the findings from the three studies comprising this dissertation, fits the findings into the conceptual framework, and presents future directions in the current research program.
CHAPTER 2: BEHAVIORAL GENETICS AND BEHAVIORAL ENDOCRINOLOGY

Behavioral Genetic Theory and Methods

The behavioral genetic approach is applied in order to understand how genetic and environmental influences shape emotion, behavior, and/or relationships, and is comprised of quantitative and molecular genetic strategies. Quantitative genetic strategies take advantage of the natural experimental design of family members who vary in degree of genetic relatedness (e.g. through twin, sibling and adoption designs). Typically, quantitative genetic studies use latent modeling techniques from a broader, top down (i.e. theory to method) approach to estimate genetic and environmental influences on behavior based on quantitative genetic theory. Quantitative genetic models are built on theoretically derived assumptions, and genetic influences are operationalized as latent factors subsuming all genetic influences from structural differences in genotypes. Molecular genetic strategies use technological advances and a bottom up (i.e. method to theory) approach to examine how specific genes (or sets of genes) influence behavior. For this dissertation I focus on quantitative genetic strategies, though molecular genetic methods will also be important for fully testing the conceptual model.

Quantitative genetic research uses family samples where members share different proportions of their segregating genes. Basic, univariate quantitative genetic studies parse the variance in any given phenotype (for example, externalizing behavior) into three variance components: genetic, shared, and nonshared environmental influences. First, genetic influences are derived based on quantitative genetic theory specifying the average proportion of segregating genes family members share. Using twins and siblings as an example, monozygotic twins share 100%, dizygotic twins and full siblings share on average 50%, half siblings and cousin pairs whose parents are monozygotic twins share on average 25%, cousin pairs whose parents are dizygotic twins or full siblings share on average 12.5% of their segregating genes, and adoptive or step siblings do not share any genes. By comparing the similarity of different types of siblings and/or family members for externalizing behavior (correlations between sibling 1’s externalizing behavior and sibling 2’s externalizing behavior in each family, compared across sibling types), the extent to which variation in genes contribute to externalizing behavior can be estimated. Shared environmental influences are a latent construct representing all nongenetic influences contributing to likeness among family members. Shared environmental influences, then, are necessarily correlated 1 between siblings across all sibling types residing in the same household and 0 for related individuals not residing together (e.g., biological parents and the child they placed for adoption). Finally, nonshared environmental influences are a latent construct representing all nongenetic influences contributing to differences in
family members, and are therefore uncorrelated between siblings. Nonshared environmental influences also include measurement error, unless measurement error is explicitly modeled.

Together, these principles drawn from quantitative genetic theory about family similarity are used to infer genetic, shared, and nonshared environmental influences on phenotypes. For example, genetic influences are operating if monozygotic twins are two times more similar for their externalizing behavior than dizygotic twins because quantitative genetic theory states that monozygotic twins share (on average) twice as many genes as dizygotic twins. Shared environmental influences are indicated by the extent to which correlations between sibling 1 and sibling 2 externalizing behavior for monozygotic twins and dizygotic twins/full siblings are equal, or externalizing behavior of genetically unrelated siblings are correlated. The extent to which monozygotic twins are not perfectly correlated indicates the contribution of nonshared environmental influences on externalizing behavior. The first empirical paper of this dissertation (Chapter 6) uses twin/sibling studies to infer genetic and environmental influences on parents’ negativity and adolescents’ externalizing behavior.

Adoption designs also use variations in the genetic relatedness of family members to disentangle genetic from environmental influences on child and/or adolescent emotions, behavior, and/or relationships. Adoption designs use the genetic (un)relatedness of family members by taking advantage of the natural break of the confound between genetic and environmental influences provided by parents to offspring. However, they differ from the quantitative genetic studies described above in the way that genetic risk is inferred—genetic influences are operationalized as birth parent characteristics that are associated with children’s behavior when they are not reared by the birth parent. Adoption designs have also been extended to study differences between adopted children and biological children raised together. Any similarity between adopted and biological children must be due to shared environmental influences because they do not share genes, but do share the same rearing environment. The extent to which parents and their biological children are more similar on the phenotype than parents and their adopted children are indicate genetic influences on the phenotype. The third empirical paper of this dissertation (Chapter 8) uses an adoption design comparing the similarity of birth parents with adopted children on internalizing and externalizing symptoms to infer genetic influences and associations between adoptive parents’ over-reactive parenting with adopted children’s internalizing and externalizing symptoms to infer environmental influences of parenting on children’s internalizing and externalizing behavior.

**Gene-environment interplay.** Considering theories of development that emphasize transactional influences among genetic, biological, and environmental influences, simply parsing the variance in phenotypes and the covariance across phenotypes falls short of testing developmental
mechanisms, though parsing variance of a phenotype into discrete categories has made a great impact on how researchers think about development (McGue, 2010). Thus, studies using a behavioral genetic approach have moved beyond measures of genetic and environmental influences in the development of internalizing and externalizing behavior (see Moffitt, 2005 for review). This dissertation focuses on this next generation of behavioral genetic studies seeking to understand how genetic and environmental influences work together (referred to as gene-environment interplay) in the development of internalizing and externalizing behavior. Indeed, an large and growing body of research demonstrates how genes and family environmental factors work together to influence child and adolescent development (Horwitz et al., 2011). Broadly, the goals of research investigating gene-environment interplay are to understand how genetic influences operate through environmental mechanisms and to understand how genetic factors may moderate the effects of frequently studied ‘environmental’ influences, or environmental influences may moderate genetic influences on behavior. Using evidence from multiple study designs (i.e., twin and sibling studies, adoption designs), it is possible to draw together converging evidence across studies and interpret the direction of effects in associations between parent and child behavior, and the underlying gene-environment interplay mechanisms of these associations (Rutter, 2007).

Gene-environment interplay occurs on multiple organizational levels. Environmental influences may moderate the functional roles of genes on behavior throughout development as well as at specific times, and gene variants may impact susceptibility to certain environmental influences. Even on a cellular level, gene expression may act to change internal environmental factors (i.e. hormone or neurotransmitter levels, e.g. Joffee & Cohen, 1998) which then can moderate the expression of other genes (e.g. through epigenetic mechanisms, see Meaney, 2010). On a broader scale, genes and environments work together through gene-environment correlation and interaction processes across development, and gene-environment correlation and interaction themselves may also moderate the effect of environmental influences on the development of later, more severe problems, particularly major depression, conduct disorder, and substance abuse. On each of these organizational levels, genes and environments have transactional influences on each other and on phenotypic outcomes over the course of development.

Three types of genotype-environment correlation (rGE) are commonly described: passive, active, and evocative (Plomin, DeFries, & Loehlin, 1977; Scarr & McCartney, 1983). Passive rGE occurs when parents pass on genes to their offspring and an environment consistent with the heritable characteristics of the offspring. For example, if parents pass on genes predisposing their offspring toward externalizing behavior, and show a lot of aggression and hostility in their parenting, their offspring will more likely develop externalizing problems both because of genetic influences and
because of modeling his/her parents’ behavior. Active rGE occurs when an individual seeks out environments consistent with their heritable characteristics, whereas evocative rGE occurs when an individual evokes responses from the environment because of their heritable characteristics (Scarr & McCartney, 1983). In the parent-child relationship, evocative rGE is more likely operating than active rGE because offspring cannot choose their parents, but may influence their parent (although it is possible that adolescents choose when and how they interact with parents, which could indicate active rGE). Thus, evocative rGE may contribute to externalizing behavior if parents pass on genes predisposing their offspring toward externalizing behavior, and then respond more negatively to the child because of those externalizing behaviors exhibited by the offspring. These different forms of rGE are not mutually exclusive, and may simultaneously affect expression of a phenotype (e.g. Narusyte et al., 2011; Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein et al., 2007).

A variety of study designs have been used to investigate how rGE operates across childhood (see Marceau & Neiderhiser, in press, and Horwitz et al., 2011 for review). Briefly, adoption studies and studies comparing adopted and biological offspring suggest that both passive and evocative rGE explain associations between child internalizing and externalizing behavior and negative parenting behaviors (e.g., Deater-Deckard & O’Connor, 2000; Ge et al., 1996). Evidence from the ECOT design directly tested for rGE and corroborated the importance of evocative rGE in explaining associations between parent behaviors and both internalizing and externalizing behavior, but also that parenting behaviors may have some additional direct environmental influence on some child behaviors (Horwitz, Marceau et al., in preparation; Marceau, Horwitz et al., in press; Narusyte et al., 2008; Narusyte et al., 2011). Studies comparing genetic and environmental influences in parent-based and child-based twin samples also suggest that both passive and evocative rGE impact parenting behavior (Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein, et al., 2007), and that parents’ and adolescents’ age moderates the extent of passive and evocative rGE (Marceau, Neiderhiser et al., under review). Specifically, evocative genotype-environment correlation was found for parental positivity among older adolescents and older parents, while evocative genotype-environment correlation was found for parental negativity among younger adolescents and younger parents, whereas passive genotype-environment correlation was found for parental positivity among younger adolescents and younger parents, while passive genotype-environment correlation was found for parental negativity among older adolescents and older parents (Marceau, Neiderhiser et al., under review). The focus of the first study (Chapter 5) is on rGE, and so detailed empirical evidence for the presence of the various forms of rGE are presented in Chapter 5.

The other commonly studied form of gene-environment interplay is genotype x environment interaction (GxE). GxE tests whether genetic factors moderate the influence of environmental factors,
or whether environmental factors moderate the influence of genes on behavior. GxE and rGE are conceptually independent, but likely occur simultaneously in development (Jaffee & Price, 2007). Because estimates of genetic and environmental influences are latent in twin and sibling studies, twin/sibling studies cannot test whether genetic influences moderate the influence of environmental factors. However, these studies can test whether genetically and environmentally influenced constructs moderate the influence of genes on behavior. Both parental negativity and warmth have been found to moderate genetic influences on aggressive and nonaggressive forms of adolescent antisocial behavior (Feinberg, Button, Neiderhiser, Reiss, & Hetherington, 2007) such that genetic influences were greater for adolescent antisocial behavior when parenting behaviors were more negative or less warm. Generally, findings from twin studies suggest that genetic influences on internalizing and externalizing spectrum disorders are greater in the presence of environmental adversity (see Meyers & Dick, 2010; South & Krueger, 2008; South & Krueger, 2011; Tuvblad, Grann, & Lichtenstein, 2006), though much less work has been done examining whether environmental adversity moderates genetic influences on internalizing behavior.

GxE can also be tested using adoption designs. In the classic example (Cadoret, Cain, & Crowe, 1983) biological parent psychopathology was considered genetic risk, and adoptive parent psychopathology was considered environmental risk. Children with both genetic and environmental risk were more likely to develop psychopathology than children with only one or no risk factors (Cadoret et al., 1983; see Reiss & Leve, 2007). Generally, adoption studies have shown that the confluence of both genetic (biological parent psychopathology) and environmental risk factors (harsh or negative parenting or family environments, adoptive parent or sibling psychopathology) confers an additional, interactive risk for developing externalizing behavior in childhood and adolescence (Reiss & Leve, 2007). There is evidence of GxE in the development of internalizing and externalizing symptoms even in infancy and toddlerhood. For example, birth parents’ substance dependence and antisocial behavior predicted higher levels of novelty seeking during a frustration task (an early predictor of externalizing behavior) in nine month olds only when adoptive parents also had higher levels of depressive and anxiety symptoms (Leve et al., 2010). Among 18 month olds, marital instability between adoptive parents earlier in infancy predicted elevated levels of toddlers’ anger and frustration only among toddlers whose birth mothers reported high levels of anger and frustration (Rhoades et al., 2011). This pattern of effects was also found when examining the interaction between parental responsiveness and genetic risk for depression in toddlerhood, such that toddlers with less responsive adoptive parents and a genetic predisposition for depression displayed the highest levels of fussiness (Natsuaki et al., 2010).
In summary, studies of gene-environment interplay suggest a highly complex relation between parenting and behavior during childhood and adolescence (represented in paths a through d of the conceptual model, Figure 1.1). Genetic and environmental influences both contribute to parenting and to child behavior. Genetic and environmental influences work together through both passive and evocative rGE at multiple points during childhood and adolescence, likely driving both the development of parent-child relationships and internalizing and externalizing behavior. Thus, rGE is a plausible mechanism for the emergence and propagation of associations between parenting and child behavior. Evidence of GxE adds further complexity, showing that parenting, as an environmental influence, can moderate the influences of genes and putative genetic risk on internalizing and externalizing behavior.

**Assumptions of quantitative genetic models.** There are several assumptions applicable to twin and sibling studies that can impact the estimates of genetic and environmental influences recovered in quantitative genetic analyses. First, quantitative genetic studies are built on the equal environments assumption: shared and nonshared environmental influences are equivalent for each sibling type. That is, monozygotic twins’ environments are not more similar than genetically unrelated siblings’ environments. Thus far, no systematic differences have been found negating the validity of the equal environments assumption (e.g. Koenig, Jacob, Haber, & Xian, 2010; Loehlin & Nichols, 1976).

Second, assortative mating can affect estimates of genetic and shared environmental influences. Assortative mating occurs when individuals choose their mates based on heritable characteristics for which they are alike. Thus, parents who have assortatively mated are more likely to pass on similar genetic influences to offspring. This compromises the theory of the relative proportion of segregating genes different sibling types share because the average percentage of genes (that likely influence externalizing behavior) shared by DZ twins and non-twin siblings would be increased. While assortative mating is generally modest for most psychological traits (e.g. Plomin, DeFries, & McClearn, 1990; Maes et al., 1998), there is evidence of moderate assortative mating for antisocial behavior (e.g. Du Fort, Boothroyd, Bland, Newman, & Kakuma, 2002), making this assumption less tenable in quantitative studies of externalizing behavior, at least for samples selected for high levels of externalizing problems or focusing on extreme groups. It is possible, however, to test for such effects if related constructs are assessed in the parents. The presence of assortative mating on traits involved in the intergenerational transmission of externalizing behavior inflates shared environmental influences at the expense of genetic influences (because MZ and DZ twins will appear more similar, reducing the contrast in correlations that would suggest genetic influences on externalizing behavior). The inclusion of genetically unrelated siblings in quantitative genetic designs helps to attenuate this
bias. Assortative mating on antisocial behavior also suggests that passive rGE is more likely contributing to the development of externalizing behavior, thus highlighting the importance of studying gene-environment interplay especially for externalizing behavior. There are also specific assumptions related to more advanced statistical models (i.e., extended children of twins model) and for adoption designs that are described in Chapters 6 and 8, respectively.

Environmental influences. It is important to note that the ‘E’ in rGE and GxE is not a truly environmental factor, except for in adoption studies. Commonly studied ‘environmental’ influences on behavior include childhood stressors like abuse, availability and access to drugs and alcohol, negative peer groups, religiosity, parental monitoring, and harsh parenting (Meyers & Dick, 2010). The confounding of genes in these environmental influences is especially problematic for family environmental influences like parenting. An ongoing goal of behavioral geneticists should be (and for many, is) better and more nuanced measurement of environmental factors. Poor measurement is a limitation common to behavioral genetic studies generally, because the number of participants needed for adequate power for behavioral genetic analyses is so high that the cost of research limits the feasibility of some measures. This limitation has likely contributed to the mixed findings found across behavioral genetic studies. For example, estimates of genetic influences on externalizing behavior recovered from basic univariate quantitative genetic studies ranged from 13% to 94% of the total variance in externalizing behavior during middle childhood, and shared environmental influences accounted for between 0 and 62% of the total variance in externalizing behavior during middle childhood across studies (Deater-Deckard & Plomin, 1999). This is a particularly wide range in variance estimates for both genetic and shared environmental influences, prompting developmental researchers to try to understand what causes the variability in estimates of genetic and environmental influences on disruptive behavior across childhood. Differences in measurement and definitions across the myriad studies examining genetic and environmental influences have been cited as plausible reasons for the range in estimates observed across studies (e.g. Burt, 2009a; Marceau, Humbad et al., 2011, reviewed below). Behavioral genetic studies can be used not only to identify rGE and GxE operating during development, but to also clarify the phenotypes of interest (Moffitt, 2005).

Behavioral Endocrinology

While rGE and GxE as described above occurs at an interpersonal level of analysis, genes must operate through biologic mechanisms in order to influence behavior and subsequent relationships. Thus, rGE and GxE can be extended downward to a within-person level of analysis. One likely biologic mechanism important for the development of rGE and GxE, and for the influence of rGE and GxE on family relationships and internalizing and externalizing behavior is the
functioning of the endocrine system. The endocrine system is primarily a regulatory system. At its most basic, the endocrine system operates through hormones traveling in the blood stream between major endocrine glands. Genetic influences are highly intertwined with endocrine functioning and development. For example, genes code for protein hormones, and those hormones change the transcription and expression of genes to regulate further hormone production through feedback mechanisms. Further, hormone regulation of gene expression is thought to be tissue-specific and time-dependent (i.e., inherently developmental; Griffin & Ojeda, 1996). Adrenal glands and gonads are comprised of cells that steroid hormones can penetrate, and thus steroid hormones modulate the expression of genes in those cells that are particularly responsive to steroid hormones. The responsiveness to steroid hormones is thought to change over the course of development, and during particularly sensitive developmental periods (Griffin & Ojeda, 1996), especially prenatally and during puberty. Thus, the prenatal period and adolescence are both sensitive periods for the development of the HPA and HPG axes (Griffin & Ojeda, 1996; Mulder et al., 2002; Phillips & Jones, 2006), and so it is crucial to include the prenatal environment along with genetic and environmental influences when investigating the development of the HPA and HPG axes, and hormone-behavior associations during adolescence (represented via paths e, c, i, and g in the conceptual model, Figure 1.1).

In my work, I focus primarily on hormone responsivity to environmental cues (i.e., stress) on two of the main endocrine axes—the Hypothalamic-Pituitary-Adrenal (HPA) and Hypothalamic-Pituitary-Gonadal (HPG) axes. For detailed reviews of the HPA and HPG axis development and functioning, see Griffin and Ojeda (1996), Grumbach (2002), Grumbach and Styne (2003), Gunner and Quevedo (2007), and Marceau, Ruttle et al., (under review). Briefly, the HPA axis is a major part of the stress response system, and thus an important biological mechanism by which genetic and family environmental influences may impact the development of internalizing and externalizing behavior. Cortisol is a steroid hormone produced by the HPA axis in response to stress, and is easily collected via saliva (e.g. Dickerson & Kemeny, 2004). Dehydroepiandrosterone (DHEA) is another steroid hormone released by the adrenal gland, often co-released with cortisol (Sapolsky, 1997), and also easily collected via saliva. Therefore, I focus primarily on cortisol and DHEA for HPA axis functioning.

The HPG axis also likely plays an important developmental role in the increase in parent-child relationship problems and both internalizing and externalizing behavior observed during adolescence (Paikoff & Brooks-Gunn, 1991). The HPG axis is a major part of the reproductive system, and a primary mechanism for pubertal development during the adolescent transition. Testosterone and estrogen are pubertal hormones primarily released by the gonads, and drive changes in genital and breast development in boys and girls, respectively (Hiort, 2002; MacGillivray,
Morishima, Conte, Grumbach, & Smith, 1998). Here I focus on testosterone to capture HPG axis functioning partially because of the ease of measuring testosterone (as opposed to estrogen which is somewhat harder to reliably measure; Dorn, Dahl, Woodward, & Biro, 2006) and because testosterone has also been shown to be stress responsive (e.g. Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Sutton, Coleman, Casey, & Lazarus, 1973) whereas estrogens have not consistently been shown to be stress responsive.

Though the HPA and HPG axes are separate systems, they are highly interactive (Grumbach, 2002). Steroid hormones involved in HPA and HPG axis functioning share several key features. First, the release of HPA and HPG hormones are controlled by the hypothalamus and the pituitary gland and undergo feedback loops to signal to the hypothalamus and pituitary to regulate the production of the relevant hormone. Second, steroid hormones of adrenal and gonadal origin have similar developmental patterns of change characterized by high activity prenatally, serving to organize physical and brain development, followed by a relative period of juvenile quiescence (though the axes remain active at a lower level), and increased activation during adolescence with puberty (Grumbach & Styne, 2003; Terasawa & Fernandez, 2001). Third, HPA and HPG steroid hormones all originate from cholesterol and share several pro-hormones like pregnenolone and progesterone (Brown et al., 2008; Viau, 2002). Further, testosterone, an HPG hormone, is metabolized in the adrenal glands as well as the gonads. DHEA is derived from the adrenal cortex, but also in the testes, and about 50% of androgens in males and 75% of estrogens in females are derived from DHEA and DHEA-S (Kroboth, Salek, Pittenger, Fabian, & Frye, 1999; Poortman et al., 1980). Thus, while adrenal and gonadal hormones have distinct primary functions, the derivation and metabolism are highly integrated.

Interestingly, increases in DHEA are also responsible for promoting HPG activity and subsequent pubertal development (Grumbach & Styne, 2003), further highlighting the integration of the two axes, biologically. Although DHEA is primarily released by the adrenal glands, some DHEA is also released by the gonads in healthy males and females. DHEA is the first hormone to show large increases at approximately age 6, precipitating adrenarche, the first major sign of puberty (Palmert et al., 2001), and rising levels of DHEA during adrenarche is typically one necessary factor for the onset of puberty. Thus, it is important to consider hormones of the HPA and HPG axis together in order to understand how hormones influence emotional and behavioral development during adolescence.

In order to understand the role of endocrine functioning throughout childhood and adolescence, I draw primarily from the organizational-activational hypothesis. The organizational-activational hypothesis was originally developed to explain the role of the HPG axis in development. This hypothesis basically states that early in life (e.g. prenatally) the hypothalamic-pituitary-gonadal (HPG) axis has an organizational effect on the developing body and brain and exerts later activational
effects when the system re-awakens with puberty (Phoenix, Goy, & Young, 1967; Romeo, 2003). That is, prenatal development of the endocrine system sets the developing fetus on a developmental trajectory consistent with the fetus’s genes and the environmental influences experienced in utero, thus exerting adaptive, organizational effects. There is evidence that prenatal testosterone exposure predicts early behavior including play behaviors and toy preferences (Berenbaum & Snyder, 1995; see Zahn-Waxler et al., 2008).

In addition to organizational effects of the prenatal period, throughout development, and particularly during pubertal development, the reactivation of the endocrine system exerts activational effects. That is, hormones exert specific influences when produced in the body, which impact physical, psychological, and social development (represented via paths f, j, and m). Recently, this theory has been extended to include HPA axis functioning to propose that both HPG (e.g. testosterone) and HPA (e.g. DHEA, cortisol) hormones exert initial organizational and later activational effects, particularly during puberty (Shirtcliff & Ruttle, 2010).

This extension of the organizational-activational hypothesis is bolstered by evidence that the HPA and HPG axes are highly interactive (e.g. Viau, 2002). For example, cortisol and other stress hormones can suppress sex hormones (e.g. testosterone), and HPG hormones and DHEA can protect the body against deleterious consequences of cortisol on the body during adulthood (e.g., Herbert, 1997; Montoya, Terberg, Bos, & van Honk, 2012). During adolescence the relations between testosterone, DHEA, and cortisol are expected to be somewhat different from these adult suppression patterns (e.g. Matchock, Dorn, & Susman, 2007) because of re-activation of the endocrine system including elevations in both stress and pubertal hormones during puberty (e.g. Grumbach & Styne, 2003; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). However, relatively few studies have examined the roles of HPA and HPG hormones together during adolescence (see Marceau, Ruttle, et al., under review for further theoretical rationale as well as empirical evidence for a dual-axis approach considering hormones of the HPA and HPG axes during adolescence). Therefore, in the second empirical paper of this dissertation (Chapter 7) I simultaneously examine the responsiveness of testosterone, DHEA, and cortisol to parent-child conflict in relation to internalizing and externalizing behavior during adolescence.

**Genetic influences on endocrine functioning.** Variations in genotype may affect how responsive genes are to hormones (e.g. cortisol) and how long the response takes (Gunnar & Quevedo, 2007). Studies have demonstrated the heritability of cortisol, especially morning levels (e.g. Bartels, Van den Berg, Sluyter, Boomsma, & de Geus, 2003; Wust et al. 2009; Van Hulle, Shirtcliff, Lemery-Chalfant, & Goldsmith, 2012). Testosterone levels have also been shown to be 50-70% heritable (e.g., Hong et al., 2001; Harris, Vernon, & Boomsma, 1998). Further, GxE studies have
shown that early maltreatment can exacerbate cortisol responses given specific genotypes (e.g. having the GG allele of the corticotrophin releasing hormone receptor gene CRHR1; Tyrka et al., 2009). Specific genes have also been identified for age at menarche, (e.g. Elks et al., 2010), and genetic influences on cortisol stress responses have been demonstrated for the serotonin transporter gene 5HTTLPR (e.g. Jabbi et al., 2007). Further, one study found a specific gene X testosterone interaction predicting cortisol stress responses such that individuals with an S allele of 5HTTLPR in combination with high testosterone showed the highest cortisol stress response to threat (Josephs et al., 2012).

Thus, there is already a growing literature establishing genetic influences on the activation of hormones (represented by path e in the conceptual model. Figure 1.1) including the cortisol stress response in boys and girls, and pubertal hormones in girls (measured via menarche).

Very few genetically informed studies have examined the influence of genes and hormones together on the development of behavior (i.e., path k in the conceptual model, Figure 1.1) despite the notion that hormones may work in part as scaffolding genetic influences (McEwen, 1997). Further, the same genes have been identified as influencing HPA axis functioning and behaviors including depression and drug initiation, use, and addiction (e.g. Jabbi et al., 2007; Kreek, Nielson, Butelman, & LaForge, 2005; Wust et al., 2004; Wust et al., 2009). Since hormones drive some changes in gene expression over time, they also likely contribute to changes observed in genetic influences on behavior over time (Gottlieb, 1996). Therefore, this dissertation addresses this key gap in the behavioral development and family literature combining theory and methods from quantitative behavioral genetics and behavioral endocrinology through the conceptual framework and in the third empirical paper (Chapter 7).
Definitions and Development

Briefly, externalizing disorders are repeated, persistent patterns of violating social rules (American Psychological Association, 1994). Externalizing behaviors encompass disinhibited, under-controlled and hostile behaviors (Cicchetti & Toth, 1991). Children with externalizing problems also often display impulsivity, hostility and callousness (Cicchetti & Toth, 1991), which can disrupt social and cognitive development. As such, externalizing problems encompass a wide variety of behaviors, and have been conceptualized in terms of aggressive (including proactive or reactive aggression, Crick & Dodge, 1996) and non-aggressive behaviors (e.g. Burt, 2009b), or as distinct disorders falling under the same spectrum: attention deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder in childhood, and later antisocial personality disorder and substance use disorders (e.g. American Psychological Association, 1994). In contrast, internalizing disorders are characterized by problems with mood and emotion (American Psychological Association, 1994; Kovacs & Devlin, 1998). Children with internalizing problems often display anxiety, withdrawal, and sadness – symptoms of anxiety and depression (Kovacs & Devlin, 1998). Internalizing problems also encompass a wide variety of moods and behaviors, and are generally conceptualized in terms of symptoms of distinct disorders including anxiety, depression, panic disorders, and eating disorders (American Psychological Association, 1994). Although these disorders are distinct according to the DSM-IV, they are often highly comorbid, especially in childhood (Cicchetti & Toth, 1991; Zahn-Waxler et al., 2008), and when taking a dimensional approach symptoms of these disorders load highly onto a single internalizing subgroup of behavior problems (e.g., Achenbach, 1991).

Internalizing and externalizing behavior emerge very early in childhood, but increase in adolescence (e.g. Lewinsohn, Hops, Roberts, Seeley, & Andrews, 1993; Zahn-Waxler, Crick, Shirkcliff, & Woods, 2006; Zahn-Waxler et al., 2008), and the increase in internalizing behavior, particularly depression, is more marked in girls compared with boys (Zahn-Waxler et al., 2006; Zahn-Waxler et al., 2008). Generally, two different onset types have also been observed: adolescent limited, characterized by children who display an increase in internalizing and/or externalizing behavior during adolescence, but then a corresponding decrease to normative levels in adulthood, and life course persistent, characterized by children who consistently show elevated levels of internalizing and/or externalizing behavior starting in early childhood and lasting throughout adulthood (e.g. Moffit & Caspi, 1991).

Internalizing and externalizing behavior as a phenotype pose a number of issues for researchers, including the multidimensionality of each phenotype, debate about whether internalizing and externalizing behavior are better described as dimensional or categorical, how best to incorporate
developmental principles, and issues surrounding levels of analysis of different factors that are associated with each type of problem (Hinshaw, 2002), as well as comorbidity of each broad phenotype. Therefore, a comprehensive model of the development of internalizing and externalizing behavior cutting across multiple levels of analysis, and including multiple predictors within a developmental framework is critical for understanding which adolescents who exhibit internalizing and/or externalizing behavior will continue to develop into more severe, long-lasting problems. In this dissertation, I conceptualize internalizing and externalizing behavior as dimensional constructs because I am interested in the normative development of these behaviors, as well as the development of subclinical and clinical levels of problem behaviors broadly defined that may put children at risk for future adjustment issues in later adolescence and adulthood, including substance use disorders. I also address multiple developmental principles and levels of analysis in the current dissertation.

Past research has identified a number of risk factors for adolescent externalizing problems, including child factors (i.e. difficult temperament, hyperactivity, and early onset disruptive behaviors), family factors (i.e. parents’ antisocial behavior and substance use, neglect and punishment, and other negative parenting, and poor parent-child relationship quality), and school, peer, and neighborhood risk factors (Hinshaw, 2002). Most of those same risk factors have also been shown to influence adolescent internalizing behavior (e.g., Galambos, Barker, & Almeida, 2003; Kovacs & Devlin, 1998; see Zahn-Waxler, Klimes-Dougan, & Slattery, 2000). Further, past psychopathology is a salient predictor of future psychopathology within disorders, within internalizing and externalizing spectrums, and across spectrums (e.g., Leve, Kim, & Pears, 2005; Gilliom & Shaw, 2004; Moffitt et al., 2007). For example, during adolescence, previous attention deficit hyperactivity disorder predicted future oppositional defiant disorder and previous conduct disorder predicted substance use in girls (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003), and depressive symptoms have been shown to be preceded by childhood anxiety symptoms (Brady & Kendall, 1992). Together, the predictive power across disorders within the internalizing and externalizing spectrums and across spectrums supports the dimensional view of internalizing and externalizing behavior taken here.

Generally, most studies explore biological and child characteristics as primary predictors of internalizing and externalizing behavior (e.g. Leve et al., 2005; Kreek et al., 2005; Meaney, Brake, & Gratton, 2002), or relationships as primary predictors of internalizing and externalizing behavior (generally using ecological theory, e.g. Cyranowski, Frank, Young, & Sheer, 2000; Dobkin, Tremblay, Masse, & Vitaro, 1995; Liddle et al., 2002). Evidence from basic and prevention and intervention research emphasizes the importance of considering biological and social factors together (Dodge & Petit, 2003; Leve et al., 2005; Iacono, Carlson, Taylor, Elkins, & McGue, 1999; Tarter et
al., 1999; Leshner, 1997; Zahn-Waxler et al., 2000; Zahn-Waxler et al., 2008). However, even the more comprehensive models of biosocial development of behavior (e.g. Dodge & Petit, 2003; Iacono et al., 1999) are very limited in describing how biological influences exert influences on early behavior (as opposed to the literature on biological influences on addiction, for example), and do not include both genetic and endocrine influences within the child on the development of early and adolescent internalizing and externalizing behavior.

**Genetic and Environmental Influences on Internalizing and Externalizing Behavior**

Univariate quantitative genetic studies consistently demonstrate that genetic influences are important for internalizing and externalizing behavior in childhood and adolescence, though there is inconsistency in the proportion of variance explained by these genetic effects (Burt, 2009a; Miles & Carey, 1997; Rhee & Waldman, 2002; Rice & Thapar, 2009). For externalizing behavior, most studies indicate that the majority of variance can be explained by genetic influences with little contribution of shared environmental influences (i.e., environmental influences that increase sibling similarity regardless of the proportion of genes shared), although some reports indicate significant and sizable shared environmental influences. For example, some studies using samples of children suggest that genetic influences on antisocial behaviors are modest, and shared environmental influences explain more variance (DiLalla & Gottesman, 1989; Rutter et al., 1990), while other findings indicate that genetic influences on antisocial and other externalizing behaviors are more robust, leaving little variance to be explained by shared environment (e.g. Dick, Viken, Kaprio, Pulkkinen, & Rose, 2005; Slutske et al., 1997). For internalizing, genetic influences on depressive symptoms varied from 11-70% and genetic influences on internalizing behavior broadly (i.e. combined depressive and anxious symptoms) ranged from 17-62% (see Rice & Thapar, 2009 for review) across 26 studies. Further, there is consistent evidence of shared and nonshared environmental influences on both depressive behavior and internalizing behavior broadly (Rice & Thapar, 2009), the magnitude of which also varies by study.

There are several explanations for differences in genetic and environmental influences on internalizing and externalizing behavior across studies, including definition specificity, age, and error (see Burt, 2009a; Marceau, Humbad et al., 2011; and Rhee & Waldman, 2002 for a discussion of these issues). The measurement of internalizing and externalizing behavior offers yet another compelling possibility, as it is now widely acknowledged that heritability estimates vary by informant (Burt, 2009a). Finally, other sample-related differences (e.g. environmental adversity, Meyers & Dick, 2010) may drive differences in genetic and environmental influences on internalizing and externalizing behavior, since estimates of genetic and environmental influences rely on variations in correlations across sibling types within a sample, and thus are particularly sample-specific. Thus, it is
important to consider age and measurement in studies of genetic and environmental influences on internalizing and externalizing behavior, and for interpreting findings from the current dissertation.

Although there is evidence that the magnitude of genetic and environmental influences can differ based on the particular behavior being assessed, there is also evidence of an underlying genetic factor common to multiple externalizing behaviors including attention deficit-hyperactivity, oppositional-defiant, and conduct disorders in boys and girls in middle childhood and adolescence (e.g. Dick et al., 2005; Eaves et al., 2000; Nadder, Rutter, Silberg, Maes, & Eaves, 2002; Tuvblad, Zheng, Raine, & Baker, 2009). This underlying genetic factor common to different externalizing disorders suggests a common genetic lability to externalizing behavior assessed using a dimensional approach, as taken in this dissertation. For internalizing, there is evidence of two separate genetic influences: one on phobia, panic disorder, and eating disorders, and the other on anxiety and depression in adulthood (Kendler et al., 1995). However, it may be that the separate genetic influences emerge later in life. In childhood, there is evidence that genetic risk for internalizing and externalizing behavior in childhood and adolescence may be nonspecific – that is, the same set of genetic influences contribute to both internalizing and externalizing behavior, but do not distinguish between which type of problem is expressed in children (Lahey, Van Hulle, Singh, Waldman, & Rathouz, 2011).

Age related changes. As the conceptual framework of this dissertation is inherently developmental, it is important to highlight evidence that genetic and environmental influences on internalizing and externalizing behavior may change over time. A second source of systematic differences found in estimates of genetic and environmental influences across studies is age, or developmental change in the child. Overall, most studies (using longitudinal and cross-sectional approaches) have shown that genetic and nonshared environmental influences increase, whereas shared environmental influences decrease, from adolescence to adulthood (i.e., as individuals mature and widen social circles beyond the home, Ulbricht & Neiderhiser, 2009, or because of gene-environment correlation that serves to differentiate DZ twins and full siblings more than MZ twins over time, Beam & Turkheimer, 2013) for internalizing (e.g., Feigon, Waldman, Levy, & Hay, 2001) and externalizing (Miles & Carey, 1997). Of note, this pattern may not be universal for every specific internalizing and externalizing symptom: genetic influences were shown to increase with age particularly for aggressive as opposed to rule-breaking behaviors in a meta-analysis (Burt, 2009b) although specific studies have shown that genetic influences on delinquency were greater among older adolescents than younger adolescents (e.g., Burt & Neiderhiser, 2009).

One mechanism hypothesized to drive age-related changes in genetic and environmental influences on internalizing and externalizing behavior relevant to the present conceptual model is
pubertal development. According to biosocial theories, puberty is viewed as a particularly sensitive and transitional period of development because of major changes in the endocrine system as well as accelerated growth and physical maturational that contribute to the metamorphosis of a child into an adolescent (Dorn & Biro, 2011). These myriad biological changes can impact adolescents’ perceptions of the social changes that occur during puberty, potentially resulting in problematic psychological and emotional functioning in some adolescents (Paikoff & Brooks-Gunn, 1991). Biometric moderation analyses typically show that advanced pubertal status and early pubertal timing moderate environmental, but not genetic influences on girls’ internalizing and externalizing behavior (Burt, McGue, DeMartel, Krueger, & Iacono, 2006; Dick, Rose, Pulkkinen, & Kaprio, 2001). Further, bivariate biometric analyses suggest that primarily the shared environment explains associations between advanced pubertal status and early pubertal timing with internalizing and externalizing behavior and substance use, particularly in girls (Burt et al., 2006; Dick, Rose, Viken, & Kaprio, 2000; Dick et al., 2001; Eriksson, Kaprio, Pulkkinen, & Rose, 2005; Marceau, Neiderhiser, Lichtenstein, & Reiss, 2012). It is yet unclear whether it is the hormone changes driving puberty or social changes surrounding puberty that drive these effects, and although it is out of the scope of the current dissertation, future work examining the role of pubertal hormones in regard to genetic and environmental influences on internalizing and externalizing behavior will help to clarify this body of literature.

In summary, there are genetic and environmental influences on internalizing and externalizing behavior in childhood and adolescence (i.e., paths a-d of the conceptual model, Figure 1.1). While the relative influence of genes and environments differ for distinct disorders, there is evidence of an underlying genetic vulnerability common to multiple types of behavior. This evidence further supports examining the development of internalizing and externalizing behavior simultaneously, as the etiology of internalizing and externalizing behaviors overlap substantially in childhood and adolescence. Further, the literature generally shows that genetic influences on internalizing and externalizing behavior increase whereas environmental influences tend to decrease from early childhood through adulthood. These age-related changes in genetic influences appear to be distinct from puberty-related changes, which may impact internalizing and externalizing problem via primarily environmental mechanisms, though the role of hormone functioning and changes have not been extensively examined within behavioral genetic studies.

**Hormonal Influences on Internalizing and Externalizing**

Models of the development of internalizing and externalizing behavior focusing on familial influences in childhood and adolescence can be strengthened by including more explicit, comprehensive measures of endocrine functioning (Susman, 2006). However, while numerous studies
have examined associations between single hormones and behavior in adolescence, the literature as a whole is largely inconclusive, indicating the need to move beyond simple hormone-behavior associations to realize the potential impact of including explicit, comprehensive measures of endocrine functioning in developmental models.

**Cortisol and internalizing and externalizing.** Cortisol has been a major focus of biological influences on behavior. Research has linked both high and low cortisol levels and release in response to a stressor to increased mental health problems. Recently, a comprehensive evolutionary biosocial model, the adaptive calibration model (ACM, see Del Giudice et al., 2011), has been proposed to explain how different profiles of stress responsivity are associated with internalizing and externalizing behavior. According to this theory, the combination of different developmental contexts and HPA responsiveness patterns lead to particular behavioral outcomes, which may differ for males and females. For example, high responsivity of the HPA axis may lead to more internalizing-type behavior, whereas low responsivity of the HPA axis may lead to more externalizing-type behavior, especially in the context of unpredictable environments across development. Further, girls with particularly sensitive stress response systems and who show high basal levels or responsivity to the environment are more likely to develop hyper-vigilance which may lead to anxiety and potentially other internalizing-type behavior, whereas for boys, sensitive stress response systems may lead to agonistic behavior or reactive aggression (Del Giudice et al., 2011). Thus, different types of responsivity patterns may predict internalizing versus externalizing behavior via different mechanisms.

Research on how cortisol has been associated with externalizing and drug use behaviors has been guided by several additional theories, including fearlessness theory (Raine, 1996), the challenge hypothesis (Archer, 2006) and stimulation seeking (Zuckerman, 1979). Generally, these biosocial theories of externalizing problems posit that youth who were exposed to prolonged stressors may experience a ‘down-regulation’ of cortisol, evidenced as low levels of cortisol and blunted reactivity in response to challenge or stress (Fries, Hesse, Hellhammer, & Hellhammer, 2005). It has been posited that children and adolescents who engage in externalizing behavior may do so, in part, in an attempt to increase or return arousal to more typical levels (Koob & Le Moal, 2001). These theories are supported by research suggesting that children and adolescents exhibiting externalizing behavior generally display blunted patterns of HPA axis reactivity (Fairchild et al., 2008; Susman, Dorn, Inoff-Germain, Nottelmann, & Chrousos, 1997; Yoon & Joormann, 2012) as well as low basal and diurnal levels of cortisol (Andersen & Teicher, 2009; Brown et al., 2008; Hastings et al., 2011; Moss, Vanyukov, & Martin, 1995; Ruttle et al., 2011), though findings remain mixed (see Alink et al., 2008; Susman et al., 1997). Interestingly, a developmental effect has been identified within the externalizing
research suggesting that increased externalizing behavior are initially associated with HPA hyperactivity and later associated with hypoactivity (Alink et al., 2008).

Research on cortisol and internalizing has been shaped by theories which suggest that certain individuals are born with a lower threshold for limbic-hypothalamic arousal in response to changes in one’s context (Kagan, Reznick, & Snidman, 1988), possibly due to elevated levels of neuroendocrine receptors located in the regions of the brain associated with fear. These individuals’ heightened sensitivity to the environment results in both hyper-physiological (e.g. cortisol) and behavioral (e.g. fear, anxiety, social withdrawal) reactions to benign perturbations in their environment. The general notion of elevated physiological arousal leading to internalizing behavior has been supported by numerous studies; for example, adolescents with internalizing behavior, anxiety, or depression generally demonstrate more pronounced and prolonged HPA responses to stressful events (Gunnar, 2001; Lopez-Duran, Kovacs, & George, 2009; see Burke, Davis, Otte, & Mohr, 2005 for review). The literature examining associations between internalizing behavior and basal and diurnal cortisol levels is complex. While the cross-sectional literature is largely divergent (Cicchetti and Rogosh, 2001; De Bellis et al., 1996; Granger et al., 1998; Kaufman, 1991; Perez-Edgar, Schmidt, Henderson, Schulkin, & Fox, 2008), longitudinal research suggests that high levels of cortisol have been found to precede the development of internalizing behavior in children and adolescents (Goodyer et al., 1996, Goodyer, Herbert, Tamplin, & Altham, 2000, Goodyer, Park, Netherton, & Herbert, 2001; Halligan, Herbert, Goodyer, & Murray, 2007; Smider et. al., 2002; Ruttle et al., 2011). Although not all studies have found the anticipated positive association between cortisol and internalizing behavior, divergence from this association has been attributed to down-regulation of the HPA axis (Feder et al., 2004; Ruttle et al., 2011). There may also be a developmental effect for internalizing behavior, such that prepubescent children with dysphoria display blunted patterns of HPA axis reactivity whereas post-pubertal adolescents and adults display increased levels of cortisol reactivity to psychosocial stressors (Hankin, Badanes, Abela, & Watamura, 2010; Luby et al., 2003; Rao, Hammen, Ortiz, Chen, & Poland, 2008).

**DHEA and internalizing and externalizing.** Compared to cortisol, less research has been devoted to understanding DHEA-behavior associations during adolescence. Studies examining associations between basal DHEA and externalizing behavior generally suggest that higher DHEA and its sulphate, DHEA-S levels have also been associated with increased aggression and externalizing behavior (Brown et al., 2008; Susman et al., 1987; van Goozen et al., 1998; van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000). Lower levels of DHEA during adolescence have been associated with more negative affect (Susman et al., 1987; Susman, Dorn, & Chrousos, 1991) and internalizing symptoms (Goodyer, Herbert, & Altham, 1998; Goodyer, Herbert,
& Tamplin, 2003) during adolescence; however, higher levels of DHEA during adolescence have also been associated with more internalizing behavior (Goodyer et al., 2000; Shirtcliff, Granger, Booth, & Johnson, 2005; Susman, Granger, Murowchick, Ponirakis, & Worrall, 1996). It may be that DHEA is associated with internalizing behavior when it is concomitant with stressful life events, potentially operating as a stress hormone (see Angold, 2003 for further discussion).

Fewer studies have examined associations between stress responsivity of DHEA and emotionality or behavior. Stress responsiveness of DHEA has been associated with increased internalizing symptoms in adolescents in response to social tasks, including parent-child conflict (Shirtcliff, Zahn-Waxler, Klimes-Dougan, & Slattery, 2007), and the DHEA stress response to venipuncture was concurrently positively associated with negative emotionality in adolescent boys (Marceau, Dorn et al., 2012). It is yet unknown whether DHEA responsivity is associated with externalizing behavior.

**Testosterone and internalizing and externalizing.** The association between testosterone and aggression in social relationships in adulthood has received substantial attention (Archer, 2006). Like studies in adults (Booth, Granger, Mazur, & Kivlighan, 2006), associations between basal testosterone levels and behavior are mixed for adolescents. This is somewhat surprising given the general acceptance of a testosterone-aggression links, and suggests that the studies thus far may be missing an important moderator or contextual force. Testosterone levels, and changes in levels across adolescence, have been associated with increased aggression during adolescence (Brooks-Gunn & Warren, 1989; Dorn et al., 2009; Inoff-Germain et al., 1988; Susman et al., 1991; Susman et al., 1996; Olweus, Mattsson, Schalling, & Low, 1988; Dabbs, Jurkovic, & Frady, 1991). Further, boys with higher basal testosterone did not respond as well to treatment for oppositional defiant disorder and conduct disorder as boys with lower basal testosterone (Shenk et al., in press). Similarly, being in more advanced stages of pubertal development and having correspondingly higher levels of testosterone for boys is generally associated with more externalizing behavior (Angold, 2003; Brown et al., 2008; Dawes et al., 1999; Flannery, Montemayor, Eberly, & Torquati, 1993; Maras et al., 2003). In one of the few longitudinal studies of aggression and testosterone, boys who had a history of high physical aggression from ages six to 12 years were found to have lower testosterone levels at age 13 compared with boys who did not have a history of high physical aggression (Schaal, Tremplay, Soussignan, & Susman, 1996). However, some studies have found that basal testosterone was not associated with aggression in young girls (Susman et al., 1987; Booth et al., 2003), and findings are generally mixed regarding testosterone and externalizing behavior (see Book, Starzyk, & Quinsey, 2001 for meta-analysis and review).
For internalizing, lower initial levels of testosterone and slower declines in testosterone across the day were associated with higher levels of anxiety and depression in pubertal youth (Granger et al., 2003) and lower testosterone has been associated with increased negative affect (Inoff-Germain et al., 1988). However, in another study, higher basal levels of testosterone were associated with higher levels of negative emotional tone concurrently in girls (Susman et al., 1991). In other studies, testosterone has not been associated with negative emotionality during puberty in girls (Susman et al., 1987; Brooks-Gunn & Warren, 1989; Booth et al., 2003). Thus, as with cortisol and DHEA, findings are mixed regarding the nature of the association between testosterone levels and adolescent emotionality and behavior.

The responsivity of testosterone to social challenges, especially during adolescence, has been associated with greater psychopathic tendencies in males (Yildirim & Derksen, 2012), and aggression, and social status-seeking behaviors in animals and humans (Eisenegger, Haushofer, & Fehr, 2011). Associations between testosterone responses to laboratory stressors and adolescent emotionality and behavior have not been demonstrated during adolescence, though two recent studies suggest that testosterone responses to venipuncture and parent-child conflict were not directly related to negative emotionality (Marceau, Dorn et al., 2012) or internalizing or externalizing problems (Marceau, Shirtcliff et al., accepted) during adolescence. More research is needed to understand whether stress responsivity of testosterone to laboratory stressors is associated adolescent mental health.

In summary, there is a substantial body of research examining associations between levels and responsivity of cortisol, DHEA, and testosterone on emotion and behavior during adolescence (i.e., paths f and m of the conceptual model, Figure 1.1). Although some specific hormone-behavior patterns tend to prevail (e.g. low cortisol is generally associated with increased externalizing behavior), the literature is plagued with inconsistent findings. One explanation for the inconsistent findings is that there is not an abundance of studies of testosterone and DHEA in young adolescents and the studies tend to use smaller samples with little power to detect stable findings. It was not until recently, and after multiple studies, that the low cortisol and externalizing behavior associations became a moderately consistent finding. The mixed findings in the literature likely indicate the presence of moderators of single hormone-behavior associations. Given the interactive nature of the HPA and HPG axes and the buffering properties of both DHEA and testosterone on the neurotoxic effects of cortisol, there is a clear need for studies examining how the HPA and HPG axes interact and bi-directionally influence each other and behavior during adolescence. Further, according to the biosocial perspective, the family context is another hypothesized, though relatively infrequently tested moderator of hormone-behavior associations.
As noted above (Chapter 2), HPA and HPG axis hormones share several commonalities including joint pathways of activation and hormone synthesis, increasing levels across adolescence, and stress responsive properties. Theory and evidence support the hypothesis that the HPA and HPG axes may work simultaneously to influence adolescent emotionality and behavior, and that hormone systems may influence behavior differently in boys and girls. Studies using ratios and interactions of multiple hormones generally suggest that high cortisol in the context of low DHEA is associated with internalizing, whereas low cortisol in the context of high testosterone is associated with externalizing behavior (see Marceau, Ruttle et al., accepted). Thus, levels or responsivity of multiple hormones contribute to a hormonal milieu that likely has important implications for behavioral correlates of each hormone. However, this literature is quite sparse and further replication is necessary in order to be sure of these conclusions (see Marceau, Ruttle et al., accepted). The biosocial perspective emphasizes that the environmental context in which a hormone is expressed is important for the behavioral correlates of the hormone (Booth, et al., 2006; Del Giudice et al., 2011). There is evidence that the hormonal milieu (i.e., endogenous context) is equally as important to consider as broader environmental contexts (i.e., exogenous contexts) for the development of internalizing and externalizing behavior during adolescence (see Marceau, Ruttle et al., accepted). Therefore, this dissertation considers both the hormonal milieu (see Chapter 6) and family environmental context (Chapter 6 and 7; represented via paths m and j in the conceptual model, Figure 1.1) for understanding the role of hormones for the development of internalizing and externalizing behavior during childhood and adolescence.
CHAPTER 4: PARENTING AND THE FAMILY CONTEXT

Development of the Parent-Child Relationship

In childhood, parents play a large role socializing and teaching their children, and thus the parent-child relationship is often assessed via parenting style or quality. Adolescence is a developmental period marked by significant change in the parent-child relationship (Steinberg & Morris, 2001; Steinberg & Silk, 2002; Steinberg et al., 2004). Increases in parent-child conflict and decreases in closeness during adolescence can lead to a number of types of problems including attention deficit hyperactive disorder symptoms, conduct disorder symptoms, oppositional defiant symptoms (e.g. Burt, Krueger, McGue, & Iacono, 2003), depressive symptoms (e.g., Noack & Puschner, 1999), and substance use (e.g., Iacono et al., 1999).

The parent-child relationship is a central relationship that typically changes as adolescents gain autonomy. The parent-child relationship provides a particularly important context for studying the development of internalizing and externalizing behavior in childhood and adolescence because parenting and parent-child relationship quality is intricately enmeshed with multiple known antecedents of internalizing and externalizing behavior, including difficult temperament and insecure attachment in early childhood (e.g. Hinshaw, 2002; Morris et al., 2002), and dysregulated hormone functioning and early timing and fast tempo of puberty in middle childhood and adolescence (e.g. Susman et al., 1991; Marceau, Ram, Houts, Grimm, & Susman, 2011). Further, poor parenting and parent-child relationship quality is directly associated with early internalizing and externalizing behavior and more severe forms of externalizing psychopathology and substance use in adolescence (e.g. Hinshaw, 2002; Morris et al., 2002).

During adolescence, the quantity of angry discussions between parents and their children increase (Steinberg & Morris, 2001), and parents and children also share more intense conflicts and negative emotionality associated with conflict during adolescence as opposed to childhood (Collins & Laursen, 2004; Zeman & Shipman, 1997). Across early adolescence, this increase in parent-child conflict is observed in tandem with a slight decrease in cohesion (Steinberg & Morris, 2001). For example, the mother-son relationship was marked by more closeness in middle childhood than during early adolescence (Starrels, 1994). In later adolescence the parent-child relationship is thought to move out of a state of disequilibrium and is redefined and marked by more cohesion, autonomy, and less conflict (Steinberg & Morris, 2001). It is important to note, however, that conflict and closeness in the parent-child relationship are not mutually exclusive, and often co-occur within the same relationship.

Studies investigating developmental change in parent-child relationship quality have shown that parent-child conflict increases and parent-child warmth/closeness decreases from middle
childhood to early adolescence (e.g. 7-12 years; Fleming, Catalano, Haggerty, & Abbott, 2010; Shanahan, McHale, Crouter, & Osgood, 2007; Shanahan, McHale, Osgood, & Crouter, 2007). Further, from early to mid-adolescence (age 11-14), youth-reported conflict with parents increased and warmth decreased, especially for girls compared with boys (McGue, Elkins, Walden, & Iacono, 2005). These increases in conflict and decreases in warmth are thought to occur primarily between middle childhood and early adolescence. However, other studies have shown that from early to mid-adolescence (e.g. 10-16), parent-child conflict decreases (e.g. Laursen, Coy, & Collins, 1998; Shanahan; McHale, Osgood et al., 2007). In all, it is still unclear whether there is a ‘strong’ developmental shape underlying change in the parent-child relationship across adolescence. It may be that in addition to smaller developmental changes, change in parent-child closeness and conflict across adolescence is mostly characterized by increased variability in relationship quality (Marceau, Ram, & Susman, under revision).

**Parent-Adolescent Relationship Quality and Internalizing and Externalizing Behavior.**

Generally, studies suggest that the increase in conflict and decrease in closeness observed during adolescence is associated with more adolescent internalizing and externalizing behavior and related temperament characteristics (Zeman & Shipman, 1997; Steinberg & Morris, 2001; Collins & Laursen, 2004; Shanahan, McHale, Crouter et al., 2007; Shanahan, McHale, Osgood et al., 2007). Child internalizing and externalizing behavior and parent-child relationship quality are likely bidirectionally influenced (e.g. Maccoby, 1992). For example, poor parenting quality in childhood predicts higher levels of negative emotionality (i.e., part of the internalizing spectrum) during adolescence (e.g. Masten et al., 1999). Further, low levels of competence in childhood (academic, conduct, and social) predict a decline in parent-child relationship quality in adolescence (Masten et al., 1999). These findings indicate that parent-child relationships are implicated in and influenced by the behavioral, emotional and social development of the adolescent.

Negative emotionality and aggressive behavior in both parents and children are hypothesized to be involved in coercive cycles leading to poor family and child mental health outcomes (Patterson & Fisher, 2002). That is, parents who respond to negative emotionality or aggression (i.e., internalizing and externalizing behavior) in their offspring with negative or harsh parenting strategies appear to exacerbate the child’s internalizing and externalizing behavior. This reciprocal negative interactive style sets the parent-child relationship on a path promoting future negative interactions, exacerbating parent and child internalizing emotions and/or externalizing behaviors and conflict in the parent-child relationship. The internalizing emotions or externalizing behaviors expressed in parent-child conflict may serve to perpetuate further conflict among family members (Collins & Laursen, 2004). Thus, poor parent-child relationship quality is proposed to lead to internalizing and
externalizing behavior, which in turn may lead to further deterioration in parent-child relationship quality (represented by path a of the conceptual model, Figure 1.1).

**Genetic and Environmental Influences on the Parent-Child Relationship**

The influence of family relationships on child internalizing and externalizing behavior poses a unique challenge to psychological researchers because of the dyadic nature of the phenotype (Deater-Deckard, Reiss, Hetherington, & Plomin, 1997). Because parents pass on genes and provide and share an environment with their offspring, traditional studies cannot distinguish whether parenting impacts adolescent behavior or adolescent behavior impacts parenting (i.e. which member of the dyad drives development of the relationship). Accordingly, family researchers have taken advantage of multiple reporters and sample designs to gain insight into genetic and environmental influences on several different aspects of the parent-child relationship (see Horwitz et al., 2011 for review). Parent-based twin designs are used to estimate the extent to which variation in parents’ own genotype and environment contribute to their parenting behavior, while child-based twin designs are used to estimate the extent to which variations in the children’s genotype and environment contributes to the parenting they receive.

Evidence from parent-based twin designs (Children of Twins; COT, Silberg & Eaves, 2004) suggest that parents’ genes and nonshared environmental influences impact positive and negative aspects of their own parenting behavior and family relationships (e.g. Kendler, 1996; Losoya, Callor, Rowe, & Goldsmith, 1997; Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein et al., 2007; Narusyte et al., 2008). There is also some evidence that negative aspects of parenting behavior and family relationships are influenced by shared environmental influences common to twin parents (Kendler, 1996), though this pattern of effects is not always found. Other studies show that positive, negative, and global measures of family relationships are influenced by variation in parents’ genotypes and nonshared, but not shared environment (e.g. Losoya et al., 1997; Perusse, Neale, Heath, & Eaves, 1994; Plomin, McClearn, Pedersen, Nesselroade, & Bergeman, 1989). Generally, parents’ genes and environments both contribute to the positive and negative aspects of how they parent.

The vast majority of research on genetic and environmental influences on parent-child relationships has examined the effects of variation in offspring’s genotypes and environments on parent behaviors and parent-child relationship quality. Though there are differences in the genetic and environmental influences on parental warmth and negativity toward the child, generally variation in children’s genotype, shared, and nonshared environmental influences all contribute to the etiology of positivity and negativity in the parent-child relationship (see McGuire, 2003; Towers, Spotts, & Neiderhiser, 2002 for review). Evidence from child-based designs also shows general trends of
change in genetic and environmental influences on parenting with child age (see McGuire, 2003; Towers et al., 2002; Ulbricht & Neiderhiser, 2009 for review). Genetic influences on parenting of infants and young children are small, while shared environmental influences large (e.g. Boivin et al., 2005), and in mid-adolescence, observed parenting behaviors during parent-adolescent interactions (e.g. warmth, involvement, anger, and coercion) were greatly influenced by youths’ shared environment, while observed adolescent behaviors were influenced primarily by variation in their genotypes (O’Connor, Hetherington, Reiss, & Plomin, 1995).

However, genetic influences on parent-son conflict, regard, involvement, and overall support were greater among 17 year olds than among 11 year olds (Elkins, McGue, & Iacono, 1997). Supporting this finding of increased genetic influences on parent-child conflict, longitudinal investigations of parent-child relationships showed that the variance in parent-child warmth and conflict increased from early to middle adolescence, and that those increases were primarily attributable to increased genetic influences (McGue et al., 2005; Reiss, Neiderhiser, Hetherington, & Plomin, 2000). Generally, reviews of the literature reveal that the influence of child’s genes on parenting seem to increase across childhood and adolescence (represented via path c in the conceptual model, Figure 1.1), while shared environmental influences remain generally consistent or decrease slightly (Ulbricht & Neiderhiser, 2009), similar to the pattern of changes in genetic and environmental influences on internalizing and externalizing behavior noted previously.

**Genetic and Environmental Influences on the Parent-Child Relationship and Internalizing and Externalizing**

Most longitudinal studies examining parent-child relationships have investigated how aspects of parent-child relationships and child behavior are associated. For example, a common genetic factor influenced adolescent aggression in middle adolescence and negative parenting and adolescent antisocial behavior in later adolescence (Narusyte, Andershed, Neiderhiser, & Lichtenstein, 2007). There was also a common shared environmental factor influencing negative parenting and concurrent adolescent antisocial behavior in later adolescence (Narusyte et al., 2007). Cross-lagged designs have suggested roughly equal genetic, shared environmental, and nonshared environmental influences explaining the association between parent-child conflict in early adolescence and youth externalizing behavior in mid-adolescence and youth externalizing behavior in early adolescence and parent-child conflict in mid-adolescence (Burt et al., 2005). Genetic and shared environmental influences also contributed to stability in parent-child conflict from early to mid-adolescence (Burt et al., 2005). Parent-child conflict in middle adolescence contributes to antisocial behavior in young adulthood and adolescent antisocial behavior in mid-adolescence contributes to parent-offspring conflict in young adolescence were primarily influenced by variation in the adolescents’ genotypes (Neiderhiser et al.,
Earlier in childhood, primarily environmental influences explained the association between parental negativity at age 4 and externalizing behavior at age 7, while genetic effects explained the association between child externalizing behavior at age 4 and parental negativity at age 7 (Larsson et al., 2008). Thus, adolescents’ genes and environments contribute to associations between parenting and externalizing behavior concurrently and longitudinally (represented via path d in the conceptual model, Figure 1.1). Although cross-lagged analyses have not been conducted for the longitudinal associations between parenting and adolescent internalizing behavior, there is evidence that genetic and environmental influences both contribute to associations between parenting and internalizing behavior concurrently and over time (Reiss et al., 2000).

There is a small but growing body of literature seeking to disentangle the direction of effects and characterize the underlying mechanisms of associations between parenting and adolescent behavior using genetically informed designs (see Horwitz et al., 2011; Marceau & Neiderhiser, in press). See Chapter 5 for a detailed review of these studies. Generally, studies have found evidence of passive and evocative gene-environment correlation in the parent-adolescent relationship (Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein et al., 2007) and in associations between parenting and adolescent behavior (see Horwitz et al., 2011; Marceau & Neiderhiser, in press). Thus, evidence from longitudinal investigations suggests mainly genetic, but also environmental influences contribute to change in positivity and negativity in the parent-adolescent relationship, and concurrent and longitudinal associations between aspects of the parent-child relationship and internalizing and externalizing behavior over time.

**Hormone Influences and Family Relationships**

According to biosocial theory, the physiological changes of puberty are hypothesized to affect the parent-child relationships during the adolescent transition. The effect of pubertal changes on parent-child relationship quality may operate through changes in both parent’s and adolescents’ behaviors because of changes in the adolescent’s secondary sex characteristics, hormonally influenced brain changes and ultimately changes in the adolescent’s behaviors, and other developmental changes in both parents and adolescents happening in concert with pubertal changes (Ge & Natsuaki, 2009; Paikoff & Brooks-Gunn, 1991; Susman et al., 1987).

Three studies suggest that testosterone levels may be associated with poor parent-adolescent relationship quality. First, lower levels of testosterone have been linked to expressions of anger during parent-adolescent conflict in boys and girls (Inoff-Germain et al., 1988). Second, higher levels of testosterone were associated with lower quality of mother-son and father-son relationships (Booth et al., 2003). Finally, increased testosterone reactivity to venipuncture was associated with increased family problems one year later for boys (Marceau, Dorn et al., 2012). These findings indicate that
changes in puberty-related hormones are potentially relevant to understanding the changes in parent-child relations during adolescence, though conclusions about the direction of effects cannot be made.

Parent-child relationships also may be changed via hormone-related changes in adolescent behavior, or via growth of secondary sex characteristics and simultaneous changes in parents and adolescents reactions to these changes (Ge & Natsuaki, 2009; Paikoff & Brooks-Gunn, 1991; Susman et al., 1987). First, hormonal changes may contribute to changes in the parent-child relationship through changes in the adolescent’s behaviors if parents react to the increased aggression, negativity, or irritability shown by their offspring. Thus, pubertal hormones that are associated with increases in aggressive affect generally and within observed parent-child interactions (e.g. Brooks-Gunn & Warren, 1989; Inoff-Germain et al., 1988; Susman et al., 1991; Susman et al., 1987) could mediate the effects of puberty on parent-child negativity and conflict, and propagate coercive cycles during adolescence. Findings showing that more advanced pubertal maturational status, as opposed to early timing of puberty is more strongly related to parent-child relationship quality (e.g. Steinberg, 1987; Steinberg, 1988) suggest that the overall levels of circulating pubertal hormones (which advance maturational status) may be particularly salient for the parent-child relationship.

Hormonal changes may also contribute to changes in the parent-child relationship through changes in secondary sex characteristics (i.e. breast and pubic hair development in girls, genital and pubic hair development in boys) and reactions to those changes by parents and adolescents. Pubertal maturation, as measured via observable changes in the adolescent (particularly being in more advanced maturational stages and experiencing earlier pubertal timing or faster tempo) as opposed to hormonal measures, has been associated with more negative affect during conflict, higher rates of conflict, and reduction of closeness in the parent-adolescent relationship, and poorer family relationship quality generally (Anderson, Hetherington, & Clingempeel, 1989; Collins & Laursen, 2004, Flannery et al., 1993; Inoff-Germain et al., 1988; Marceau, Ram, & Susman, under review; Steinberg, 1987; Steinberg, 1988; see Laursen et al., 1998 for meta-analysis) as well as internalizing and externalizing behavior (e.g. Mendel, Turkheimer, & Emery, 2007; Marceau, Ram et al., 2011). When adolescents develop quickly, parents and adolescents may not have adequate time to adjust to changes in the adolescent. Despite evidence that the timing and tempo of puberty assessed via secondary sex characteristics is associated with internalizing and externalizing behavior and family relationships, more work investigating the timing or tempo of re-activation of hormone changes (both the monotonically increasing change of pubertal development and the relative increase in the responsivity of both sex and stress hormones to stressors) occurring during puberty in relation to internalizing and externalizing behavior and family relationships is needed. Increased responsivity (or deviations from normative increases in responsivity) may provide another mechanism for changes in
parent-child relationship quality in association with internalizing and externalizing behavior (Marceau, Dorn et al., 2012).

While the theory and evidence presented above explicates how hormones may influence parent-child relationships during adolescence, a substantial body of research has also shown that parenting and parent-child relationships are particularly important for the development of the endocrine system in early childhood. Life history theory emphasizes that life experiences within salient domains, particularly the early rearing environment, influence HPA and HPG axis functioning and development during adolescence (e.g. Del Giudice et al., 2011). This theory has been primarily used to explain the development of HPA axis functioning, though it can also be extended to HPG axis development (Shirtcliff & Ruttle, 2010).

Early adverse experiences, especially early maltreatment and insecure attachment, and early rearing environments marked by high family conflict, appear particularly salient for organizing HPA axis functioning, including functioning during adolescence (Del Giudice et al., 2011; Essex, Klein, Cho & Kalin, 2002; Gunnar, Morison, Chisholm, & Schuder, 2001; Halligan, Herbert, Goodyer, & Murray, 2004; Heim et al., 2002; Tarullo & Gunnar, 2006; see Matthews, 2002 for review). For example, studies of children adopted from Russian and Romanian orphanages show that children who had profound deprivation and neglect early in life had elevated basal cortisol levels in middle childhood, and elongated patterns of activation after stress (Wismer-Fries, Shirtcliff, & Pollak, 2008). Foster children placed in infancy showed smaller diurnal changes in cortisol than children not in foster care (Dozier et al., 2006). This effect is probably explained by lower morning cortisol (Fisher, Gunnar, Dozier, Bruce, & Pears, 2006), which is likely due to early maltreatment and neglect (Bruce, Fisher, Pears, & Levine, 2009). History of depression and parent hostility also predicted more reactive cortisol in preschool aged children (Dougherty, Klein, Rose, & Laptook, 2011). Even more normative forms of negative parenting (e.g. spanking, maternal withdrawal) in infancy can have lasting effects on HPA functioning (Bugental, Marotell, & Barraza, 2003). Given the moderately consistent associations between cortisol functioning and internalizing and externalizing behavior, this evidence suggests that one potential mechanism of parenting influences on internalizing and externalizing behavior is through the development of the endocrine system.

The degree of the buffering of the stress responsiveness of the HPA axis in the juvenile period also depends to some extent on early stressful experiences and the quality of parent-child interactions (Gunnar & Donzella, 2002). Access to supporting parenting relationships helps buffer the HPA stress response in childhood because parents may be regulators of the child’s HPA system during childhood (Gunnar & Quevedo, 2007). For example, children aged 2-4 years old showed increases in cortisol over days in which they were in child care but not during days they are at home
(Gunnar & Donzella, 2002 for review). As noted above, the juvenile buffered period is an important period of development, thought to allow children to develop in different ways before reaching reproductive adulthood. However, even during the juvenile buffered period, increased levels of cortisol has been related to daily traumatic family events compared with levels of cortisol within the same children on days in which there were not negative family events (Flinn & England, 1997).

Less work has been done examining early experience on HPG axis functioning. However there is some evidence that the HPG axis is tuned to early family environmental experiences. Early family environmental factors including father absence, and particularly chaotic and negative rearing environments before age 5 years are also thought to impact the timing of onset and duration of pubertal development (i.e. HPG axis functioning) and particularly the timing of the increase of DHEA in middle childhood/adolescence associated with puberty (Belsky, Steinberg, & Draper, 1991; Belsky, Bakersman-Kranenburg, & van Ijzendoorn, 2007; Ellis, Shirmeliff, Boyce, Deardorff, & Essex, 2011; Ellis & Essex, 2007; see Ellis, 2004 for review). Thus, while harsh parenting and dysregulated neuroendocrine functioning are risk factors for internalizing and externalizing behavior, they are not likely independent. The early rearing environment affects the development of multiple hormone systems (see also Susman & Pollak, in press), and early negative rearing environments (i.e. harsh parenting) predict cortisol, DHEA, and testosterone dysregulation (Shirtcliff & Ruttle, 2010 for review). Evidence that hormone functioning further predicts parent-child relationship quality (e.g. Booth et al., 2003; Inoff-Germain et al., 1988; Marceau, Dorn et al., 2012) suggests that the association between hormones and parent-child relationships is bidirectional (represented via path j in the conceptual model, Figure 1.1).

Finally, several studies have demonstrated that parent-adolescent relationship quality can moderate the association between adolescent hormone levels and behavior (e.g., Booth et al., 2003; Dorn et al., 2009; Fang et al., 2009, represented via path m in the conceptual model, Figure 1.1). For boys, higher levels of testosterone were associated with more risk taking behaviors and lower levels of testosterone were associated with more depressive symptoms and delinquent behavior only among families reporting lower parent-adolescent relationship quality, vs. higher relationship quality (Booth et al., 2003; Fang et al., 2009). However, for girls, lower levels of testosterone were associated with more risk taking behaviors, delinquency, and depressive symptoms only among families reporting lower relationship quality (Booth et al., 2003; Fang et al., 2009). Contextual family variables also moderated the extent to which boys with disruptive behavior disorders and controls varied in levels of DHEA and cortisol (Dorn et al., 2009). Notably, these studies examine basal levels, and not responsivity of hormones. In this dissertation I expand on this literature by examining whether the family context also moderates associations between hormone responsivity and behavior (Chapter 6).
Stress responsivity of HPA and HPG hormones in reaction to parent-child conflict likely changes child behavior during the conflict by increasing negative affect or aggressive and hostile behaviors, thus affecting subsequent patterns of parent-child interactions. In this way, stress reactivity in hormones may reinforce cycles of problematic family relationships through increases in problematic mood or behavior in the adolescent. Based on the reviewed findings, I propose that stress-related reactivity in stress and puberty related hormones represents a second mechanism, in addition to overall levels of hormones, for how stress and puberty related hormones may be associated with family relationships during adolescence and adolescent internalizing and externalizing behavior. The stress-reactivity of cortisol, testosterone, and DHEA hormones may make adolescents more sensitive to stressful family interactions, resulting in poorer quality of parent-adolescent relationships during puberty (which is also a risk factor for internalizing and externalizing behavior).

There is some evidence supporting this hypothesis. First, hypo-responsivity of hormones was implicated in the intergenerational transmission of substance use disorder in men and their sons (Moss et al., 1995). Additionally, we recently found that for boys, higher testosterone responsivity to a venipuncture paradigm during early adolescence predicted more family problems one year later, whereas higher in DHEA responsivity predicted more negative emotionality concurrently at the six month and one year visits (e.g. Marceau, Dorn et al., 2012). Although zero-order correlations showed cortisol responsivity in the morning of the initial visit predicted more family problems one year later, cortisol responsivity did not predict later negative emotionality or family problems in the full cross-lagged model testing predictions of cortisol responsivity, negative emotionality, and family problems concurrently and longitudinally at three time points. However, responsivity of cortisol, testosterone, and DHEA were unrelated to negative emotionality or family problems in girls after controlling on pubertal status and age. As negative emotionality is primarily an internalizing trait, these findings support the hypothesis that blunted activational effects contribute to externalizing behaviors while exaggerated activational effects contribute to internalizing behavior.

Together, the body of literature examining family context and hormone functioning suggests that the link between parent-child relationship quality and endocrine functioning is highly complex and transactional over time (i.e., path j of the conceptual model, Figure 1.1), and that the combination of negative parenting and dysregulated endocrine functioning may exacerbate risk for internalizing and externalizing behavior (i.e., path m of the conceptual model, Figure 1.1). That is, this evidence suggests that the family context is implicated in hormone-behavior associations during adolescence via a number of different mechanisms. There is evidence of transactional associations between hormone functioning and the parenting environment which may be mediated by or have implications.
for behavioral development. However, studies examining the role of the parent-child relationship in conjunction with pubertal hormones on internalizing and externalizing behavior have primarily examined the role of circulating levels of pubertal hormones, not stress responsivity, despite evidence that testosterone and DHEA are stress responsive, and the associations between responsivity of stress and puberty related hormones and internalizing and externalizing behavior. Therefore, in this dissertation I focus primarily on responsivity of each of these three hormones when possible, in conjunction with the family context (Chapters 6 and 7).

**Summary and Integration**

In summary, the associations between parent-child relationship quality and internalizing and externalizing behavior appear to be bidirectional over time, and changes in associations between aspects of the parent-child relationship and children’s internalizing and externalizing behavior is likely explained by multiple mechanisms (i.e., child behavioral development, development of the parent-child relationship, gene-environment correlation and interaction, and hormone-environment correlations and interactions). The family context changes over time, marked by small decreases in closeness and increases in conflict during adolescence, and fluctuations in relationship quality. Genetic and environmental influences of parents and children contribute to positive and negative aspects of the parent-child relationship, and the influence of children’s genes on parenting tends to increase across childhood and into adolescence, suggesting the potential for an increased role of evocative gene-environment correlation underlying associations between parent and youth behavior during adolescence. Similarly, there are correlations between stress and puberty related hormones and parent-child relationship quality, including evidence of transactional associations between HPA and HPG hormones and the rearing environment (i.e., hormone-environment correlation) across childhood and adolescence.

Two potential mechanisms linking parent-child relationships and hormone functioning conceptually map onto the commonly studied mechanisms of gene-environment interplay. The literature on associations between hormones and family relationships is conceptually very similar to the literature on gene-environment correlation, highlighting that the parenting environment adolescents receive is often correlated with the physiological underpinnings of behavior. This could be akin to passive gene-environment correlation, if parents provide environments that influence the development of the endocrine system as well as genetic or epigenetic influences that are consistent with the provided rearing environment. Or, hormone-parenting associations could be evocative, if parents react to hormonally influenced behaviors during adolescence. There is also evidence that the parent-adolescent relationship quality may moderate hormone-behavior associations during adolescence (i.e., hormone-environment interactions). In this way, studies examining whether the
parent-adolescent relationship moderates hormone-behavior associations extend the logic of gene-environment interaction to show that there are potentially other physiological-environment interactions important for the development of internalizing and externalizing behavior. Since the steroid hormones reviewed here can change gene expression, these hormone-environment correlations and interactions could represent biological mechanisms through which gene-environment correlation and interactions in part operate. Thus, it is important to not only examine gene-environment correlation (i.e., Chapter 5) and/or interaction, but also hormone-environment interaction (i.e., Chapter 6) and/or correlation, and to examine both gene- and hormone- environment correlations (i.e., Chapter 7) and/or interactions together in the same developmental model of the development of internalizing and externalizing behavior.
CHAPTER 5: PAPER 1: GENE-ENVIRONMENT CORRELATION UNDERLYING THE ASSOCIATION BETWEEN PARENTAL NEGATIVITY AND ADOLESCENT EXTERNALIZING PROBLEMS

Throughout the opening sections of this dissertation, I have presented the conceptual framework guiding my program of research, and provided background and evidence for the basic tenants of this framework from behavioral genetic and endocrinology approaches. This chapter begins the set of empirical articles testing portions of the conceptual framework outlined in Chapter 1. The first paper explores the association between parental negativity and adolescent externalizing behavior using novel behavioral genetic methodology. Thus, in the first paper I test a model that draws on paths a through d of Figure 1.1.

Citation

Abstract
Studies of adolescent or parent-based twins suggest that gene-environment correlation (rGE) is an important mechanism underlying parent-adolescent relationships. However, information on how parents’ and children’s genes and environments influence correlated parent and child behaviors is needed to distinguish types of rGE. The present study used the novel Extended Children of Twins model to distinguish types of rGE underlying associations between negative parenting and adolescent (age 11-22 years) externalizing problems with a Swedish sample of 909 twin parents and their adolescent offspring and a US-based sample of 405 adolescent siblings and their parents. Results suggest that evocative rGE, not passive rGE or direct environmental effects of parenting on adolescent externalizing, explains associations between maternal and paternal negativity and adolescent externalizing problems.

Introduction
The association between negative parenting behaviors (i.e., harsh discipline, criticism, conflict, parental rejection) and child and adolescent externalizing problems (i.e., acting out, delinquency, aggression) is well established (e.g., Baumrind, 1991; Stice & Barrera, 1995) and there has been longstanding interest in understanding the mechanisms underlying this association (e.g.,
Traditionally, researchers have assumed a unidirectional model, such that children who are exposed to greater parental negativity, form insecure attachment bonds, or receive poor socialization from parents are at greater risk of developing externalizing problems (e.g., Hirschi, 1969). More recently, theories of transactional processes have been used to explain associations between parental negativity and children’s externalizing problems, such that parenting affects children’s externalizing problems, but that child externalizing problems also impact parenting behaviors (e.g., Lansford et al., 2011; Lytton, 1990; Stice & Barrera, 1995).

To date, few studies examining transactional processes have utilized genetically informed study designs capable of testing the contributions of parents’ and children’s genes on the association between parental negativity and adolescent externalizing problems. The Extended Children of Twins (ECOT) design is a novel approach that combines a sample of parents who are twins and their adolescent children with a sample of adolescents who are twins and siblings and their parents (Narusyte et al., 2008; Narusyte et al., 2011). This combination assesses genetic and environmental contributions from both adolescents and parents to both parenting and adolescent behavior, which is integral for understanding the mechanisms that underlie the associations between them, and enables researchers to test the direction of effects (i.e. parent-to-child and or child-to-parent) of the associations. Thus, the present study uses the ECOT approach to examine the extent to which adolescent- and or parent-driven effects account for the association between parental negativity and adolescent externalizing problems.

Transactional Theories of Parental Negativity and Adolescent Externalizing

Theorists have postulated that both child- and parent-based effects contribute to associations between parental negativity and child and adolescent externalizing problems (e.g., Shaw & Bell, 1993). Transactional theories have been used to explain associations between positive parenting and normative development (e.g., Greenberg & Speltz, 1988; Greenberg, Speltz, & Deklyen, 1993) as well as associations between negative parenting and child behavior problems. Patterson and Fisher (2002) posited that parental negativity and child externalizing problems are associated transactionally, via coercive cycles, such that parents who respond negatively to their child’s problematic behaviors appear to exacerbate the child’s emotional and behavioral problems through environmental mechanisms. This reciprocal negative interactive style sets the parent-child relationship on a downward spiral of continual negative interactions, resulting in the exacerbation of both negative parenting and offspring externalizing problems. This model was originally developed in regard to the early development of conduct disorder in boys on very short time-scales (i.e. over the course of specific parent-son interactions). The coercive cycles theory has made great contributions in terms of highlighting the transactional influences of parenting and child behaviors in the development of
externalizing problems, but did not anticipate the importance of child-based genetic influences in perpetuating negative cycles and for longer-term bidirectional influences across the lifespan.

Children’s characteristics have been hypothesized to elicit negative responses from others in interpersonal relationships throughout the lifespan, thereby contributing to the continuity of behavior problems over time (Moffit, 1993). Theories of transactional or bidirectional influences between parents and children have generally been supported using non-genetically informed, longitudinal studies across childhood and adolescence most often examining one child per family (e.g., Deater-Deckard & Dodge, 1997; Dishion, Patterson, Stoolmiller, & Skinner, 1991; Pettit & Arsiwalla, 2008; Zadeh, Jenkins, & Peplar, 2010). These studies have typically shown that earlier child externalizing problems are associated with later negative parenting and that earlier negative parenting is associated with later child externalizing problems via cross-lagged methods. However, these non-genetically informed studies cannot account for the influence of genes shared by children and their parents, or the influence of children’s genes on how they are parented. There is substantial evidence of genetic influences on negative parenting (e.g., Kendler, 1996; Losoya, Callor, Rowe, & Goldsmith, 1997; Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein, Spotts, & Ganiban, 2007) and on child and adolescent externalizing problems (e.g., Burt, 2009a; Miles & Carey, 1997; Rhee & Waldman, 2002). Therefore, it is possible that adolescents’ genes contribute to both parenting and externalizing problems.

Reciprocal interactions could reflect the direct environmental influences between parents and children implicit in many theories of development. However, because genes and environments are correlated, especially in families, passive rGE (i.e., parents and children share genes which may influence how parents parent and children behave) and/or evocative rGE (i.e., when parenting is a response to children’s genetically influenced behavior) may also exacerbate the association between negative parenting and offspring externalizing problems (Plomin, DeFries, & Loehlin, 1977; Scarr & McCartney, 1983). Therefore, understanding whether rGE contributes to the association between negative parenting and adolescent externalizing problems, and if so, which type, can help to clarify the mechanism of bidirectional influences between parenting and adolescent behavior. Drawing from the transactional theories of parent-child relationships and methodological and theoretical frameworks that distinguish the influence of adolescents’ and parents’ genes on the association between parental negativity and adolescent externalizing problems (i.e., ECOT) we examine the contributions of rGE to associations between parental negativity and adolescent externalizing problems.

**Influence of Parent Genes**

Negative parenting behaviors may be associated with adolescents’ externalizing problems via passive rGE if children and parents share genes predisposing the child to have externalizing problems
and predisposing the parent to use negative parenting behaviors. Samples of parents who are twins (Children of Twins design, COT) provide a useful test of passive rGE by using the contrast in genetic relatedness of MZ and DZ twin parents to estimate the influence of parents’ genes and environments on parenting behavior, child externalizing problems, and or associations between parenting behaviors and child behaviors (see Horwitz, Marceau, & Neiderhiser, 2011).

COT studies have been used to examine several types of negative parenting behaviors, including exposure to harsh discipline, family conflict, and marital conflict. Findings suggest that while exposure to harsh parenting and conflict is an environmental risk factor, parents’ genes are also implicated in associations between family conflict and adolescents’ externalizing and substance use problems (D’Onofrio et al., 2007; Knopik et al., 2006; Lynch et al., 2006; Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein et al., 2007; Schermerhorn et al., 2011; Silberg, Maes, & Eaves, 2010; Silberg, Maes, & Eaves, 2011). Studies comparing adopted and biological children also suggest that passive rGE operates in infancy, early and middle childhood, and adolescence (Braungart-Rieker, Rende, Plomin, DeFries, & Fulker, 1995; McGue, Sharma, & Benson, 1996), and that family environmental influences (e.g., maternal depression, parent-child conflict) contribute to stability of antisocial behavior (Burt, McGue, & Iacono, 2010; Klahr, McGue, Iacono, & Burt, 2011; Tully, Iacono, & McGue, 2008). Together, this evidence suggests that parenting and offspring development are associated via passive rGE while causal, environmental mechanisms are likely also operating. While COT studies have permitted a careful examination of the role of passive rGE in influencing child and adolescent outcomes, they are underpowered for identifying evocative rGE because of limited variability in the genetic relatedness of offspring. Thus, to detect evocative rGE, studies have investigated the influence of adolescents’ genes on their own behavior and on their parents’ behavior.

**Influence of Child Genes**

Adolescents’ heritable characteristics may shape negative parenting via evocative rGE, such that adolescents with more externalizing problems may evoke more negative responses from their parents. For example, there is evidence that variation in adolescents’ genotypes contribute to the parenting they receive (e.g., McGue, Elkins, Walden & Iacono, 2005; Narusyte, Andershed, Neiderhiser, & Lichtenstein, 2007; Neiderhiser, Reiss, Hetherington, & Plomin, 1999; Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein et al., 2007; Plomin, 1994 ). Genetically-informed studies of twin and sibling children have examined transactional influences between child externalizing problems and parental negativity. Generally, findings support bidirectional influences, showing that child externalizing problems predict parental negativity and that parental negativity predicts later externalizing problems in early to middle childhood (e.g., age 4-7, Larsson, Viding, Rijssdijk, & Plomin, 2008) and during adolescence (Burt, McGue, Krueger, & Iacono, 2005; Neiderhiser Reiss,
Hetherington, & Plomin, 1999). These findings suggest that mainly the influence of children’s genes, but also children’s environmental influences, contribute to change in positivity and negativity in the parent-child relationship.

The findings described above represent evocative rGE, as the power to detect evocative rGE is greater in child-based designs, i.e., when adolescent siblings vary in genetic relatedness. Findings from adoption studies corroborate the role of evocative rGE in explaining associations between negative family environmental influences (e.g., parenting, parent-child conflict) and children’s externalizing symptoms (Deater-Deckard & O’Connor, 2000; Ge et al., 1996; O’Connor, Deater-Deckard, Fulker, Rutter, & Plomin, 1998). However, studies of adolescents who are twins and siblings lack the ability to definitively separate passive from evocative rGE when examining the association between parenting behavior and adolescent characteristics because only genetic and environmental influences of the adolescent can be estimated. Information on genetic and environmental influences on both parent and adolescent behavior is needed to distinguish passive from evocative rGE in associations between parent and adolescent behavior (e.g., Heath, Kendler, Eaves, & Markell, 1985).

**Extended Children of Twins Design (ECOT)**

A novel approach for understanding the direction of effects (parents influencing offspring vs. offspring influencing parents) and rGE underlying the association between parent and adolescent behavior is the Extended Children of Twins (ECOT) design (Narusyte et al., 2008, Narusyte et al., 2011; see also Silberg & Eaves, 2004). ECOT combines two studies, a parent-based and a child-based twin design, with comparable measures of parent and adolescent behavior within the same nested model. Therefore, the model includes multiple genetic and environmental sources, allowing for tests of reciprocal effects, and an identified model with unbiased estimates (Heath et al., 1993). The power to detect evocative rGE lies in the child-based design, which estimates the influence of children’s genes on their behavior and on their parents’ behavior. The ability to detect passive rGE lies in the parent-based design, which estimates the influence of parents’ genes and environmental influences on their behavior and their child’s behavior. By combining the two studies in the same statistical model using similarly measured constructs, the ECOT design allows researchers to examine three possible mechanisms explaining associations between parent and child characteristics: a) direct environmental effects of parenting behavior on child behavior, free of genetic influences of the parent or child, b) passive rGE, suggesting that parents’ genes influence both their parenting and their child’s behavior, and c) evocative rGE, suggesting that children’s genes influence both their externalizing problems and the way they are parented.
Only two studies have used the ECOT design to disentangle the contributions of passive \( r_{GE} \), evocative \( r_{GE} \), and direct environmental parenting influences on the association between aspects of parenting behaviors and child adjustment problems (Narusyte et al., 2008; Narusyte et al., 2011). In the first study, the ECOT design was applied to examine the association between maternal emotional overinvolvement and adolescent internalizing problems. Findings showed that evocative \( r_{GE} \) completely accounted for the association between maternal overinvolvement and adolescent internalizing problems, highlighting the role of adolescents’ heritable characteristics for shaping parents’ behaviors (Narusyte et al., 2008). More recently, the ECOT model was applied to the association between maternal and paternal criticism and adolescent externalizing problems. Findings revealed that the association between maternal criticism and adolescent externalizing problems was explained by evocative \( r_{GE} \), whereas there was only direct environmental influence of paternal criticism on child externalizing problems (Narusyte et al., 2011).

These novel studies have contributed to the literature by demonstrating the role of children’s genes in the evocative effects proposed in theories of parental contributions to children’s behavior problems. Though Narusyte et al. (2011) found differences in mechanisms underlying associations between mothering vs. fathering and adolescent adjustment problems, there was limited power to detect evocative \( r_{GE} \) in the association between paternal criticism and adolescent externalizing due to a low response rate by fathers in the child-based sample used. Because of this limitation it is unclear whether the observed differences between mother- and father-adolescent relationships actually reflect a lack of power to detect evocative \( r_{GE} \) in the fathering sample. Further, it is unclear whether findings are specific to parental criticism, or if evocative \( r_{GE} \) and environmental parenting effects also drive the associations across different features of parental negativity (i.e. criticism, punishment, and conflict) and adolescent externalizing problems.

**Present Study**

Using the ECOT approach, we examined the association between a broad rating of parental negativity (including negativity, conflict, and harsh discipline) and adolescent externalizing problems using the same parent-based sample as used in the two previous ECOT studies (Narusyte et al., 2011; 2008), and a different sample of adolescent twins and siblings. The inclusion of a wider range of negative parenting behaviors, all of which have been highlighted in the literature to have strong and consistent effects across studies (e.g., Loeber & Dishion, 1983), will help to clarify whether the mechanisms underlying the associations between parental negativity and adolescent externalizing problems identified by Narusyte et al., (2011) are specific to parental criticism, or if there is a more general effect of negative parenting. The inclusion of a twin and sibling sample with a large number of
fathers will help to clarify whether there are differences in the rGE underlying associations between maternal vs. paternal negativity and adolescent externalizing problems.

**Hypotheses.** Drawing from the literature suggesting that adolescents’ genes impact the parenting they receive, we hypothesized that the association between parental negativity and externalizing problems in adolescence would arise primarily because parents are responding to adolescents’ genetically influenced externalizing problems (i.e. evocative rGE) in such a way that contributes to higher levels of parental negativity, broadly. We also expect that parental negativity (particularly fathers’ negativity) would exert an environmental influence on adolescent externalizing problems, based on findings from Narusyte et al., (2011), and evidence of passive rGE for fathers’ but not mothers’ negativity in Neiderhiser et al., (2004) and (2007).
Method

Participants and Procedures

The present study examines how genetic and environmental influences are correlated for the association between parental negativity and adolescent externalizing problems using the US-based Nonshared Environment in Adolescent Development study (NEAD; Neiderhiser, Reiss, & Hetherington, 2007; Reiss, Neiderhiser, Hetherington, & Plomin, 2000), and the Swedish-based Twin and Offspring Study in Sweden (TOSS; Neiderhiser & Lichtenstein, 2008). TOSS was designed in part to mirror NEAD. Indeed, this pair of studies is the only instance of such a design. In TOSS the twins are parents, whereas in NEAD the twins are adolescents. Great care was taken to use identical measures of parenting in both. Because of its unique design, TOSS could only be conducted in a country with a very large, well-documented twin registry. At the time the studies were designed, only the Swedish twin registry was large enough. To maintain the integrity of the mirror-image design across nationalities, careful attention was paid to potential cultural confounds and to translating instruments from English to Swedish (Reiss et al., 2001). Previous reports have found the US and Swedish samples to be comparable on a number of key demographic and substantive variables including negative parenting and externalizing problems (Neiderhiser et al., 2004; Neiderhiser, Reiss, Lichtenstein et al., 2007). While TOSS has been used for ECOT models in the past (Narusyte et al., 2008; 2011), the inclusion of NEAD as the child-based sample is unique to this study. By adding nontwin siblings with the twins in the child-based design, the ECOT model is able to more precisely detect evocative rGE effects, and is more generalizable to multiple family types, not only twins.

The Nonshared Environment in Adolescence Study (NEAD). The NEAD sample consisted of 721 predominantly White (94%) families of twins and siblings who participated in the first wave of data collection in the NEAD project (Neiderhiser, Reiss, & Hetherington, 2007). Most of the families were recruited through a national market survey of 675,000 families, though some were recruited through random digit dialing of 10,000 telephone numbers throughout the United States. Zygosity was established using a validated questionnaire for which adolescent twins were rated for physical similarity (Nichols & Bilbro, 1966). The agreement of this particular questionnaire with genotyping has been estimated at over 90% (e.g., Goldsmith, 1991).

The twins and siblings who still resided primarily at home were also assessed approximately three years after the initial assessment. Data were drawn from the 408 twin and siblings who participated in the second assessment in order to match the ages of adolescents in both samples. The analysis sample consists of 405 families falling into one of six sibling categories in two family types: 63 same-sex monozygotic twins (MZ), 75 dizygotic twins (DZ), and 58 full siblings (FI) in non-divorced families, and 95 full siblings (FS), 60 half siblings (HS), and 44 genetically unrelated
siblings (US) in stepfamilies. Stepfamilies were together for at least 5 years at the time of the first assessment to avoid periods of instability due to family formation. The analysis sample is slightly reduced (by 3 pairs) because of missing information on zygosity. Adolescents were 11 to 22 years old ($M = 15.5$ years; $SD = 2$ years). Siblings were within 4 years of age of each other ($M = 1.6$ years; $SD = 1.3$ years), and lived in the same two-parent household at least 50% of the time for at least 5 years.

**The Twin and Offspring Study in Sweden (TOSS).** The TOSS sample consisted of 909 White pairs of twin parents, their spouse or partner, and their adolescent child (Neiderhiser & Lichtenstein, 2008). The TOSS sample was obtained through the use of the Swedish Twin Registry. Zygosity was established using the same validated questionnaire as used in the NEAD study, for which adolescent twins were rated for physical similarity (Nichols & Bilbro, 1966). The analysis sample consists of 854 families for whom we have zygosity information (126 MZ fathers, 188 DZ fathers, 258 MZ mothers, 282 DZ mothers). As in the NEAD study, adolescents were aged 11 to 22 years old ($M = 15.7$ years, $SD = 2.5$ years). All adolescent cousin pairs were the same sex and were within 4 years of age of each other ($M = 1.8$ years; $SD = 1.5$ years).

**Measures**

**Parental negativity.** Parental negativity was measured in each study by mother, father, and adolescent report using identical composite scores including the conflict subscale (e.g., how much do you yell at the child after you’ve had a bad day) of the Parent Child Relationships questionnaire (Hetherington & Clingempeel, 1992), and the coercive (e.g., brought up a lot of the child’s faults when the two of you argued) and punitiveness (e.g., punished you more severely than usual for bad behavior) subscales of the Parent Discipline Behavior Inventory (Hetherington & Clingempeel, 1992; $a > .61$ across reporters in both samples). Mother and adolescent reports of mothers’ negativity, and father and adolescent reports of fathers’ negativity on each subscale were standardized and summed to create the negativity composites ($a > .74$ for both samples). Composites were created in this way to be consistent with previous reports (i.e., Neiderhiser et al., 2004; 2007) and to avoid single-measure bias (Bank, Duncan, Patterson, & Reid, 1993). Each composite score was ranked to normalize distribution, consistent with previous studies using these data (Neiderhiser et al., 2004; 2007).

**Adolescent externalizing problems.** Adolescent externalizing problems were measured using multi-rater composite scores in each study. In NEAD, mother, father, and adolescents reported externalizing problems on the Zill Behavior Problems Inventory (ZIL: Zill, 1988). The ZIL externalizing problems subscale is comprised of 20 items (e.g., Breaks things on purpose, deliberately destroys his or her own or other’s things; Is disobedient at home) on a 1 (often true) to 3 (never true) scale over the past three months ($a > .87$ for each reporter). Items are reversed and then summed so higher scores indicate more externalizing problems. In TOSS, mother, father, and adolescent reported...
externalizing problems on the child behavior checklist (CBCL, Achenbach, 1991) were summed to create an externalizing composite. The CBCL externalizing subscale is comprised of thirty items (e.g., I destroy my own things; I disobey my parents) on a 1 (not true) to 3 (often true) scale over the past six months. The composite externalizing scores were acceptably reliable in both studies (α > .62 for both siblings in NEAD; α > .70 for both siblings in TOSS). Again, each composite score was ranked to normalize the distributions.

**Analytic Strategy**

**Twin and sibling studies.** Quantitative genetic analyses take advantage of similarities and differences between twin and siblings with varying degrees of genetic relatedness to parse the variance in a particular phenotype into additive genetic (A), shared environmental (C) and nonshared environmental (E) components. By definition, twins and siblings share 100% of their shared environment (nongenetic influences that make family members similar) and none of their nonshared environment (nongenetic influences that make family members different including measurement error). Different sibling types share different average proportions of their segregating genes: monozygotic (MZ) twins share 100% of their genes, dizygotic (DZ) twins and full siblings share an average of 50% of their segregating genes, half-siblings and offspring of MZ twins share an average of 25% of their segregating genes, offspring of DZ twins share an average of 12.5% of their segregating genes, and step-siblings are genetically unrelated.

Estimates of A, C, and E can be obtained by comparing the sizes of correlations between sibling 1 and sibling 2 across sibling types. For example, if the correlation of sibling 1’s externalizing problems and sibling 2’s externalizing problems among MZ twins is twice that of the correlation among DZ twins, genetic influences are indicated (because MZ twins share twice as many segregating genes as DZ twins). Shared environmental influences are indicated to the extent that the correlations between sibling 1 and sibling 2’s externalizing problems among each different sibling type are similar. Nonshared environmental influences are indicated to the extent that MZ twins’ externalizing problems are not perfectly correlated. For a more detailed explanation of quantitative genetic theory and expectations from twin correlations see Marceau & Neiderhiser, in press, and Narusyte et al., 2008.

**ECOT model.** In order to test whether the association between parental negativity and externalizing problems in middle-late adolescence arises because of evocative rGE, passive rGE, or environmental effects free from genetic confounds we used the Extended Children of Twins Model (Narusyte et al., 2008; 2011; ECOT, Figure 5.1). The ECOT model adds the power of a classic twin design to the power of a children-of-twins design. The lower box on the left of Figure 5.1 represents the classic twin sample, while the larger box on the right represents the children-of-twins sample.
This quantitative genetic model is built in a structural equation modeling framework using Mx (Neale, 1999), which parses the variance in parental negativity into the influence of parents’ genes and the nonshared environment. These are represented as A1 (genetic influences) and E1 (nonshared environmental influences) along the top of Figure 5.1. The influence of shared environment on parental negativity for twin parents was not included in the initial model for two reasons. First, the intra-class correlations suggested that effects from the shared environment were not present on maternal and paternal negativity (see Table 5.1). This is consistent with other studies that have examined genetic and environmental influences on twin parents (e.g., Lynch et al., 2006; Knopik et al., 2006; Narusyte et al., 2008; 2011). Second, the ECOT model performs better when shared environmental influences are estimated on only the child phenotype rather than on both the parent and child phenotype because a larger sample size is needed to detect small shared environmental effects on the parent phenotype (Narusyte et al., 2008). This is in part because there is less power in twins-only designs (as opposed to twin and sibling designs) to detect shared environmental influences. Furthermore, shared environmental influences on parenting are likely to be small in COT designs since they reflect either the lasting impact of the twin parents’ own rearing environment or current contact.

In accordance with quantitative genetic theory and the average percentage of genes shared between different types of siblings, the correlation between A1 for twin parent 1 and twin parent 2 is constrained to 1 for MZ twin parents and .5 for DZ twin parents. Genetic transmission is also included in the model using the latent factor A1’. The correlation between A1 (influence of parents’ genes on their own parenting) and A1’ (influence of those parenting genes on adolescent externalizing problems) is set to .5 (because children inherit half of their genes from their parents), and the influence of those genes that parents and offspring share on externalizing problems are freely estimated (A1’).

The variance in adolescent externalizing problems is parsed into the influence of adolescents’ genes, shared and nonshared environments. These are represented as A1’ (parent and child shared genes for parental negativity and adolescent externalizing problems) A2 (unique genetic influences on externalizing) C2 (shared environmental influences on externalizing) and E2 (nonshared environmental influences on externalizing) along the bottom of Figure 5.1. Because twins and siblings are used in the child-based design (NEAD), the correlation between A2 for adolescent sibling 1 and adolescent sibling 2 is constrained to 1 for MZ twins, .5 for DZ twins and full siblings, .25 for half siblings, and 0 for genetically unrelated step siblings.
Figure 5.1. Extended Children of Twins Model. This is a representation of the path diagram used to fit the ECOT model. The lower left-hand box represents the child-based sample. Parent negativity is correlated 1 for twin 1 and twin 2 because in the child-based sample the adolescent siblings share parents. The larger right-hand box represents the parent-based (COT) sample. A1 represents latent genetic influences of parents on their parenting, E1 represents latent nonshared environmental influences of parents on their parenting. A2 represents latent genetic influences of adolescents on their externalizing problems, C2 represents latent shared environmental influences of adolescents on their externalizing problems, E2 represents latent nonshared environmental influences of adolescents on their externalizing problems. A1' represents the effect of genes shared by parents and adolescents on adolescents' externalizing problems. Path m represents direct environmental effects of parenting on adolescents' externalizing problems while path n represents child evocative effects of adolescents’ externalizing problems on parenting. Path s represents the influence of shared genes of parents and adolescents; significant path s and m signifies passive rGE while significant path n and either A2 or s signifies evocative rGE. Measurement error is estimated as ε1 and ε2, and constrained to be equal during model fitting.

Correlations between the influence of adolescents’ genes and parents’ genes are tested, along with direct causal paths from parental negativity to adolescent externalizing problems and paths from adolescent externalizing problems to parental negativity. Correlations between parents and adolescents’ genetic influences in combination with genetic influences on parental negativity in TOSS and genetic influences on externalizing problems in NEAD (path s) and a significant path from parental negativity to adolescent externalizing problems (path m) together suggest parent-based
genetic effects (i.e., passive rGE). Only a significant path from parental negativity to adolescent externalizing problems (path m) without a significant genetic association (path s) indicates a direct environmental effect of parental negativity on adolescents’ externalizing problems. A significant path from adolescent externalizing problems to parental negativity (path n) in combination with genetic influences on externalizing problems in NEAD (as indicated by a significant path from A1’ or A2 on adolescent externalizing problems) indicates the influence of child-based genes (i.e., evocative rGE).

Finally, measurement error is estimated separately (ε₁ and ε₂ in Figure 5.1). Detailed information about the specifications and power of the ECOT model can be found in Narusyte et al., 2008. Because this model is conducted using raw data in a structural equation modeling framework, missing data is handled using full information maximum likelihood data estimation procedures. All analyses were conducted after controlling for age, sex, and age difference (for nontwin siblings in NEAD).

Model Fitting. We took an informed approach to model fitting, starting with a full model estimating all paths, and culminating in a best-fitting model favoring parsimony. First, 95% confidence intervals were used to determine significance of path estimates. Then, we conducted a series of nested models in order to determine if there was a significant decrement in model fit when dropping paths. We systematically dropped paths m (passive rGE) and n (evocative rGE) in order to verify the significance of each of these paths. Then, we dropped each path deemed non-significant based on confidence intervals separately, and as a group. The model with the least number of parameters without a significant decrement of model fit was judged to be the best-fitting, most parsimonious model.

Assumptions. Twin models are built on several assumptions that can impact the estimates of genetic and environmental influences recovered in analyses. First, the equal environments assumption is that shared and nonshared environmental influences are equivalent for each sibling type (i.e., monozygotic twins’ environments are no more similar than genetically unrelated siblings’ environments). No systematic differences have been found negating the validity of the equal environments assumption (Loehlin & Nichols, 1976; Neiderhiser et al., 2004; Reiss et al., 2000). Second, it is assumed that assortative mating is limited (i.e. individuals do not systematically choose their mates based on genetically influenced characteristics). While assortative mating is generally modest for most psychological traits (e.g. Plomin, DeFries, & McClearn, 1990), there is evidence of moderate assortative mating for antisocial behavior (e.g. DuFort, Boothroyd, Bland, Newman, & Kakuma, 2002). The presence of assortative mating on traits involved in the intergenerational transmission of externalizing problems inflates shared environmental influences at the expense of genetic influences. This is because both parents are more likely to pass on genes influencing externalizing problems, so DZ twins would likely share more than 50% of their segregating genes for
externalizing problems and would have a higher sibling concordance, more similar to that of MZ twins. This would reduce the contrast in correlations between MZ and DZ twins, and models using the standard fixed path coefficients for correlations between genetic influences of MZ (1) and DZ (.5) twins would underestimate genetic but overestimate shared environmental influences. However, the inclusion of genetically unrelated siblings in the child-based design in the present study helps to attenuate this bias. Assortative mating on antisocial behavior also suggests that passive rGE is more likely contributing to externalizing problems, inflating the likelihood of finding passive rGE here.

There are also several assumptions specific to the ECOT model (see also Narusyte et al., 2008 and Naruyste et al., 2011). Namely, the samples are assumed to be equivalent. Specifically, we assume no systematic differences in genetic and environmental influences on parenting or on adolescent externalizing problems across the two samples. We also assume that the measurement error does not differ across phenotypes (i.e., ε₁ = ε₂), across samples in order for the model to be identified. Finally, in the present study we assume that rGE processes underlying the association between parenting and adolescent externalizing problems do not differ in the Swedish and US populations represented in TOSS and NEAD, as we obtain one estimate using both samples.

Results

Intra-Class Correlations

Intra-class correlations for mothers’ and fathers’ negativity and adolescents’ externalizing problems are presented in Table 5.1. These correlations provided preliminary indications of the extent to which parental negativity and adolescent externalizing problems are explained by passive and evocative rGE effects and environmental effects free from genetic confounds. Intra-class correlations on parenting in NEAD suggest some influence of adolescent’s genetic (approximately 0-8% of the variance for mothers, 10-34% of the variance for fathers), shared (approximately 12-73% of the variance for mothers, 26-64% of the variance for fathers), and nonshared environmental influences (approximately 15% for mothers, 26% for fathers) on maternal and paternal negativity. The presence of genetic influences suggests the potential for evocative rGE. Intra-class correlations on parenting in TOSS suggest primarily nonshared environmental influences (approximately 71-73% of the variance for mothers and fathers), with some influence of mothers’ genes and fathers’ shared environmental influences on their own parenting. Because large shared environmental influences were not indicated, they were excluded from the ECOT model for parsimony and model performance.
Table 5.1

Intra-class Correlations for Parental Negativity and Child Externalizing in Each Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>MZ</th>
<th>DZ</th>
<th>FI</th>
<th>FS</th>
<th>HS</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>126</td>
<td>148</td>
<td>116</td>
<td>190</td>
<td>120</td>
<td>88</td>
</tr>
<tr>
<td>Externalizing</td>
<td>.67</td>
<td>.47</td>
<td>.29</td>
<td>.39</td>
<td>.19</td>
<td>.08</td>
</tr>
<tr>
<td>Maternal Negativity</td>
<td>.63</td>
<td>.68</td>
<td>.54</td>
<td>.46</td>
<td>.34</td>
<td>.12</td>
</tr>
<tr>
<td>Paternal Negativity</td>
<td>.74</td>
<td>.69</td>
<td>.24</td>
<td>.55</td>
<td>.39</td>
<td>.27</td>
</tr>
<tr>
<td>Externalizing – Maternal Neg.</td>
<td>.44</td>
<td>.31</td>
<td>.24</td>
<td>.27</td>
<td>.03</td>
<td>.05</td>
</tr>
<tr>
<td>Externalizing – Paternal Neg.</td>
<td>.32</td>
<td>.28</td>
<td>.10</td>
<td>.23</td>
<td>.15</td>
<td>.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TOSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N Mothers</td>
<td>516</td>
<td>564</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Externalizing (adolescents of twin mothers)</td>
<td>.48</td>
<td>.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Negativity</td>
<td>.29</td>
<td>.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Externalizing – Maternal Neg.</td>
<td>.24</td>
<td>.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N Fathers</td>
<td>252</td>
<td>376</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Externalizing (adolescents of twin fathers)</td>
<td>.38</td>
<td>.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal Negativity</td>
<td>.27</td>
<td>.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Externalizing – Paternal Neg.</td>
<td>.17</td>
<td>.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


For NEAD, intra-class correlations suggest that adolescent externalizing problems were influenced by the adolescents’ genetic, shared, and nonshared environmental factors. Intra-class correlations suggest genetic influences explain between 29 and 40% of the variance in externalizing...
problems, shared environmental influences account for approximately 27 to 38%, and nonshared environmental influences account for about 33% of the variance in externalizing problems. Intra-class correlations on externalizing problems in TOSS suggest the influence of mothers’ shared (approximately 40% of the variance, for mothers, and no variance for fathers), and parents’ nonshared environmental influences (approximately 52% of the variance for mothers and 62% of the variance for fathers), but little to no influence of mothers’ genes, and moderate influences of fathers genes on adolescent externalizing problems. Thus, there is potential for an environmental influence of parenting on externalizing problems, but the influence of shared genetic influences (A1’) indicative of passive rGE is unlikely.

Maternal negativity and adolescent externalizing problems were moderately correlated in both samples, with similar magnitudes ($r = .58$ for NEAD, $r = .52$ for TOSS), as were paternal negativity and adolescent externalizing problems ($r = .49$ for NEAD, $r = .46$ for TOSS). We conducted cross-twin cross-trait correlations (see Table 1) in order to gauge whether adolescents’ genes and environments and parents’ genes and environments contributed to these associations. Cross-twin cross-trait correlations are limited by the phenotypic correlation. Therefore, in NEAD, adolescent-based genetic influences likely contribute the majority of the variance to the association between maternal and paternal negativity and externalizing problems (MZ correlations were .44 and .32, respectively), with some potential contribution of adolescent-based shared and nonshared environmental influences. This suggests that we will find evidence of evocative rGE in both mother and father models. In TOSS, parent-based environmental influences likely contribute to the covariation between parental negativity and externalizing problems, with potentially some mother-based genetic influences (see Table 5.1). Therefore, we could also find some, relatively small, environmental influences of parents on adolescents.

**ECOT Model**

**Maternal negativity and adolescent externalizing problems.** Results for the full model for maternal negativity and adolescent externalizing problems are presented in Figure 5.2. Measurement error was modest across both phenotypes. There were significant influences of mothers’ genes and nonshared environment on maternal negativity. There were also significant influences of adolescents’ genes on their own externalizing problems. Paths n (evocative rGE) and A2 (adolescents’ genes) were significant, suggesting evocative rGE explains the correlation between adolescent externalizing problems and maternal negativity, while path m and s were both nonsignificant, suggesting no passive rGE or direct environmental effects of maternal negativity on adolescent externalizing problems.
Figure 5.2. Results for Maternal Negativity ECOT Model. This figure is reduced from Figure 5.1 in order to more succinctly present results. Unstandardized path estimates and 95% confidence intervals (in brackets) are provided for each estimated path. Significant paths are emboldened. Model fit statistics are provided in the lower left. A1 represents latent genetic influences of parents on their parenting, E1 represents latent nonshared environmental influences of parents on their parenting. A2 represents latent genetic influences of adolescents on their externalizing problems, C2 represents latent shared environmental influences of adolescents on their externalizing problems, E2 represents latent nonshared environmental influences of adolescents on their externalizing problems. A1’ represents the effect of genes shared by parents and adolescents on adolescents’ externalizing problems. Path m represents direct environmental effects of parenting on adolescents’ externalizing problems while path n represents child evocative effects of adolescents’ externalizing problems on parenting. Path s represents the influence of shared genes of parents and adolescents; significant path s and m signifies passive rGE while significant path n and either A2 or s signifies evocative rGE. e1 and e2 are the measurement error, and constrained to be equal.
Nested model fitting results confirmed results from the full model. Path m could be dropped without a decrement in model fit (Full model: $-2\text{Lnl} = 8187.5$, df = 3185, AIC = 1807.1; Without path m: $-2\text{Lnl} = 8175.5$, df = 3185, AIC = 1805.1, $\Delta\chi^2 (1) = 0.0$, $p > .05$). However, dropping path n resulted in a decrement in model fit (Without path n: $-2\text{Lnl} = 8187.5$, df = 3185, AIC = 1817.5, $\Delta\chi^2 (1) = 12.48$, $p < .05$). Dropping each of the non-significant paths separately or together did not result in a decrement in model fit. Thus the most parsimonious model included effects of A1, E1, N, and A2 ($-2\text{Lnl} = 8175.1$, df = 3188, AIC = 1799.1, $\Delta\chi^2 (4) = 0.0$, $p > .05$). As such, evocative rGE explained the correlation between maternal negativity and adolescent externalizing problems.

**Paternal negativity and adolescent externalizing problems.** Results for the full model for paternal negativity and adolescent externalizing problems are presented in Figure 5.3. Measurement error was modest across both phenotypes. Results for paternal negativity were remarkably consistent with results for maternal negativity. As with mothers, evocative rGE explained the correlation between paternal negativity and adolescent externalizing problems. Nested model fitting results generally confirmed results from the full model. Path m could be dropped without a significant decrement in model fit (Full model: $-2\text{Lnl} = 6332.7$, df = 2498, AIC = 1336.7; Without path m: $-2\text{Lnl} = 6332.7$, df = 2499, AIC = 1334.7, $\Delta\chi^2 (1) = 0$, $p > .05$). However, dropping path n resulted in a decrement in model fit (Without path n: $-2\text{Lnl} = 6621.9$, df = 2499, AIC = 1623.9, $\Delta\chi^2 (1) = 289.2$, $p < .05$). Dropping each of the non-significant paths from the full model separately or together did not result in a decrement in model fit. Thus the most parsimonious model included effects of A1, E1, N, and A2 ($-2\text{Lnl} = 6337.4$, df = 2502, AIC = 1333.4, $\Delta\chi^2 (4) = 4.7$, $p > .05$).
Figure 5.3. Results for Paternal Negativity ECOT Model. This figure is reduced from Figure 5.1 in order to more succinctly present results. Unstandardized path estimates and 95% confidence intervals (in brackets) are provided for each estimated path. Significant paths are emboldened. Model fit statistics are provided in the lower left. A1 represents latent genetic influences of parents on their parenting, E1 represents latent nonshared environmental influences of parents on their parenting. A2 represents latent genetic influences of adolescents on their externalizing problems, C2 represents latent shared environmental influences of adolescents on their externalizing problems, E2 represents latent nonshared environmental influences of adolescents on their externalizing problems. A1’ represents the effect of genes shared by parents and adolescents on adolescents’ externalizing problems. Path m represents direct environmental effects of parenting on adolescents’ externalizing problems while path n represents child evocative effects of adolescents’ externalizing problems on parenting. Path s represents the influence of shared genes of parents and adolescents; significant path s and m signifies passive rGE while significant path n and either A2 or s signifies evocative rGE. $e_1$ and $e_2$ are the measurement error, and constrained to be equal.
Discussion

The present study found that evocative rGE explained the association between parental negativity and adolescent externalizing problems for both mothers and fathers. This suggests that parental negativity is partially a response to adolescents’ heritable externalizing problems. This study builds on previous studies by examining how adolescent externalizing problems contribute to rGE effects. Previous findings using the same samples and parenting measures used in the current study, but not employing the newly developed ECOT approach, suggested that evocative rGE contributed to maternal negativity, while passive and evocative rGE contributed to paternal negativity (Neiderhiser et al., 2004; 2007). Our findings extend these conclusions by showing that adolescent’s genetically influenced externalizing problems contributed to the evocative rGE underlying maternal and paternal negativity identified in Neiderhiser et al., 2004 and 2007.

Across studies there is consistent evidence of evocative rGE for maternal negativity and the association between different forms of negative mothering behaviors and adolescent externalizing problems, despite differences in measurement and sample inclusions. Findings for fathers are somewhat less consistent. Like Neiderhiser et al., (2007), the present study suggests that evocative rGE plays an important role in paternal negativity and the association between paternal negativity and externalizing problems. However, there is evidence that paternal criticism has a direct environmental influence on externalizing problems (Narusyte et al., 2011). Narusyte et al., (2011) used the same parent-based twin sample and a different sample of adolescent twins to examine the role of rGE in a similar correlation: the associations between maternal and paternal criticism and adolescent externalizing problems. The present study differed from and expanded on Narusyte et al., (2011) in several ways. First, the present study used multi-measure multi-rater composites for parental negativity and multi-rater composites for externalizing problems instead of parent and youth self report. Multi-measure, multi-rater composites avoid single-measure bias (Bank et al., 1993) and may result in more general results capturing a broader spectrum of negative parenting and adolescent externalizing problems than the specific parenting behavior (parental criticism) and adolescent self reported externalizing problems assessed in Narusyte et al., (2011).

Second, the present study used an adolescent sample of twins and siblings instead of an adolescent sample of only twins. The addition of nontwin siblings in the child-based twin design increased the power to detect shared environmental influences on adolescent externalizing problems, which, if present but un-estimated, could inflate the estimate of genetic influences on externalizing problems. Thus, the present study provided a more conservative test of evocative rGE than Narusyte et al., (2011). The addition of nontwin siblings also increased the generalizability of findings beyond families of twins to non-divorced and stepfamilies of twins and nontwin siblings. Narusyte et al.,
(2011) reported limited power to detect evocative GE (65% accuracy) in the association between fathering and externalizing problems because of the limited number of fathers who participated in the child-based twin sample. The present study included a larger sample of fathers in the child-based sample (approximately 400 vs. approximately 130 in Narusyte et al., 2011), increasing our power to detect evocative GE for fathering compared with Narusyte et al., (2011) while maintaining a more conservative test because of the inclusion on nontwin siblings.

Thus, differences in findings for fathers could reflect power differences between samples, or may suggest that particular fathering behaviors have different effects on adolescent externalizing problems. Unfortunately, we do not have appropriate measures of parental criticism available in the NEAD study, and are unable to definitively test whether differences in findings reflect differences in types of parenting behaviors. Further investigation using larger sample sizes and multiple measures of parenting are needed to fully understand these differences in findings across studies.

**Transactions between Parenting and Child Behavior**

The present findings add to the growing evidence suggesting that the mechanism underlying associations between parenting and adolescent behavior problems is not a purely environmental mechanism, but instead is propagated through genotype-environment correlation processes (e.g., Narusyte et al., 2011). The present study was limited in testing theories of bidirectional influence between parents and adolescents because we had only a single measure of parental negativity and adolescent externalizing problems. Longitudinal ECOT models are called for in order to test the changing role of GE in associations between negative parenting and adolescent externalizing problems. There is evidence that child-based genetic effects increase from childhood to adolescence (e.g., Burt & Neiderhiser, 2009). Further, recent evidence suggests that the role of passive GE on negative mothering may increase with children’s age, and that parents’ age also likely plays an important role in the GE processes underlying negative parenting (Marceau et al., 2011, March). Together this evidence suggests that parents’ and children’s genes are implicated in parent-child relationships across adolescence, though the present study only captures a snapshot of a dynamic developmental process.

**Implications**

The role of evocative GE in associations between parental negativity and adolescent externalizing problems suggests that children’s genes, but not parents’ genes, are particularly influential for parent-adolescent relationships. That is, parental influence as a main effect was not important for the negative parent-adolescent relationship here. Theories of parental influence on child behaviors (i.e., social learning theories and coercive cycles) should be expanded to incorporate genotype-environment correlation as a mechanism of the developing negative parent-child
relationship. The present findings also have practical implications. Finding evocative \( r_{GE} \) and not that parenting exerts an environmental influence on externalizing problems during adolescence suggests that parents’ responsivity to, and relationship with adolescents may be the optimal target for interventions aimed to reduce antisocial behavior, rather than simply targeting a reduction in negative parenting behaviors (Feinberg et al., 2001). For example, therapeutic interventions might be aimed at helping parents keep their cool by promoting positive coping strategies particularly when provoked by their adolescents as a way of reducing parent-adolescent relationship problems and adolescent externalizing problems, as opposed to aiming to reduce negative parenting behaviors generally, at all times.

**Limitations and Future Directions**

Evidence from molecular genetic and adoption studies suggests that gene-environment interaction may also be operating for the parent-adolescent relationship and adolescent externalizing problems (e.g., Beaver & Belsky, 2012; Delisi, Beaver, Vaughn, & Wright, 2009; Ge et al., 1996), though we could not test for it with this model. Similarly, there is evidence that assortative mating is particularly important to consider when studying offspring externalizing problems (e.g., Krueger et al., 1998), but we could not control for assortative mating because the current formulation of the ECOT model only incorporates one parent at a time. So, results should be interpreted with caution, as the ECOT model simplifies the transactional processes and gene-environment interplay driving the association between parenting and externalizing problems. Similarly, although we tested direction of effects, the data are cross-sectional and correlational, therefore the findings should be interpreted with caution regarding causality. Finally, while we were able to use multi-rater composites, we were unable to include observer reports of parenting or externalizing problems, which could strengthen or change results. Observer reports were available in the NEAD study, but not for fathers in TOSS. Nonetheless, the present study makes an important contribution to the literature examining associations between parenting and child behavior by showing that parental negativity in adolescence is primarily a response to adolescent’s genetically influenced externalizing problems.
CHAPTER 6: NEUROENDOCRINE RESPONSES TO CONFLICT AND BEHAVIOR PROBLEMS: THE MODERATING ROLE OF PARENT-ADOLESCENT CONFLICT FOR GIRLS’ AND BOYS’ ADJUSTMENT

The first study of this dissertation (Chapter 5) showed that evocative gene-environment correlation is particularly important for explaining associations between parents’ negativity and adolescents’ externalizing behavior during adolescence. However, quantitative genetic studies are limited in explaining how genes actually exert influence on behavior and subsequent relationships. In this case, though results suggest that some genetic predisposition toward externalizing behavior eventually arise in adolescents exhibiting externalizing behavior that parents then respond negatively to, the biological process by which genes are encoded and transmit signals to the brain ultimately resulting in the expressed behavior is unaccounted for. In this dissertation, I focus on hormones as one likely biological mechanism linking genes with behavior because of evidence that hormones, particularly steroid hormones, in part regulate gene expression, and are also genetically influenced (Joffe & Cohen, 1998; Meaney, 2010). In work related to this dissertation, I showed that for boys, testosterone responsivity is associated with an increase in family problems over the course of a year (Marceau, Dorn, et al., 2012). This potentially suggests that at least for boys, testosterone reactivity could contribute to the evocative gene-environment correlation found in the first paper of this dissertation, though more work is certainly required. Indeed, other studies have uncovered correlations between hormones and the family environment (Booth et al., 2003; Inoff-Germain et al., 1988).

In addition to gene-environment correlation, gene-environment interaction has also gained traction for understanding how genetic and environmental influences work together to influence behavior. Quantitative genetic studies using twin methodology similar to that used in the first study of this dissertation suggests that the parenting environment can moderate the strength of genetic influences on behavior (Marceau & Neiderhiser, in press). Thus, extending theory and evidence from quantitative genetic studies of gene-environment interplay while drawing on theories of hormone-behavior associations from the behavioral endocrinology literature (Chapter 3), I hypothesize that the family context may also moderate hormone-behavior associations, as has previously been shown by studies examining testosterone levels and family conflict and cohesion (Booth et al., 2003; Dorn et al., 2009; Fang et al., 2009). Thus, parallel to gene-environment interplay, there is evidence of hormone-environment interplay such that hormone functioning is associated with parenting and there may also be evidence of hormone-parenting interactions in association with adolescent behavior. Because of the dearth of research examining hormone-environment interplay during adolescence, in
the second paper (this chapter) I examine ways in which hormone responses of three steroid hormones (capable of regulating gene expression): cortisol, dehydroepiandrosterone, and testosterone, respond to mother-adolescent conflict, and whether associations between hormone responses to conflict and internalizing and externalizing behavior are moderated by family conflict.

**Citation**


**Abstract**

Several developmental theories highlight transactional roles of physiology and parenting for behavior problems. However, studies examining hormone-behavior associations during adolescence rarely consider the moderating role of family context. The present study addresses these issues using a sample of 213 adolescents (age 11–16, M = 13.7 years, 49% boys, ranging from normative to clinical levels of psychopathology symptoms) and their parents. We examined associations of cortisol, dehydroepiandrosterone, and testosterone responses to a conflict discussion paradigm with internalizing and externalizing problems, and whether parent-adolescent conflict moderated hormone responsivity-behavior problem associations. Hormone responses did not directly predict behavior problems. However, proximal maternal negativity moderated associations of girls’ testosterone and cortisol responses with internalizing and externalizing problems, respectively, whereas global maternal and paternal conflict intensity moderated associations of boys’ cortisol and DHEA responses with externalizing problems. Findings highlight differential hormone-behavior relationships depending on affective context, and suggest that family conflict represents an important context for understanding hormone-behavior associations in adolescents.

**Introduction**

Adolescence is a sensitive period of physiological, social, and psychological development, encompassing changes in hormones and growth associated with puberty (Grumbach & Styne, 2003) and parent-child relationships (Steinberg & Silk, 2002), and increases in psychopathology symptoms (e.g., Zahn-Waxler, Shircliff, & Marceau, 2008). Evidence that multiple hormones (i.e., cortisol, testosterone, dehydroepiandrosterone [DHEA]) generally become less responsive to acute stressors during middle childhood (age 6–10 years; Shirtcliff & Ruttle, 2010) but become more responsive
again during adolescence (age 11–18 years, Dahl & Gunnar, 2009; Gunnar, Wewerka, Frenn, Long, & Griggs, 2009), and that hormones are associated with behavior problems (Susman, 2006) suggests that endocrine responsivity to various stressors, including conflict, frustration, and competition during adolescence may contribute to adolescent behavior problems. Developmental models of psychopathology emphasize interactions among multiple levels of influence, including endocrine functioning and family processes (Cicchetti & Toth, 2009), as salient for understanding risk and etiology. Thus, including explicit measures of the family context may strengthen models of hormone contributions to adolescent behavior problems.

Though hormone changes have long been hypothesized to impact behavior and behavior problems, surprisingly few systematic hormone-behavior associations have been found (e.g., Alink et al., 2008; Booth, Granger, Mazur, & Kivlighan, 2006), suggesting that salient contextual moderators may have been missed (Boyce & Ellis, 2005). Here, three understudied facets of hormone-behavior associations are addressed. First, suggestions that it is important to consider how the family context might influence the links between hormones and behavior problems (e.g., Dorn et al., 2009; Granger & Kivlighan, 2003) have been infrequently addressed. Here, we provide a theoretical rationale for why the family context would be expected to moderate hormone-behavior associations, and also test whether the family context moderates the associations of three different hormones with behavior problems. Second, studies of behavior problems have less often considered the shorter-term activational effects of testosterone and DHEA, or stress responsivity to environmental cues, than basal hormone levels. Therefore, we examine stress responsivity of three hormones, cortisol, testosterone, and DHEA, in the present study. Finally, although there is substantial theoretical rationale for examining multiple hormones simultaneously when examining hormone-behavior associations, this is relatively infrequently done in the literature (Marceau, Ruttle, Shirtcliff, Essex, & Susman, under review). Therefore, the present study addresses these gaps by examining cortisol, testosterone, and DHEA responsivity to mother-youth conflict simultaneously in relation to adolescent internalizing and externalizing problems, with consideration of the moderating role of parent-adolescent conflict on these associations.

**Stress and Pubertal Hormones and Behavior Problems**

There is a large literature on hormone-behavior associations in adolescence (Alink et al., 2008). This literature is notoriously mixed, with many contradictory and null findings. Here, we provide a selective review highlighting moderately consistent findings across the field.

**Cortisol.** Cortisol, the primary steroid hormone of the hypothalamic-pituitary-adrenal (HPA) axis and stress response system, has been a major focus of biological influences on behavior problems. Biosocial theories of how cortisol influences behavior suggest that youth exposed to
prolonged stressors may experience a “down-regulation” of cortisol, evidenced as blunted reactivity or low basal levels, and this down-regulation would predict psychopathology symptoms (e.g., Fries, Hesse, Hellhammer, & Hellhammer, 2005) such as externalizing problems, in part because youth with low basal cortisol or responsivity engage in externalizing or stimulation seeking behaviors in an attempt to increase arousal to more typical levels (Raine, 1996; Zuckerman, 1979; Koob & Le Moal, 2001). Conversely, although chronic stress experiences also are associated with internalizing problems, most studies have linked adolescents’ internalizing problems and associated psychopathology symptoms to having higher basal cortisol levels or hyper-responsivity to acute stressors (Guerry & Hastings, 2011).

There are some inconsistencies in the existing literature. The association between lower cortisol responsivity and externalizing behavior is, perhaps, least clear, and may change with age (Alink et al., 2008). Some studies have found that lower cortisol responsivity to laboratory stressors was associated with more externalizing problems (e.g., Snoek, van Goozen, Matthys, Buitelaar, & van Engeland 2004; van Goozen et al., 1998; van Goozen, Fairchild, Snoek, & Harold, 2007). However, other studies have linked increased cortisol responsivity with more externalizing problems (e.g., Granger, Weisz, & Kauneckis, 1994; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Sontag-Padilla et al., 2012; Susman et al., 2010). One source of this inconsistency might be the nature of the “stressors” used across studies (i.e., social performance, conflict); there might be particular stimuli or contexts to which youths with externalizing problems manifest greater adrenocortical responsivity. Further, many studies examine these associations in typically developing samples with few behavior problems, so investigating cortisol responsivity in a high-risk sample may help clarify these associations.

**Dehydroepiandrosterone (DHEA).** DHEA is an adrenal androgen closely linked with cortisol: DHEA is in part metabolized into cortisol (Brown et al., 2008), is co-released with cortisol from the adrenal gland during the HPA stress response (Sapolsky, 2003), stimulated in part by adrenocorticotropic hormone (ACTH; Wolf & Kirschbaum, 1999), and is responsive to a variety of laboratory stressors including venipuncture, MRI, parent-child conflict, and social performance tasks (e.g., Eatough, Shirtcliff, Hanson, & Pollak, 2009; Marceau, Dorn, & Susman, 2012; Shirtcliff et al., 2007). DHEA also plays an important permissive role in pubertal development, increasing in middle childhood precipitating adrenarche (Palmert et al., 2001). DHEA is sometimes thought of as a protective hormone because there is evidence that the simultaneous release of DHEA may be able to buffer against the deleterious effects of cortisol (Herbert, 1997). Lower basal DHEA and its sulfate, DHEA-S, have been associated with internalizing problems, and higher basal DHEA and DHEA-S have been associated with externalizing problems during adolescence (e.g., Brooks-Gunn & Warren,
Moreover, heightened stress responsivity of DHEA was associated with internalizing symptoms in girls in response to social tasks, including parent-child conflict (Shirtcliff et al., 2007), and with negative emotionality in boys in response to venipuncture (Marceau et al., 2012), raising the possibility that this “protective” hormone may not necessarily be protective in an acute stress context (Shirtcliff et al., 2007). It is yet unknown whether DHEA responsivity is associated with externalizing problems, though levels and responsivity of other adrenal androgens have been linked with externalizing problems (Susman et al., 1987; Susman, Nottelmann, Dorn, Inoff-Germain, & Chrousos, 1988; van Goozen et al., 1998).

Testosterone. Testosterone is a primary gonadal steroid hormone that increases during puberty in girls and especially in boys (Grumbach & Styne, 2003). The association between testosterone and aggression and antisocial behavior has been given much attention. Much of the literature on testosterone-aggression associations is built on the evolutionary challenge hypothesis, stating that during and after puberty, increases in testosterone to social challenges facilitate competition and aggression in boys and girls (Archer, 2006). Recently, experimental literature suggests that increases in testosterone may also diminish trust and empathy, and prosocial behaviors, leading to increases in antisocial behavior, callousness, aggression, and status seeking (Bos, Terberg, & Van Honk, in press; Johnson & Breedlove, in press).

Meta-analyses suggest that for boys and girls, there is a weak, positive association between basal testosterone and aggression behaviors (Archer, 1991; Book, Starzyk, & Quinsey, 2001) although like cortisol-externalizing associations, testosterone-aggression associations are mixed. Some studies find that basal testosterone is positively associated with externalizing problems (e.g., Dabbs, Jurkovic, & Frady, 1991; Granger et al., 2003), whereas other studies find no association between basal testosterone and psychopathology symptoms (e.g., Booth, Johnson, Granger, Crouter, & McHale, 2003; Brooks-Gunn & Warren, 1989; Susman et al., 1987).

The responsivity of testosterone to social challenges, especially during adolescence, has been associated with greater psychopathic tendencies in males (Yildirim & Derksen, 2012). Although testosterone administration studies have shown that increases in testosterone are associated with increases in aggression and externalizing-type problems even in adolescence (Yildirim & Derksen, 2012), and testosterone has been shown to be responsive to multiple types of stressors or challenges including venipuncture, MRI, exercise, and competition (e.g., Bateup, Booth, Shirtcliff, & Granger, 2002; Eatough et al., 2009; Elloumi, Maso, Michaux, Robert, & Lac, 2003; Kivlighan, Granger, & Booth, 2005; Kraemer et al., 2001; Marceau et al., 2012), associations between testosterone responses...
to laboratory stressors and behavior problems have not been demonstrated during adolescence. One study found that testosterone response to venipuncture was unrelated to negative emotionality during adolescence (Marceau et al., 2012). Thus, more research is needed to understand whether testosterone responsivity to common laboratory stressors is associated with adolescent behavior.

In all, the literature is mixed or sparse regarding the direction of the association between hormone responsivity to stressors and behavior problems during adolescence. As highlighted above, a few general, moderately consistent findings emerge. Increased cortisol responsivity appears to be associated with more internalizing problems. More tentatively, increased DHEA responsivity might be associated with more internalizing problems, whereas increased testosterone responsivity has often been associated with more externalizing problems. The literature was inconsistent regarding the presence and direction of associations between cortisol responsivity and externalizing problems, and the literature was too sparse to draw conclusions regarding the presence of associations between DHEA and testosterone responsivity with externalizing problems.

Inconsistencies in findings could be in part due to potential differences in which hormones respond to which types of stressors within individuals, and differences in sampling procedures across studies. HPA and hypothalamus-pituitary-gonadal (HPG) functioning have both been shown to be context dependent (e.g., Ellis, 2004; Gunnar & Quevedo, 2007). Therefore, the mixed findings for hormone-behavior associations potentially indicate the presence of contextual moderators of this association. Below, we focus on the family as a salient social context, potentially capable of moderating hormone-behavior relationships.

The Parent-Adolescent Relationship

The parent-child relationship typically changes as adolescents gain autonomy (Steinberg & Silk, 2002), and negative parenting and lower parent-child relationship quality is related to early behavior problems and more severe forms of psychopathology and substance use in adolescence (e.g., Hinshaw, 2002; Steinberg & Morris, 2001). The parent-child relationship appears to be an important contextual factor for hormone-behavior associations across multiple time scales, for example in regard to hormone levels across development and in response to stressors (Adam, 2012). First, a substantial body of research has demonstrated that the early rearing environment affects the development of multiple hormone systems (e.g., Susman & Pollak, in press) such that early experience (i.e. harsh parenting) predicts cortisol, DHEA, and testosterone dysregulation (see Del Guidice, Ellis, & Shirtcliff, 2011; Ellis, 2004; Shirtcliff & Ruttle, 2010 for reviews). Second, parent-adolescent conflict during specific interactions has been shown to elicit hormone responsivity (e.g., Klimes-Dougan et al., 2001; Shirtcliff et al., 2007), potentially because the child may view parental negativity directed toward the child as a stressor or as a social challenge. Third, lower parent-
adolescent relationship quality, broadly, has also been concurrently associated with higher levels of testosterone and adrenal androgens (e.g., Booth et al., 2003; Inoff-Germain et al., 1988). For boys but not girls, higher testosterone responses to venipuncture during adolescence predicted more family problems one year later, and DHEA responses were concurrently associated with negative emotionality, which subsequently predicted family problems (Marceau et al., 2012). Together, evidence suggests that the link between parent-child relationship quality and endocrine functioning is complex and likely transactional over time.

One theoretical framework that considers both family context and hormonal functioning is exemplified by emerging evolutionary developmental biosocial theories, which suggest that physiological profiles can be adaptive or maladaptive depending on whether the environmental context is supportive versus unsupportive, or predictable versus unpredictable (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011). Two strengths of evolutionary developmental biosocial models are their specific predictions about which physiological stress-response profiles lead to internalizing versus externalizing problems given different types of rearing environments, and their explicit consideration of concurrent moderation of hormone-behavior associations by environmental context (Del Giudice et al. 2011).

These theories have conceptualized biological influences as moderators of environmental influences on behavior, but it is just as likely that environmental contexts might also moderate the influence of biological factors on behavior (Sapolsky, 2004). For example, theories of gene-environment interaction posit that the environment may moderate the extent to which genetic predispositions influence behavior, such that in more restrictive environments (i.e., high parental monitoring) genetic influences play less of a role whereas in more open environments (i.e., little parental monitoring) genetic influences play a stronger role in adolescent behavior (e.g., Marceau & Neiderhiser, in press; Meyers & Dick, 2010).

Combining and extending both evolutionary developmental theories, which posit that hormonal influences in combination with environmental influences are important for behavioral development, with theories of gene-environment interaction, which posit that environmental influences can moderate biological influences on behavior, we hypothesize that the family context may moderate hormone-behavior associations. For example, cortisol responsivity in the context of a low-stress environment should be unrelated to behavior problems, but cortisol responsivity in the context of an unpredictable or adverse environment may lead to internalizing problems in girls, and externalizing problems in boys. Together, these theories suggest that the parent-adolescent relationship, conceptualized as a larger-scale contextual factor, moderates concurrent hormone-behavior associations (Belsky & Pluess, 2009; Del Giudice et al., 2011; Meyers & Dick, 2010).
Combined with studies demonstrating hormone responses to specific parent-adolescent conflicts, one could expect that hormone-behavior associations could be moderated by both proximal indicators of parent-adolescent relationship quality, such as the affective tone of discrete interactions, and by more distal or global indicators, such as the typical levels of rancor or disagreement in the home (e.g., Sturge-Apple, Davies, Cicchetti, & Manning, 2012).

**Family context as a moderator of hormone influences on behavior.** There have been a few recent studies demonstrating that parent-adolescent relationship quality can moderate the association between adolescents’ basal hormone levels and their behavioral and emotional adjustment (e.g., Booth et al., 2003; Dorn et al., 2009; Fang et al., 2009). Importantly, these have reported different patterns of findings for boys and girls. Booth and colleagues (2003) reported that associations between testosterone and adjustment only existed for youths from families with lower parent-adolescent relationship quality. For boys, higher levels of testosterone were associated with more risk taking behaviors and lower levels of testosterone were associated with more depressive symptoms, whereas for girls, lower levels of testosterone were associated with both more risk taking behaviors and more depressive symptoms (Booth et al., 2003). Similarly, under conditions of low family cohesion, higher testosterone has been associated with more delinquent behavior among boys, and lower levels of testosterone with more delinquent behavior among girls (Fang et al., 2009). Although these findings provocatively suggest that variations in family context might have contributed to past inconsistencies in the documented links between hormones and adjustment, it is important to note that comparable studies of adolescents’ hormonal responsivity to stressors have not been conducted. Thus, the present study will expand on this literature by examining whether the family context also moderates associations between hormone responsivity and behavior problems in girls and boys.

These studies also examined only one hormone, or used separate analyses to examine multiple hormones. Dual-axis theories highlight the importance of considering hormones of the HPA and HPG axes together because of similarities in production and regulation, and because of cross-talk such that hormones of each axis can influence the production and changes in the other (e.g., Romeo, 2005; Stratakis and Chrousos, 1995). For example, cortisol, DHEA, and testosterone are all controlled by the hypothalamus and the pituitary gland and undergo feedback loops to regulate production, and each originates from cholesterol and share several pro-hormones, including pregnenolone and progesterone (e.g., Grumbach, 2002; Romeo, 2005; see Marceau et al., under review). Thus, the present study extends this literature, and the majority of the literature on hormone-behavior associations during adolescence, by simultaneously examining all three hormones within the same models.
In the present study, we examined whether parental negativity moderated associations of responsivity of cortisol, testosterone, and DHEA to conflict with internalizing and externalizing problems in a sample of adolescents with a range of psychopathology symptoms. We examined intensity of conflict with mothers and fathers separately because of differences in the links between mothering versus fathering and adolescent behavior (e.g., Collins & Russell, 1991; Kawabata, Alink, van IJzendoorn, & Crick, 2011) and physiology (e.g., Essex et al., 2011). We also examined boys and girls separately, as is common in studies of hormones/puberty and behavior (e.g., Granger et al., 2003; Nottleman et al., 1987; Marceau, Ram, Houts, Grimm, & Susman, 2011; Susman et al., 1991), because of gender differences in rates of internalizing and externalizing behavior problems during adolescence (Zahn-Waxler et al., 2008), and differences in pubertal hormones in boys and girls. In boys testosterone is released in the gonads, whereas in girls testosterone is released (in much smaller quantities) primarily from the adrenal gland (Grumbach & Styne, 2003). Further, statistical controls may not adequately test for gender differences in hormone-behavior associations because of invariance in hormone levels across boys and girls, which can lead to misspecification of gender differences (Shirtcliff & Granger, 2001; Granger et al, 2003).

**Hypotheses.** We hypothesized that mother- and father-adolescent conflict/negativity would moderate the association between hormone responsivity to stress and internalizing and externalizing problems during adolescence, following the literature showing that family conflict moderated associations between testosterone levels and depressive symptoms, and testosterone, cortisol, and DHEA and externalizing problems (e.g., Booth et al., 2003; Dorn et al., 2009; Fang et al., 2009). Drawing from developmental evolutionary and other biosocial theories, we expect the predominant findings in the literature described above (low cortisol responsivity with more externalizing – especially for boys; high cortisol responsivity with more internalizing – especially for girls; high DHEA responsivity with more internalizing; high testosterone responsivity with more externalizing) to be stronger in the context of higher versus lower parental negativity.

**Methods**

**Participants and Procedures**

Our sample consists of a subset of 213 adolescents who provided saliva samples out of a total sample of 221 adolescents and their parent(s) who participated in a longitudinal investigation of the role of emotion in the development of behavior problems (Klimes-Dougan et al., 2001). Participants were recruited via announcements in newspapers and flyers in the Washington DC metropolitan area. Adolescents were oversampled for internalizing and externalizing problems. Approximately 1/3 of the adolescents were in the normal range of problems, 1/3 had sub-clinical problems, and 1/3 had
clinical problems. Participants were balanced during recruitment for approximately equal proportions of youth with internalizing, externalizing, and comorbid internalizing and externalizing problems among those with sub-clinical and clinical levels of psychopathology symptoms (Klimes-Dougan et al., 2001). Youth ranged in age from 11–16 years ($M = 13.7, SD = 1.5$ years), and were about 50% male ($N$ male = 106). Families were predominantly middle- to upper-middle class according to the Hollingshead Four-Factor Index (1975). Specifically, 4% of the sample were considered working class (Hollingshead scores between 20 and 29), 6% were lower middle class (scores between 30 and 39), 38% were middle class (scores between 40 and 54), and 52% were considered upper middle to upper class (scores between 55 and 66). Most mothers (63.9%) and fathers (77.8%) had completed at least a college degree, and the majority of the sample (70%) was Caucasian, with a larger proportion of African American (16.9%) families than families of other ethnicities (Hispanic, 2.1%, Asian American, 3.6%, and mixed race or other, 7.7%).

Families were visited in their homes, where parent-youth dyads filled out questionnaires and participated in a Conflict Discussion Paradigm (CDP), a six-minute task designed to elicit conflict. First, mothers and adolescents filled out an issues checklist (see measures section below). Then, examiners chose the topic rated by both the parent and adolescent as the most intense and frequently discussed. Later, participants were asked to discuss the identified problem and come up with a solution. The mother-youth CDP was completed first, and after working separately on questionnaires for about an hour (including the father-adolescent issues checklist), the father-youth CDP was completed for two-parent families in which the father agreed to participate ($N = 141$). Importantly, saliva samples collected in the home just prior to mother-youth CDP (i.e., baseline), and 20 minutes later, after the CDP was completed, were used in the current report. The first saliva collection occurred at approximately 4:10pm, on average ($SD = 40$ min), and the second at 5:00pm, on average ($SD = 41$ min; see Klimes-Dougan et al., 2001 for additional description). Saliva was not collected prior to or in response to the father-youth CDP, which occurred later in the visit.

**Missing data.** About 1/3 of families did not have participating fathers. Of families without participating fathers, 48 families were single-mother families (20% of the entire sample) and in 24 families (10% of the entire sample) fathers were present but chose not to participate in the study. Families with and without participating fathers did not differ for child ethnicity, mother, or father education, $t$’s(211–166) < 1.74, $p$’s > .05. Families with participating fathers were higher on SES than families without participating fathers, $t$(211) = -2.73, $p < .05$. Because of this difference, SES was included as an auxiliary variable in the imputation model, along with all other study variables, to reduce bias (e.g., Graham, 2009). Importantly, families where fathers did and did not participate did
not differ on youth behavior problems, hormone responses, or parent-child relationship variables,
\( t's(211) < 1.92, p's > .05. \)

Following recommendations by missing data theorists (e.g., Graham et al., 2007; Little, 1988; Rubin, 1987; Shafer & Graham, 2002), we imputed missing data (N = 40 imputations) including values for father reports in order to reduce bias, using SAS PROC MI under standard missing-at-random assumptions, resulting in an analysis sample of N = 106 for boys and 107 for girls. Our sample size and amount of missing data was well within parameters for optimal performance of multiple imputation (see Graham, 2009).

**Measures**

**Behavior problems.** Internalizing and externalizing behavior problems were measured using the parent and youth report internalizing and externalizing subscales on the Child Behavior Checklist/Youth Self Report (Achenbach, 1991). T-scores for mother, father, and youth-reported internalizing and externalizing problems are presented in Table 6.1. T-scores below 60 are considered to be in the normal range, T-scores between 60 and 63 are considered sub-clinical, and T-scores above 63 are considered to reach clinical levels of problems.

Principal component weights were used to create composite internalizing and externalizing scores using all three informants to generate a score where all three reporters converged on the same score. In order to obtain these composite scores to be used in hypothesis testing, two separate principal component analyses were conducted to create an internalizing problem score and an externalizing problem score. In the first model, mother, father, and youth reported internalizing problems were entered and a single factor was extracted. This factor explained 56.3% of the variance in internalizing problems, \( Eigenvalue = 1.69, \) with relatively equal loadings for mother (.77), father (.79) and youth reported (.68) internalizing problems. In the second model mother, father, and youth reported externalizing problems were entered and a single factor was extracted. This factor explained 62.8% of the variance in externalizing problems, \( Eigenvalue = 1.88, \) with slightly higher loadings for mother (.87) and father (.85) than youth reported (.64) externalizing problems.

**Hormone response to CDP.** Cortisol, testosterone, and DHEA were assayed from saliva in response to the mother-adolescent CDP. Saliva was collected in the home through expectorating approximately 5 ml of saliva into a test tube, and supervised by research assistants. Participants did not eat during the 30 minutes prior to each saliva collection. The saliva was stored in the tubes at -25°C. After being shipped overnight on dry ice to The Pennsylvania State University Behavioral Endocrinology Laboratory, saliva was stored at -86°C until assayed in duplicate. Measures of each were reliable; detailed information on assays for this sample can be found elsewhere (Granger et al., 2003; Klimes-Dougan et al., 2001; Shirtcliff et al., 2007). Youth with hormone levels over 2.5 SD of
the within-sex sample mean were winsorized to the 2.5 SD value, and then log-transformed. We created responsivity scores using a difference score measuring the change in each hormone from the baseline sample in the home prior to the CDP and the post-task sample, approximately 20 minutes after baseline, after the CDP was completed; therefore, higher scores indicate a greater hormone rise. Each hormone was responsive to the CDP in a subset of youth (See Table 6.1).

**Maternal negativity.** To assess a proximal measure of how conflict was associated with hormone responsivity to the CDP, maternal negativity was measured through observed maternal anger and hostility during the CDP. Trainee observers attended home visits with investigators and senior staff until they reached ICC ≥ .80 on affect ratings; thereafter, they were approved to conduct home visits in teams of two. Through real-time, live observations in the home, the two examiners independently rated the extent to which mothers displayed anger and hostility separately on 5-point Likert-like scales ranging from “not at all” to “very much” during the conflict discussion task. After the home visit, observers discussed the scores to resolve discrepancies, and the final, consensus ratings were used in all analyses. Scores for maternal anger and hostility were summed, resulting in a 10-point scale in which higher values indicate more observed maternal negativity ($r = .51$, $p < .05$). More than 55% of mothers showed negativity during the CDP. Thus, proximal maternal negativity was measured at the same time as the hormone changes were occurring. This proximal measure reflects negativity that could act as an immediate contextual stressor contributing to hormonal responses. We were unable to assess a parallel, proximal measure of paternal negativity because saliva was not collected from youth during the CDP with fathers. Therefore, only maternal negativity could be considered a proximal measure of how conflict was associated with hormone responsivity to the CDP.
Table 6.1
Sex Specific Descriptive Statistics for Study Variables

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th>Girls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection 1</td>
<td>25.97 (19.24)</td>
<td>11.86</td>
<td>5.37</td>
<td></td>
</tr>
<tr>
<td>Collection 2</td>
<td>24.47 (19.47)</td>
<td>10.10</td>
<td>5.07</td>
<td></td>
</tr>
<tr>
<td>Response^T (Δ 0-20 minutes)</td>
<td>-.07 (.20)</td>
<td>-.12</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td>No increase (n)</td>
<td>73 (69%)</td>
<td></td>
<td>81 (76%)</td>
<td></td>
</tr>
<tr>
<td>Increase (n)</td>
<td>33 (31%)</td>
<td></td>
<td>26 (24%)</td>
<td></td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection 1</td>
<td>.18 (.09)</td>
<td>.16</td>
<td>.07</td>
<td></td>
</tr>
<tr>
<td>Collection 2</td>
<td>.13 (.06)</td>
<td>.10</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>Response^T (Δ 0-20 minutes)</td>
<td>-.01 (.02)</td>
<td>-.01</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>No increase (n)</td>
<td>87 (82%)</td>
<td></td>
<td>96 (90%)</td>
<td></td>
</tr>
<tr>
<td>Increase (n)</td>
<td>19 (18%)</td>
<td></td>
<td>11 (10%)</td>
<td></td>
</tr>
<tr>
<td>DHEA (ng/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection 1</td>
<td>63.24 (26.54)</td>
<td>83.00</td>
<td>41.78</td>
<td></td>
</tr>
<tr>
<td>Collection 2</td>
<td>56.27 (24.38)</td>
<td>69.29</td>
<td>33.19</td>
<td></td>
</tr>
<tr>
<td>Response^T (Δ 0-20 minutes)</td>
<td>-.11 (.29)</td>
<td>-.16</td>
<td>.22</td>
<td></td>
</tr>
<tr>
<td>No increase (n)</td>
<td>73 (69%)</td>
<td></td>
<td>84 (78%)</td>
<td></td>
</tr>
<tr>
<td>Increase (n)</td>
<td>33 (31%)</td>
<td></td>
<td>23 (22%)</td>
<td></td>
</tr>
<tr>
<td>Observed Maternal Negativity</td>
<td>1.31 (1.51)</td>
<td></td>
<td>1.18 (1.32)</td>
<td></td>
</tr>
<tr>
<td>Mother Conflict Intensity</td>
<td>4.06 (1.12)</td>
<td></td>
<td>4.23 (1.78)</td>
<td></td>
</tr>
<tr>
<td>Father Conflict Intensity</td>
<td>4.41 (1.18)</td>
<td></td>
<td>4.56 (1.21)</td>
<td></td>
</tr>
<tr>
<td>Externalizing T-scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother report</td>
<td>52.71 (11.04)</td>
<td>52.05</td>
<td>(10.29)</td>
<td></td>
</tr>
<tr>
<td>Father report</td>
<td>51.23 (10.18)</td>
<td>51.37</td>
<td>(8.73)</td>
<td></td>
</tr>
<tr>
<td>Youth self report</td>
<td>51.56 (10.46)</td>
<td>53.24</td>
<td>(9.40)</td>
<td></td>
</tr>
<tr>
<td>Internalizing T-scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother report</td>
<td>52.34 (10.59)</td>
<td>52.18</td>
<td>(10.31)</td>
<td></td>
</tr>
<tr>
<td>Father report</td>
<td>51.23 (11.38)</td>
<td>49.67</td>
<td>(9.53)</td>
<td></td>
</tr>
<tr>
<td>Youth self report</td>
<td>49.04 (12.15)</td>
<td>52.26</td>
<td>(10.13)</td>
<td></td>
</tr>
<tr>
<td>Pubertal Status</td>
<td>3.58 (.93)</td>
<td></td>
<td>3.79 (1.06)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>13.65 (1.46)</td>
<td></td>
<td>13.66 (1.60)</td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Raw, windsorized hormone data is presented for each collection; hormone responses presented are change scores after log transformation.
**Parent-child conflict intensity.** As a more distal or global measure of parent-adolescent relationship quality for both mother-adolescent and father-adolescent dyads, the number and intensity of issues eliciting parent-adolescent conflict was measured using an issues checklist (see also Klimes-Dougan et al., 2001; Shirtcliff et al., 2007). Parents and adolescents separately rated whether (yes/no) they had argued about 20 items (i.e., bedtimes, money/allowance/jobs, problems at school, privacy, friends) in the past four weeks, and then rated the intensity of the conflicts on the topics they endorsed on a scale of 1 to 5. Parent-child conflict intensity was operationalized as the summed intensity level across items endorsed by mothers, fathers, and youth as something the dyad (i.e. mothers and adolescents; fathers and adolescents) had fought about (α’s > .80). Father-adolescent conflict intensity was operationalized as the average of father and adolescent reported summed intensity of conflict with fathers, and mother-adolescent conflict intensity was operationalized as the average of mother and adolescent reported summed intensity of conflict with mothers. Father and youth reports on father-adolescent conflict intensity were moderately correlated, \( r = .41, p < .05 \), as were mother and youth reported mother-adolescent conflict intensity, \( r = .44, p < .05 \).

**Physical development.** Youth reported their development using the Morris and Udry line drawings of Tanner stages (Marshall & Tanner 1969; 1970; Morris & Udry, 1980). The average of youth report of genital/breast and pubic hair development was taken to get one pubertal development score (see also Shirtcliff et al., 2007). For boys, genital and pubic hair ratings were highly correlated, \( r = .86, p < .05 \), \( M = 3.58, SD = .93 \). For girls, breast and pubic hair ratings were also highly correlated, \( r = .73, p < .05 \), \( M = 3.79, SD = 1.09 \). Tanner stages were used as control variables in this study.

**Analytic Strategy**

Sample descriptive statistics for all study variables are presented in Table 6.1. Hypotheses were tested using four sets of two hierarchical linear regressions; one set each for the prediction of boys’ internalizing problems, girls’ internalizing problems, boys’ externalizing problems, and girls’ externalizing problems. In every model, baseline hormone levels, operationalized as the log-transformed score at the first (pre-task) saliva sample, were entered as controls to ensure that effects were driven by within-person hormone responsivity and not shared variance with overall hormone levels. Age, puberty and SES were also controlled. Finally, externalizing problems were controlled in models predicting internalizing problems, and internalizing problems were controlled in models predicting externalizing problems to establish specificity of findings for each problem type because a substantial portion of our sample was comorbid for internalizing and externalizing problems.
For each outcome, two separate models were tested: one testing the moderating effect of proximal maternal negativity on hormone response-behavior associations, and one testing the moderating effect of global mother and father conflict intensity on hormone response-behavior associations. Thus, in the models testing the effect of proximal maternal negativity there were four main effects - one for each measure of hormone responses and observed maternal negativity, and three interactions - between each hormone response and observed maternal negativity predicting the four outcomes. In the models testing the effect of global mother and father conflict intensity, there were five main effects – the three hormone responses, mother conflict intensity and father conflict intensity, and six interactions - between each hormone response with mother conflict intensity and each hormone response with father conflict intensity predicting each outcome. Only two-way interactions between measures of hormone responsivity and family context were included, as these were the effects central to hypotheses.

Significant interactions were explored using Johnson-Neyman significance regions (Hayes & Matthes, 2009; Johnson & Neyman, 1936). This method tests the significance of the model-based slope of a predictor (i.e., hormone responsivity) at every level of the moderator (i.e., parenting), accounting for other predictors in the model. Thus, we determined empirically at which level of the moderator the focal variable had a significant effect on the outcome.

Results

Correlations among control and study variables. Before hypothesis testing, we examined associations among targeted study variables, and between the study variables and the control variables pubertal maturation, age, and baseline hormone levels (see Table 6.2). For control variables, pubertal status and age were associated with observed maternal negativity during the CDP for boys, but not girls. Age was associated with externalizing problems for boys, but not girls. Socio-economic status was not associated with any of the study variables. Baseline hormones were negatively or non-significantly associated with hormone responses in boys and girls, except for boys’ testosterone. Observed maternal negativity was associated with baseline cortisol and testosterone for boys only. Baseline hormone levels were not associated with behavior problems for boys or girls.

Correlations among study variables. Responsivity of cortisol with testosterone, responsivity of cortisol with DHEA, and responsivity of testosterone with DHEA were all positively associated for boys and girls (see Table 6.2). Maternal conflict intensity was associated with observed maternal negativity and father conflict intensity for boys and girls. Observed maternal negativity was associated with increased testosterone responsivity in boys and girls, and increased DHEA responsivity in girls. For boys only, testosterone and DHEA responsivity were negatively associated with paternal conflict intensity. Hormone responses were not directly associated with internalizing or
externalizing behavior problems. Each measure of maternal negativity and parental conflict intensity was significantly and positively associated with both externalizing and internalizing problems for both boys and girls, with the exception of the non-significant association between paternal conflict intensity and internalizing problems for girls.
Table 6.2
Correlations among Study Variables

<table>
<thead>
<tr>
<th>Hormone Response to Stress</th>
<th>Parental Negativity</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR</td>
<td>TR</td>
</tr>
<tr>
<td>Cortisol responsivity (CR)</td>
<td></td>
<td>.25*</td>
</tr>
<tr>
<td>Testosterone responsivity (TR)</td>
<td>.23*</td>
<td>.44*</td>
</tr>
<tr>
<td>DHEA responsivity (DR)</td>
<td>.46*</td>
<td>.40*</td>
</tr>
<tr>
<td>Observed Maternal Negativity</td>
<td></td>
<td>-0.17</td>
</tr>
<tr>
<td>Mother Conflict Intensity</td>
<td>-.002</td>
<td>-.16</td>
</tr>
<tr>
<td>Father Conflict Intensity</td>
<td></td>
<td>.04</td>
</tr>
<tr>
<td>Externalizing (Ext)</td>
<td></td>
<td>-0.06</td>
</tr>
<tr>
<td>Internalizing (Int)</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Pubertal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>-.18+</td>
<td>.06</td>
</tr>
<tr>
<td>Girls</td>
<td>.07</td>
<td>-.04</td>
</tr>
<tr>
<td>Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>-.18+</td>
<td>.05</td>
</tr>
<tr>
<td>Girls</td>
<td>.03</td>
<td>-.02</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>-.11</td>
<td>.17+</td>
</tr>
<tr>
<td>Girls</td>
<td>-.11</td>
<td>-.01</td>
</tr>
<tr>
<td>SES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>-.73*</td>
<td>-.13</td>
</tr>
<tr>
<td>Girls</td>
<td>-.68*</td>
<td>-.11</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>-.15</td>
<td>.03</td>
</tr>
<tr>
<td>Girls</td>
<td>.18+</td>
<td>-.25*</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>-.27*</td>
<td>.04</td>
</tr>
<tr>
<td>Girls</td>
<td>-.11</td>
<td>-.12</td>
</tr>
</tbody>
</table>

Note. For study variables (top), girls are presented above the diagonal, boys are presented below the diagonal. * p < .05; + p < .10.
Predictions from Observed Maternal Negativity and Hormone Responses

The four regression models predicting boys’ and girls’ internalizing and externalizing problems from observed maternal negativity and hormone responses to the CDP all accounted for a significant portion of the variance in behavior problems (see Table 6.3). There were no significant direct associations between either observed maternal negativity or any of the three hormone responses and adolescents’ internalizing or externalizing problems. However, in the model predicting girls’ externalizing problems, the final step of the model approached significance, $F_{\text{Change}}(3, 92) = 1.76$, $p = .16$, $R^2_{\text{Change}} = .03$, which was driven by two borderline-significant interactions ($p < .10$). Observed maternal negativity tended to moderate the associations between both cortisol and DHEA responses and girls’ externalizing problems.

Although these effects were only observed at trend-level, we proceeded to probe the interactions. There was no conditional effect of observed maternal negativity on the association between cortisol responses and girls’ externalizing problems. Then, we probed the opposite direction, and found that there was no conditional effect of cortisol responsivity on the association between observed maternal negativity and girls’ externalizing problems. Thus we conclude that this was a null effect. For the interaction between DHEA responsivity and observed maternal negativity in relation to girls’ externalizing problems, there was a conditional effect of cortisol responsivity on the association between observed maternal negativity and girls’ externalizing problems. The raw data and simple slopes at +/- 1 standard deviation of the mean on cortisol responsivity are presented in Figure 6.1. Among girls with average and blunted cortisol responses to conflict (53% of the sample), maternal negativity during the CDP was associated with girls’ externalizing problems, however, among girls with higher cortisol responsivity (47% of the sample), maternal negativity during the CDP was unrelated to girls’ externalizing problems. There was no conditional effect of observed maternal negativity on the association between cortisol responsivity and girls’ externalizing problems.

Predictions from Parental Conflict Intensity and Hormone Responses

The four regression models predicting boys’ and girls’ internalizing and externalizing problems from parental conflict and hormone responses to the CDP all accounted for a significant portion of the variance in behavior problems (see Table 6.4). There were significant direct associations and interaction effects in one model: The prediction of boys’ externalizing problems. Boys had more externalizing problems when they experienced more intense conflicts with their mothers, and maternal conflict intensity also tended to moderate the association between DHEA responses and boys’ externalizing problems. In addition, paternal conflict significantly moderated the associations between both cortisol and DHEA responses and boys’ externalizing problems (see Table 6.4).
Table 6.3

Parameter Estimates for Models including Proximal, Observed Maternal Negativity and Hormone Responses

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intercept</td>
<td>.45*</td>
<td>.46*</td>
<td>.42*</td>
<td>.42*</td>
<td>.42*</td>
<td>.42*</td>
<td>.42*</td>
<td>.42*</td>
</tr>
<tr>
<td></td>
<td>Basal Cortisol</td>
<td>.02</td>
<td>.08</td>
<td>.04</td>
<td>.01</td>
<td>-12.41+</td>
<td>3.46</td>
<td>-6.34</td>
<td>-2.59</td>
</tr>
<tr>
<td></td>
<td>Basal Testosterone</td>
<td>.04</td>
<td>-0.04</td>
<td>-0.05</td>
<td>.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal DHEA</td>
<td>.02</td>
<td>.18</td>
<td>.10</td>
<td>-3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Puberty</td>
<td>.11</td>
<td>-0.13</td>
<td>-0.09</td>
<td>.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-.14+</td>
<td>.18*</td>
<td>.04</td>
<td>-.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SES</td>
<td>.12</td>
<td>-.09</td>
<td>-.12</td>
<td>-.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-target Problem</td>
<td>.61*</td>
<td>.65*</td>
<td>.61*</td>
<td>.55*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cortisol Response</td>
<td>.47</td>
<td>.47</td>
<td>.43</td>
<td>.44</td>
<td>-4.90</td>
<td>-.08</td>
<td>-10.90</td>
<td>.96</td>
</tr>
<tr>
<td></td>
<td>Testos Response</td>
<td>.15</td>
<td>-.38</td>
<td>.17</td>
<td>-.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DHEA Response</td>
<td>-.21</td>
<td>-.27</td>
<td>-.17</td>
<td>-.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal Negativity</td>
<td>.07</td>
<td>.10</td>
<td>.00</td>
<td>.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Maternal Negativity</td>
<td>.47</td>
<td>.49</td>
<td>.44</td>
<td>.47</td>
<td>1.40</td>
<td>.10</td>
<td>1.81</td>
<td>-11.75+</td>
</tr>
<tr>
<td></td>
<td>X Cortisol Response</td>
<td>1.40</td>
<td>.10</td>
<td>1.81</td>
<td>-11.75+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X Testos Response</td>
<td>.04</td>
<td>-.38</td>
<td>-.58</td>
<td>-.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X DHEA Response</td>
<td>.11</td>
<td>-.13</td>
<td>.29</td>
<td>.48+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model fit:

Adjusted $R^2 = .39* | .42* | .35* | .39*  
F(14,105/106) = 5.81*| 6.35*| 5.10*| 5.77*  

Note. Unstandardized beta-weights from the full model are presented.* p < .05. + p < .10
Figure 6.1. Cortisol Responsivity Moderates Maternal Negativity-Externalizing Association for Girls. On the left, asterisks depict individual adolescents with cortisol responses lower than the threshold for the model-based significance region (53% of the sample). The long-dashed line depicts the beta-weight for the association between maternal negativity during the CDP and girls’ externalizing problems at -1 standard deviation of the sample mean for DHEA responsivity (a significant positive slope). On the right, dots depict individual adolescents with DHEA responses higher than the threshold for the model-based significance region (47% of the sample). The solid line depicts the beta-weight for the association between maternal negativity during the CDP and boys’ externalizing problems at 1 standard deviation above the sample mean for DHEA responsivity (a non-significant slope).
We first probed the interaction between father-son conflict intensity and cortisol responsivity, and found a conditional effect of father-son conflict intensity on the association between cortisol responsivity and boys’ externalizing problems. The raw data and simple slopes at +/- 1 standard deviation of the mean and the mean on father-son conflict intensity are presented in Figure 6.2. Model-based significance tests revealed that for boys with the least intense conflicts with fathers (12% of the sample), higher cortisol responsivity was associated with more externalizing problems, however for boys with the most intense conflict with fathers, (17% of the sample), cortisol responsivity was associated with blunted cortisol responsivity was associated with more externalizing problems. For the majority of boys (73% of the sample), cortisol responsivity was unrelated to externalizing problems. The pattern of effects was somewhat different for DHEA, though we also found a conditional effect of father-son conflict intensity on the association between DHEA responsivity and boys’ externalizing problems. The raw data and simple slopes at +/- 1 standard deviation of the mean on father-son conflict intensity are presented in Figure 6.3. For boys with average and below average levels of father-son conflict intensity (81% of the sample), DHEA responsivity was unrelated to externalizing problems, however, for boys with the most intense conflicts with fathers (19% of the sample), higher DHEA responsivity predicted more externalizing problems.

Finally, we probed the trend-level interaction of mother-son conflict intensity and DHEA responsivity in relation to boys’ externalizing problems. As with the trend-level finding for cortisol responsivity and observed negativity for girls, there was a conditional effect of DHEA responsivity on the association between mother-son conflict intensity and boys’ externalizing problems but not a conditional effect of mother-son conflict intensity on the association between DHEA responsivity and boys’ externalizing problems as hypothesized. The raw data and simple slopes at +/- 1 standard deviation of the mean on DHEA responsivity are presented in Figure 6.3. Among boys with blunted DHEA responsivity (36% of the sample), mother-son conflict intensity was associated with more externalizing problems, whereas among boys with average and higher DHEA responsivity (64% of the sample), mother-son conflict intensity was unrelated to boys’ externalizing problems.
Figure 6.2. Father-Son Conflict Moderates Cortisol Responsivity-Externalizing Association for Boys. On the left, open circles depict individual children with father-son conflict intensity levels higher than the threshold for the model-based significance region (12% of the sample). The short-dashed line depicts the beta-weight for the association between cortisol responsivity and boys’ externalizing problems at -1 standard deviation of the sample mean for father-son conflict intensity (a significant negative slope). In the center, asterisks depict individual children with father-son conflict intensity levels in the center of the distribution, which the model-based tests determined was a non-significant region (73% of the sample). The long-dashed line depicts the beta-weight for the association between cortisol responsivity and boys’ externalizing problems at the sample mean for father-son conflict intensity (a non-significant slope). Finally, on the right, dots depict individual children with father-son conflict intensity levels lower than the threshold for the second model-based significance region (17% of the sample). The solid line depicts the beta-weight for the association between cortisol responsivity and boys’ externalizing problems at +1 standard deviation of the sample mean for father-son conflict intensity (a significant positive slope).
### Table 6.4

**Parameter Estimates for Models including Global Mother-Adolescent and Father-Adolescent Conflict Intensity and Hormone Responses**

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IP R²</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Intercept</td>
<td>.45*</td>
<td>.46*</td>
</tr>
<tr>
<td></td>
<td>Basal Cortisol</td>
<td>-.15.75*</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>Basal Testosterone</td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal DHEA</td>
<td>.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Puberty</td>
<td>.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>-.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SES</td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-target Problem</td>
<td>.57*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cortisol Response</td>
<td>-.10.24</td>
<td>.54*</td>
</tr>
<tr>
<td></td>
<td>Testos Response</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DHEA Response</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal Conflict</td>
<td>.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paternal Conflict</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Maternal Conflict</td>
<td></td>
<td>.51</td>
</tr>
<tr>
<td></td>
<td>X Cortisol Response</td>
<td>2.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X Testos Response</td>
<td>-.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X DHEA Response</td>
<td>-.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paternal Conflict</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X Cortisol Response</td>
<td>-.100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X Testos Response</td>
<td>-.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X DHEA Response</td>
<td>-.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model fit:</td>
<td>Adjusted R²=</td>
<td>.41*</td>
</tr>
<tr>
<td></td>
<td>F(14,105/106)=</td>
<td>5.07*</td>
<td></td>
</tr>
</tbody>
</table>

Note. Unstandardized beta-weights from the full model are presented. * p < .05. + p < .10
Figure 6.3. Father-Son Conflict Moderates DHEA Responsivity-Externalizing Association for Boys. On the left, asterisks depict individual adolescents with father-son conflict intensity lower than the threshold for the model-based significance region (81% of the sample). The long-dashed line depicts the beta-weight for the association between DHEA responsivity and boys’ externalizing problems at -1 standard deviation of the sample mean for father-son conflict intensity (a non-significant slope). On the right, dots depict individual adolescents with father-son conflict intensity levels higher than the threshold for the model-based significance region (19% of the sample). The solid line depicts the beta-weight for the association between cortisol responsivity and boys’ externalizing problems at 1 standard deviation above the sample mean for father-son conflict intensity (a significant positive slope).
Figure 6.4. DHEA Responsivity Moderates Mother-Son Conflict Intensity-Externalizing Association. On the left, asterisks depict individual adolescents with DHEA responses lower than the threshold for the model-based significance region (36% of the sample). The long-dashed line depicts the beta-weight for the association between mother-son conflict intensity and boys’ externalizing problems at -1 standard deviation of the sample mean for DHEA responsivity (a significant positive slope). On the right, dots depict individual adolescents with DHEA responses higher than the threshold for the model-based significance region (64% of the sample). The solid line depicts the beta-weight for the association between mother-son conflict intensity and boys’ externalizing problems at 1 standard deviation above the sample mean for DHEA responsivity (a non-significant slope).
Discussion

In the present study, we examined associations between responsivity of stress and pubertal hormones to a mother-child conflict paradigm and internalizing and externalizing problems in boys and girls, and tested the moderating role of proximal observed maternal negativity during the conflict paradigm and more global measures of mother-adolescent and father-adolescent conflict intensity for these associations. Findings illustrated that cortisol, testosterone, and DHEA responsivity to mother-child conflict were not directly related to adolescent internalizing or externalizing problems. However, associations between hormone responsivity and boys’ externalizing problems differed in the context of high and low global conflict intensity with fathers.

There were also trend-level findings suggesting that DHEA responsivity may moderate associations between global conflict intensity with mothers and boys’ externalizing problems, and that cortisol responsivity may moderate associations between observed maternal negativity and girls’ externalizing problems. There are two tentative but provocative implications of these findings. The first stems from the tendency for the biobehavioral adjustment of male versus female adolescents to be more closely linked, respectively, to distal or global versus proximal or specific cues of adversity in the family environment. The second is that hypotheses drawn from emerging evolutionary theories (i.e., that hormone responsivity moderates context-behavior associations instead of the family context as the moderator as we focus on here) may potentially be more applicable to mother-youth relationships than father-youth relationships. However, as these are drawn in part from trend-level findings, and because gender differences were not explicitly tested in the current study, we present them only as potential hypotheses to be tested in future research, and focus on implications of the significant findings in this discussion.

The Parent-Adolescent Relationship

Although the present moderation findings give some preliminary evidence of differential effects of mothering vs. fathering on boys’ behavior (e.g., Rothbaum & Weisz, 1994), associations between parenting and child behavior showed little evidence of gendered dyad specificity. Consistent with past research (e.g., Baumrind, 1991; Steinberg & Morris, 2001), each parenting measure was associated with internalizing and externalizing problems for boys, and externalizing problems for girls (with the exception that proximal maternal negativity was not associated with girls’ internalizing problems). Thus, in this sample we found no evidence of dyadic sex differences in associations between parenting and internalizing and externalizing problems, even without explicitly testing for sex differences.

Associations between parenting measures and hormone responses suggest contextual differences in perceptions of conflict. Especially considering the generally low levels of “responders”,
it could be that the CDP task didn’t elicit stress per-se but instead elicited other emotional responses resulting in the individual differences in hormone changes observed in this study. Observed maternal negativity was associated with increased testosterone responsivity in boys and girls, and with increased testosterone and DHEA responsivity in girls. Therefore some adolescents may view the CDP as a social challenge and respond to maternal negativity with increases in testosterone, as opposed to with increasing cortisol expected if it were operating as a stressor. The CDP has also been described as a frustration task (e.g., Granger et al., 1994), so a subset of adolescents may have viewed the CDP as frustrating and initiated a physiological response to deal with frustration rather than to cope with social challenge or conflict adrenal androgens such as DHEA may be especially suited for challenges involving frustration or provocation (e.g., Azurmendi et al., 2006; Golubchik, Mozes, Maayan, & Weizman, 2009).

Further, higher global father-son conflict intensity was associated with decreased testosterone and DHEA responsivity to the CDP in boys. Given findings suggesting that fathers engage in more disciplinary and punitive types of parenting behaviors than mothers (e.g., Marsiglio et al., 2000), the intensity of father-son conflict, globally, may be particularly salient for how boys perceive other conflicts (e.g., the CDP), and therefore may affect how boys encode environmental cues via hormone responses. Although few other papers have examined mothers and fathers separately, those that have often find opposite effects for mothers vs. fathers on children’s hormone-behavior associations. Generally, findings suggest that fathers affect youth in a more global manner as part of the context that youth may more easily avoid directly dealing with on a day-to-day basis, but mother-child relationships are closer and buffer youth from other conflict (Ellis & Essex, 2007; Essex et al., 2011). Therefore, disruptions in the mother-youth relationship may have a greater impact on children’s immediate hormone functioning, but disruptions in the father-youth relationship may have a greater impact on general hormone functioning. Alternatively, as evidenced by animal and human research on dominance and testosterone, losing conflicts leads to testosterone suppression in males (Archer, 2006). Therefore, boys in highly conflicted father-son relationships may often lose conflicts or feel oppressed or subordinate to their fathers and have weaker testosterone responses to conflict, even with mothers, than do dominant boys.

**Family context as a moderator of hormone influences on behavior.** In addition to these associations, we found some evidence that the conflict intensity with fathers can moderate associations of the responsivity stress hormones and puberty-related hormones on behavior problems for boys. Overall, findings pointed to family context as a moderator of hormone responses predicting externalizing problems in particular (vs. internalizing problems).
The global measures of father-son conflict intensity moderated associations between cortisol and DHEA hormone responsivity and externalizing problems, controlling for internalizing problems. Notably, these effects were present in models of internalizing problems not controlling on externalizing problems (data available on request) but disappeared when controlling for externalizing, so effects in internalizing models were driven by the high degree of comorbid externalizing problems in this sample, highlighting the specificity of results to externalizing problems.

Low cortisol responsivity predicted externalizing problems in the context of high father conflict intensity, whereas high cortisol responsivity predicted externalizing problems in the context of low father conflict intensity. A different pattern was found for DHEA. High DHEA responsivity predicted externalizing problems in the context of high father conflict intensity, but among boys with average and below-average levels of father conflict intensity, there was no association between DHEA responsivity and externalizing problems. Thus, different HPA response-externalizing associations appear to be particularly context dependent for boys, although we did not specifically test of sex differences and therefore cannot conclude that these patterns differed for boys and girls. Nonetheless, different HPA response-externalizing associations depending on family context may help to explain the inconsistent direction of effects for these particular associations in the literature, though these findings need to be replicated before strong conclusions are drawn. It will be important for future studies to consider both cortisol and DHEA release in conjunction with one another to investigate when and why children release one adrenal hormone over another.

According to the evolutionary biosocial theories, high physiological responsivity to stress indicates that the individual is “open” to experience, actively engaging social cues from the environment, whereas low stress responsive physiological activity suggests the individual is filtering out their current context (e.g., Del Giudice et al., 2011). Although the analyses were not meant to test the evolutionary biosocial theories, findings are reminiscent of these theories in that they suggest that low cortisol/high DHEA is problematic in one context but potentially adaptive in another. We found that, high father-son conflict intensity reveals the low cortisol-externalizing problem association sometimes demonstrated in the literature, consistent with expectations based on down-regulation, fearlessness, or stimulation seeking theories (Fries et al., 2005; Raine, 1996; van Goozen et al., 2007). Therefore, in the context of father-son relationships, low cortisol responsivity (not high) appears to be the salient physiological profile for externalizing problems. This extension of evolutionary biosocial theories and principles of gene-environment interaction suggests that various physiological profiles (not only high cortisol responsivity) are more salient in different contexts, may also apply to the other HPA hormone, DHEA, which is co-released with cortisol in the body (Wolf & Kirschbaum, 1999).
The effects of cortisol and DHEA may be opposite within the context of high father-son conflict intensity because of protective, oppositional effects of DHEA to cortisol (e.g. Brown et al., 2008).

**Stress and Pubertal Hormones and Behavior Problems**

Consistent with recent biosocial models (e.g., Raine, 1996; Susman, 2006), we found little evidence of direct associations between hormone responsivity to mother-child conflict and adolescent behavior problems, a common conclusion in the literature. Notably, direct hormone-behavior associations have been found in this sample when assessing different types of hormone changes. Diurnal changes in cortisol and DHEA were associated with internalizing problems (Klimes-Dougan et al., 2001; Shirtcliff et al., 2007) and diurnal changes in testosterone were associated with internalizing and externalizing problems (Granger et al., 2003).

Further, responsivity of cortisol, when assessed via response patterns across 0–20 and 20–40 minutes, and responsivity of DHEA from 0–40 minutes, after the conflict discussion as well as a social performance paradigm were sometimes associated with internalizing problems in boys and girls in this sample (Klimes-Dougan et al., 2001; Shirtcliff et al., 2007), as opposed to the change values from 0–20 minutes assessed here. Thus, differences in measurement of hormone changes impact results and contribute to inconsistent findings in the literature. Change from 0–20 minutes was used here because of its simple interpretation, common use in the literature, and ease of comparability to past and future studies (see Marceau et al. 2012), although it is certainly not the gold-standard measure for reactivity because of the infrequency of saliva samples. Despite failing to replicate direct associations found using other methods, we found new evidence of different mechanisms by which parenting can moderate hormone response-behavior associations depending on which hormone is examined, and for proximal vs. more global measures of negativity/conflict intensity, which differed by parent and child gender.

**Limitations and Future Directions**

There are several limitations of the present study that are important to consider when interpreting results. First, we were only able to assess a proximal measure of maternal negativity, not paternal negativity, because saliva samples were only available in response to the mother-adolescent CDP. Therefore, we cannot generalize to hormone changes in response to father-adolescent conflict. Given the potentially gender-specific findings, understanding hormone changes in response to conflict with mothers and with fathers will be important for future research. Second, because consensus ratings were used while in the home, the reliability of the measure is unknown. This is a serious limitation, although the fact that the same interviewers achieved consensus on every CDP suggests that this is an adequate measure. Nonetheless, we believe that the contrast between proximal and global indexes of family context is conceptually meaningful. Despite our imperfect measurement,
we showed hints for an interesting distinction between proximal and global measures of negativity and conflict. It is our hope that this will provide a platform for future research using data better suited to the question.

There were several limitations in regard to the hormone assessment. The majority of youth did not respond to the CDP with hormone increases. Mild laboratory stressors have been shown to maximize individual differences such that for most tasks, hormone increases are observed within about 50% of individuals (Pruessner et al., 2010). We observed increases in only 23–31% of adolescents for DHEA and testosterone, and 10–18% of adolescents for cortisol from 0-20 minutes. More frequent assessments (i.e., every 10 minutes) would afford a better measure of hormone responsivity, and would also allow us to adequately assess recovery, an important component of the stress response which we were unable to focus on in the current study. Further, there may have been an anticipatory effect due to the researchers coming into the home, which we could not capture in the current study, which may have served to reduce variability of any hormone responses to the task. We were unfortunately unable to ascertain exactly how much time passed from when researchers arrived in the home to the baseline saliva sample. Similarly, we cannot tell whether hormone responses to the CDP reflect responses to stress, frustration, competition, or whether hormone responses to conflict represent a unique source of hormone fluctuation.

All constructs were measured at approximately the same time, so our findings reflect concurrent associations. Although we examined behavior problems as the outcome, it may actually be a contextual factor for endocrine functioning, and exert an influence on parental negativity. For example, recent longitudinal studies suggest that early externalizing problems predict later lower morning cortisol, blunted diurnal patterns, and reduced responsivity to stressors (e.g., Haltigan, Roisman, Susman, Barnett-Walker, & Monahan, 2011; Ruttle et al., 2011). Certainly, there are bidirectional influences, and we merely capture a snapshot of a very complex process.

Although patterns of hormone-behavior associations illustrate opposite findings for cortisol and DHEA with externalizing behavior for boys, responsivity of testosterone, cortisol, and DHEA to the CDP were all positively associated with each other in boys and girls, consistent with recent evidence examining a different stressor (venipuncture) in another adolescent sample (Marceau et al., 2012) as well as recent findings on basal hormones in adolescents (Matchock et al., 2007; Popma et al., 2007; Susman et al., 1987). These positive associations support the hypotheses that adolescence may be a sensitive period for organizational and activational effects of HPA and HPG axis hormones (e.g. Buchanan et al., 1992) as such positive associations are not typically observed in adults (e.g., Booth et al., 2006; Viau, 2002). Though it was not a primary focus of the present study, we did test for interactions between cortisol, DHEA, and testosterone responses to the CDP and behavior
problems and the measures parenting as moderators of hormone interactions, but these analyses yielded no significant effects (data available on request). It may be that a nuanced, within-person approach to examining correlated changes in HPA and HPG axis responsivity would better capture HPA-HPG cross talk and the potential influence of HPA-HPG cross-talk on behavior than between-person interactions of change scores for each hormone. Examining correlated changes in HPA and HPG functioning across development, especially incorporating the moderating role of the family context may advance our understanding of behavior problems in adolescence (Cicchetti, 2008).

In all, findings from the current study showing positive associations between activational effects of HPA and HPG axis hormones, and the moderating role of father-son conflict intensity for associations between HPA hormone responsivity to conflict open the door to many exciting directions in need of research. Particularly exciting is the observation that the context of parent-child relationships is still salient for adolescents (Steinberg, 2000), and furthermore, that the context of father-child and mother-child associations may exert different influences on the biobehavioral development of youth. Emerging theoretical perspectives like the ACM emphasize that it is impossible to understand the influence of the environment on mental health in youth without an appreciation of their biological sensitivity to context. The present study underscores this from a different angle. We illustrate here that the hormone-behavior links are also often tenuous unless the context of those hormonal changes are taken into account. Thus, we show that biological sensitivity to context very likely has divergent implications if that context is characterized by low or high maternal negativity or conflict intensity. Furthermore, by examining three different biological measures, we illustrate that these contextual forces are more or less salient for responsivity of one hormone than another, displaying not only different thresholds for activation (e.g., Obradovic, Bush, Stamperdahl, Alder, & Boyce, 2010), but also being attuned to different components of the environment.
CHAPTER 7: COMBINED INFLUENCES OF GENES, PRENATAL ENVIRONMENT, CORTISOL, AND PARENTING ON THE DEVELOPMENT OF CHILDREN’S INTERNALIZING VS. EXTERNALIZING PROBLEMS.

In the first two studies I’ve focused on gene-environment interplay and hormone-environment interplay during adolescence. However, behavior problems rarely begin in adolescence, and among the best predictors of adolescent problems are behavior problems during childhood. Further, many studies have found evidence of genetic, hormone, and environmental influences on behavior problems in childhood (see Chapters 3). In the first paper of this dissertation, I showed that evocative gene-environment correlation is important for explaining associations between parenting and adolescent externalizing behavior. In the second paper of this dissertation, I showed that family context was important for understanding how hormones are associated with behavior. However, a major tenant of the proposed conceptual model not addressed in these two papers is whether hormone function may act as mechanisms of genetic and/or environmental influences on behavior, or whether each of these factors represent separate influences on behavioral development. Thus, in the final paper of this dissertation, I integrate behavioral genetic and endocrinology approaches to test whether a single hormone – cortisol – may transmit genetic or environmental influences.

Citation

Abstract
Research has shown that genetic, prenatal, cortisol, and parenting environmental influences across development individually contribute to internalizing and externalizing problems in children. The present study tests the contributions of genetic, prenatal drug use and internalizing symptoms, cortisol at age 4.5 years, and over-reactive parenting influences across childhood together on 6-year-old children’s internalizing and externalizing problems using an adoption design using 351 families from a longitudinal US domestic adoption sample. Model fitting results suggest genetic and environmental influences each contributed uniquely to externalizing problems, but that prenatal risk and cortisol did not mediate genetic or parenting influences. However, genetic and parenting influences had indirect effect on internalizing problems through increased prenatal risk and
subsequent morning cortisol, as well as exerting unique main effects. Results suggest that prenatal and physiological influences are mechanisms of genetic and environmental influences on internalizing, but not externalizing problems in childhood.

**Introduction**

Biosocial approaches suggest that hypothalamic-pituitary-adrenal (HPA) axis functioning may be a mechanism transmitting genetic, prenatal, and parenting environmental influences on the development of behavior problems (e.g., Belsky & Pluess, 2009; DelGiudice, Ellis, & Shirtcliff, 2011). The HPA axis is a major component of the stress response system, and cortisol, a primary HPA steroid hormone involved in the stress response and maintaining homeostatic balance within individuals, has been conceptualized as a biomarker indicating the extent to which individuals are open to environmental influences (e.g., DelGuidice et al., 2011; Gunnar & Quevedo, 2007). Cortisol functioning has been shown to be influenced by genes (e.g., Bartels, Van den Berg, Sluyter, Boomsma, & de Geus, 2003; Van Hulle, Shirtcliff, Lemery-Chalfant, & Goldsmith, 2012), the prenatal environment (e.g., Phillip & Jones, 2006; Murgatroyd & Spengler, 2011), and the postnatal parenting environment (e.g., Hunter, Minnis & Wilson, 2011). Therefore, cortisol may be a mechanism of genetic, prenatal, and/or parenting influences on behavior. However, relatively few studies have examined genetic, prenatal, HPA (i.e., cortisol), and parenting influences together for the development of behavior problems in childhood.

Only two studies have tested whether cortisol mediated associations between prenatal risk and externalizing problems. One study found that child cortisol did not mediate associations between obstetric complications and externalizing problems in 10-12 year old children (Marsman, Rosmalen, Oldehinkel, Ormel, & Buitelaar, 2009), and the other found that cortisol stress reactivity did not mediate associations between prenatal and parenting influences on reactive aggression in 8-10 year old children (Ryan, Schechter, & Brennan, 2012). The present study builds on and extends this work by (1) testing whether cortisol mediates genetic influences in addition to prenatal and parenting environmental influences on internalizing and externalizing problems using an adoption design, (2) investigating whether the mediation pathways occur earlier in development (i.e., age 6 years), and (3) testing whether the hypothesized mediation pathways emerge when examining internalizing and externalizing problems while controlling on the other type of problem, as internalizing and externalizing problems are often highly comorbid in children (e.g., Zahn-Waxler, Shirtcliff, & Marceau, 2008).

**Genetic and Environmental Influences on Child Behavior Problems**
There is evidence of genetic and environmental influences on internalizing and externalizing problems during middle childhood (e.g., Rhee & Waldman, 2002; Rice & Thapar, 2009). Intergenerational studies suggest that externalizing and substance use problems in the parent generation lead to externalizing problems in children (e.g., Bailey, Hill, Oesterle, & Hawkins, 2009; D’Onofrio et al., 2007), and internalizing problems in parents lead to internalizing problems in children (Pettit, Olino, Roberts, Seeley, & Lewinsohn, 2008) via mainly genetic and in part environmental pathways. However, there is also evidence that genetic risk for internalizing and externalizing problems in childhood and adolescence may be nonspecific – that is, the same set of genetic influences contribute to both internalizing and externalizing problems, but do not distinguish between which type of problem is expressed in children (Lahey, Van Hulle, Singh, Waldman, & Rathouz, 2011). Therefore, parents’ externalizing, substance use, and/or internalizing problems could lead to internalizing and/or externalizing problems in children via genetic pathways.

In addition to genetic influences, environmental influences have been shown to be particularly important for the development of internalizing and externalizing problems in childhood. Specifically, middle childhood appears to be the most salient developmental period for the influence of shared environmental factors, or family-level nongenetic influences that make family members more similar, on internalizing and externalizing problems, as opposed to younger children or adolescents (Burt, 2009a). There is evidence that both mothers’ and fathers’ over-reactive parenting and parenting inconsistency are associated with internalizing and externalizing problems across childhood (e.g., Lipscomb et al., 2011; Marchand-Reilly, 2012; McKee et al., 2007).

Socialization theorists have historically considered the influence of parenting on child behavior to be indicative of environmental influences (e.g., Hirschi, 1969). However, an issue with most of the studies examining parenting influences on child behavior problems is that when parents and children share genes and the family environment, it is impossible to tell whether parenting behaviors represent environmental influences, or whether gene-environment correlation (rGE) is occurring. That is, associations between parenting behaviors and child behavior problems could arise if parents pass on genes to children predisposing them to develop behavior problems and simultaneously provide a negative parenting environment (passive rGE). It is also possible that negative parenting behaviors represent a response to children’s genetically influenced behavior problems and do not exert additional influence (evocative rGE). Thus, it is important to use genetically informed designs to study associations between family characteristics and child behavior in order to clarify family environmental influences from genetic influences. Here, we use an adoption design where children were adopted at birth. In this design, associations between parenting and child
behavior must be environmental influences because adoptive parents and the adopted child do not share genes or prenatal influences, and passive rGE is controlled for.

There is substantial evidence of transactional associations between parenting behaviors and child problems (e.g., Lansford et al., 2011; Larsson, Viding, Rijsdijk, & Plomin, 2008) and of rGE underlying associations between parenting and child and adolescent behavior (e.g., Narusyte et al., 2008; Marceau, Horwitz et al., in press; see Horwitz, Marceau, & Neiderhiser, 2011). However, evidence from adoption designs suggests that parenting behaviors can also exert an environmental influence on child behavior (e.g., Lipscomb et al., 2011; Pemberton et al., 2011). Thus, we expect that over-reactive and/or inconsistent parenting of mothers and fathers should exert an environmental influence on child internalizing and externalizing problems. One area in need of more research is in identifying biological mechanisms by which genes and the parenting environment may influence the development of internalizing and externalizing problems in children. In the current study we focus on prenatal risk and cortisol functioning as potential biological mechanisms mediating genetic and parenting influences on children’s internalizing and externalizing problems.

**Prenatal Risk and Child Behavior Problems**

Prenatal risk factors are important and frequently studied predictors of children’s behavior problems (e.g., Allen, Lewinsohn, & Seeley, 1998; McNeil, 1995; Williams & Ross, 2007). For example, substance use (i.e. nicotine, cocaine, alcohol), maternal anxiety and depression symptoms during pregnancy, and neonatal complications have been associated with children’s internalizing problems (e.g., Gutteling et al., 2005; Mattson & Riley, 2000; Knopik, 2009), and externalizing problems (e.g., Ben Amor et al., 2005; Gutteling et al., 2005; Goldschmidt, Day, & Richardson, 2000; Knopik, 2009; Mill & Petronis, 2008). There is also evidence that genetic and prenatal environmental influences are linked. For example, some twin studies suggest that genetic influences and maternal smoking during pregnancy predict overlapping variance in behavior problems during childhood, (see Knopik, 2009 for review). Further, evidence from adoption studies suggest that genetic influences on child behavior (i.e., birth parent characteristics) are associated with experiencing prenatal risk (e.g., Natsuaki et al., 2010; Pemberton et al., 2011), and there is evidence that prenatal risk can mediate genetic influences on toddler behavior (e.g., Neiderhiser et al., 2007; Marceau, Hajal et al., in press). However, there is also evidence of unique prenatal influence on children’s behavior; for example, other studies suggest that maternal smoking during pregnancy exerts additional influence above genetic influences on child ADHD outcomes (Knopik, 2009). Together this literature suggests that prenatal risk may partially mediate genetic influences on internalizing and externalizing problems in childhood.
Child HPA Functioning and Behavior Problems

The stress response system has been a primary biological mechanism hypothesized to contribute to the development of behavior problems. According to evolutionary biosocial theories, the combination of different developmental contexts and HPA activity patterns lead to particular behavioral outcomes (e.g., Del Giudice et al., 2011). For example, high HPA axis activity may lead to more internalizing-type problems, because children with a lower threshold for limbic-hypothalamic arousal would show exaggerated responses (including behavioral responses) to environmental influences or changes (Kagan, Reznick, & Snidman, 1988). However, low responsivity of the HPA axis may lead to more externalizing-type problems because children with lower HPA activity (i.e., a higher threshold for limbic-hypothalamic arousal) may engage in externalizing behaviors in an attempt to increase arousal (e.g., Archer, 2006; Fries, Hesse, Hellhammer, & Hellhammer, 2005; Raine, 1996; Zuckerman, 1979). Generally, empirical evidence supports these hypotheses: low basal cortisol and blunted responsivity are often associated with externalizing problems, whereas elevated basal cortisol and increased responsivity are often associated with internalizing problems, although the literature is quite mixed (e.g., Alink et al., 2008; Dickerson & Kemeny, 2004; Marceau, Ruttle, Shirtcliff, Essex, & Susman, under review).

Cortisol influences on behavior may not be distinct from genetic, prenatal, and parenting influences on children’s behavior problems. Evidence from behavioral genetic studies suggests that morning cortisol is particularly heritable, whereas evening levels tend to be influenced by family environmental factors (e.g., Bartels et al., 2003; Van Hulle et al., 2012). Further, the prenatal period is a sensitive period of development for offspring’s HPA functioning (Phillips & Jones, 2006) in part because mother’s biological responses to her environment shape the fetus’s stress response system so that the child is best prepared for the types of environmental challenges he or she is likely to experience after birth (DelGiudice, 2012). Specifically, exposure to risks prenatally may help set the limbic-hypothalamic arousal threshold, increasing the likelihood that the child develops higher baseline cortisol or a heightened responsivity to stress (Pluess & Belsky, 2011). Elevated levels of prenatal risk (i.e., drug exposure and maternal anxiety/depression) has been associated with higher levels of morning cortisol (e.g., O’Connor et al., 2005), and greater cortisol responsivity to stressors in childhood (e.g., Dozier et al., 2006; Fisher, Gunnar, Dozier, Bruce, & Pears, 2006; Jacobson, Bihun, & Chiodo, 1999; Zhang, Sliwowska, & Weinberg, 2005), even when controlling for other obstetric complications and postnatal levels of parental anxiety and depression (e.g., Glover, O’Connor, & O’Donnell, 2010; O’Connor et al., 2005). Taken together, this body of literature suggests that genetic influences and prenatal risk may be implicated in how children’s HPA functioning is associated with their behavior problems.
Early postnatal experiences have also been shown to have long-lasting impact on the development of the HPA axis (Del Giudice et al., 2011; Essex, Klein, Cho, & Kalin, 2002; Gunnar, Morison, Chisholm, & Schuder, 2001; Halligan, Herbert, Goodyer, & Murray, 2004; Heim et al., 2002; Tarullo & Gunnar, 2006), such that early negative rearing environments can impact HPA axis functioning in childhood through adolescence (Flinn & England, 1997; see Repetti, Taylor, & Seeman, 2002 for review). This may be because parents can act as regulators of the child’s HPA system during childhood (Gunnar & Quevedo, 2007) and juvenile period (Flinn & England, 1997). In particular, over-reactive parenting has been implicated in later HPA functioning (e.g., Azar, Paquette, Zoccolillo, Baltzer, & Tremblay, 2007; Flinn & England, 1997; Hastings et al., 2011; Marceau, Ram et al., under review). Thus, in addition to genetic influences and prenatal risk, over-reactive parenting and/or inconsistent parenting may influence children’s behavior problems directly and/or through children’s HPA functioning.

Present Study

In the present study we hypothesized that prenatal risk and cortisol functioning would mediate genetic influences on children’s internalizing and externalizing problems, and that cortisol functioning would also mediate parenting influences on children’s internalizing and externalizing problems. We tested these hypotheses using structural equation modeling of data from a prospective longitudinal adoption design. The longitudinal adoption design (when children are adopted at birth) is uniquely suited to test models including genetic, prenatal, physiological, and parenting environmental influences across development. Birth mothers and adopted children share genes and the prenatal environment but not postnatal environments, so any correlation between birth mother characteristics and child characteristics must be due to genetic or prenatal influences. Carefully measuring birth mother characteristics and prenatal complications separately can help clarify how genetic and prenatal pathways influence child development, as has been done to distinguish pre- and post-natal influences on child development (e.g., Marsman et al., 2009; Robinson et al., 2009; Ryan et al., 2012). Adoptive parents and adopted children share only postnatal environments, so correlations between adoptive parent characteristics and child characteristics are most likely due to postnatal environmental influences. Therefore, adoption designs provide an opportunity to examine developmental pathways leading to child behavior problems more cleanly than is possible in families where parents and children share both genes and environments.

Our specific hypotheses were that birth mothers’ lifetime psychopathology symptoms would predict internalizing and externalizing problems in adopted children, representing genetic influences on the development of behavior problems in children. We also hypothesized that higher overall levels of adoptive mothers’ and fathers’ over-reactive parenting, and more inconsistent over-reactive
parenting across childhood would predict internalizing and externalizing problems in adopted children, representing environmental influences on the development of behavior problems in children. We hypothesized that birth mother psychopathology symptoms would be associated with more prenatal risk exposure, and that more prenatal risk exposure would predict higher morning cortisol, more HPA flexibility (i.e., a construct tapping into responsivity to environmental stressors), representing higher HPA activity, and internalizing and externalizing problems in children. We also predicted that higher morning cortisol and more HPA flexibility would be associated with more internalizing problems, whereas lower morning cortisol and less HPA flexibility would be associated with more externalizing problems. Together, we predicted that prenatal risk and cortisol functioning would mediate genetic influences on children’s behavior problems. Finally, we hypothesized that more overall over-reactive parenting would predict higher morning cortisol, and inconsistent over-reactive parenting would predict higher morning cortisol and more HPA flexibility. Therefore, we also predicted that cortisol functioning (HPA activity) would mediate parenting influences on child behavior problems.

**Method**

**Participants and procedures**

Participants for the present study were drawn from the first cohort of the Early Growth and Development Study, a multisite longitudinal study of adopted children and their birth and adoptive parents (Leve et al., 2013), consisting of 361 linked birth mothers (BM) and adoptive families. Participants were recruited via thirty-three adoption agencies in 10 states across the US (see Leve et al., 2013 for further description). Participants were eligible for participation if (1) the adoption was domestic, (2) the child was placed with a non-relative adoptive family (3) and prior to 3 months of age ($M = 7.11$ days postpartum, $SD = 13.28$), (4) the child had no known major medical conditions, and (5) the BM and adoptive parents could read or understand English at least at an eighth-grade level. Sample characteristics are given in Table 7.1.

Over time, the sample (with some attrition) participated in a series of in person interviews. BMs were interviewed (usually in their homes) at approximately 4 months ($n = 360$), 18 months ($n = 333$) and 48 months ($n = 318$) postpartum. Adoptive families were interviewed when their child was 9 months ($n = 358$), 18 months ($n = 353$), 27 months ($n = 340$), 4.5 years ($n = 304$), and 6 years ($n = 308$) old. Information on attrition and missing data are presented below.
Table 7.1
Sample Descriptive Statistics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Birth Mother</th>
<th>Adoptive Parent 1</th>
<th>Adoptive Parent 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at child birth</td>
<td>24.35 (6.03)</td>
<td>37.4 (5.57)</td>
<td>38.24 (5.85)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>70.9%</td>
<td>92.2%</td>
<td>90.9%</td>
</tr>
<tr>
<td>African-American</td>
<td>13.7%</td>
<td>3.6%</td>
<td>4.7%</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>6.3%</td>
<td>1.8%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Multietnic</td>
<td>4.1%</td>
<td>1.1%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Other</td>
<td>5.0%</td>
<td>1.3%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Median Education Level</td>
<td>High School</td>
<td>4-year college</td>
<td>4-year college</td>
</tr>
<tr>
<td>Median Annual Income</td>
<td>&lt; $15,000</td>
<td>$125,000 - $150,000</td>
<td></td>
</tr>
<tr>
<td>Employment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full Time</td>
<td>35.1%</td>
<td>32.1%</td>
<td>73.9%</td>
</tr>
<tr>
<td>Part Time</td>
<td>13.3%</td>
<td>18.0%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Unemployed but looking for work</td>
<td>19.6%</td>
<td>0.4%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Full-time homemaker</td>
<td>7.4%</td>
<td>30.5%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Other</td>
<td>24.6%</td>
<td>19.0%</td>
<td>21.2%</td>
</tr>
</tbody>
</table>

Note. Adoptive Parent 1 was typically the mother. Sample descriptive statistics represent the first cohort of EGDS, N=361.

Measures

**BM psychopathology symptoms.** We assessed adopted children’s genetic risk for behavior problems through BMs’ psychopathology symptoms using the Composite International Diagnostic Interview (CIDI, Kessler & Ustun, 2004) and the Diagnostic Interview Schedule (DIS, Robins, Helzer, Croughan, & Ratcliff, 1981) at the 18 month assessment. BM substance use disorders was operationalized as the number of symptoms she reported from the substance disorders (i.e., alcohol dependence and abuse, drug dependence and abuse, and tobacco dependence) measured by the CIDI (n = 333; α = .92 across substance use diagnoses), resulting in a possible range of 0 to 31 for substance use symptoms. BM internalizing disorders was operationalized as the number of symptoms she reported from the internalizing disorders (i.e., agoraphobia, agoraphobia without panic, adult separation anxiety, dysthymia, generalized anxiety disorder, major depressive episode, panic disorder, recurrent brief depression, separation anxiety, social phobia, and specific phobias) she met using the CIDI (n = 333; α = .80 across internalizing diagnoses), resulting in a possible range of 0 to 37 for internalizing symptoms. Finally, BM externalizing disorders was operationalized as the number of symptoms she reported from the externalizing diagnoses (i.e., conduct disorder and antisocial
personality) using the DIS \((n = 303; \alpha = .76\) across externalizing diagnoses), resulting in a possible range of 0 to 50 for externalizing symptoms (See Table 6.2).

**Prenatal risk.** Prenatal risk exposure was quantified from information BMs provided at the 4-month assessment on a pregnancy history calendar (adapted version of the life history calendar, Caspi et al., 1996) and a pregnancy screener that focused specifically on the prenatal period. Relevant to the present study, BMs reported on use of alcohol, cigarettes and illegal drugs during pregnancy, 7 items from the Beck Depression Inventory (BDI; Beck, Steer, & Brown, 1996) and 5 items from the Beck Anxiety Inventory (BAI; Beck & Steer, 1993). Responses were scored using a coding system (see Marceau, Hajal et al., in press for details) based on variety of risk indices (e.g., McNeil, Cantor-Grace, & Sjostrom, 1994; Kotelchuck, 1994; Williams & Ross, 2007; Van den Bergh, Mulder, Mennes, & Glover, 2005).

*Anxiety and Depression* were the sum of anxiety and depressive symptoms, respectively, that BMs reported experiencing during pregnancy, scored by creating quartile scores identifying the rank of anxiety or depressive symptoms. The bottom 25% of the sample were given a risk score of 1, 25% to 50% = 2, 50%-75% = 3, 75%-85% = 4, and 85%-100% = 5. Approximately 30% of the BMs reached risk levels (i.e., more than moderate risk to the fetus, according to the McNeil et al., 1994 scaling criteria). *Drug use* included serious use of cigarettes, alcohol, eight illegal drugs, and prescription painkillers used illegally. Different weights were given to amounts of different drugs used in accordance with the McNeil-Sjostrom Scale; approximately 37% of BMs reached risk levels. Following the assignment of risk scores for each variable, we created weighted risk scores. If the risk score on a variable (i.e., anxiety, depression, cigarettes, alcohol, each drug) was 3 or greater (as per the McNeil-Sjostrom Scale), the risk score = 1 (prenatal risk present), if the risk score on a variable was 2 or less, the cutoff risk score = 0 (no risk). The individual risk cutoff scores for anxiety, depression, and each drug were summed to create a final prenatal risk score. Approximately 52% of the sample \((n = 360)\) reached minimum risk levels of prenatal risk considering internalizing symptoms and drug use together. Sample descriptive statistics are provided in Table 7.2.

**Postnatal over-reactive parenting.** Adoptive mothers’ and fathers’ level of over-reactive parenting was measured at the 9 \((n = 330)\), 18 \((n = 331)\), and 27 \((n = 310)\) month, and 4.5 \((n = 258)\) year assessments using the over-reactivity subscale of The Parenting Scale (Arnold, O’Leary, Wolf, & Acker, 1993; \(\alpha’ > .75\) for mothers and fathers at each assessment). Higher scores reflected more over-reactivity (i.e., displays of anger, meanness, and irritability), and lower scores reflected more appropriate responses to children’s misbehavior. Missing values were imputed (see below), and then mothers’ and fathers’ *overall over-reactive parenting* was computed as the average of each mother’s or father’s four scores over time. *Inconsistency of over-reactive parenting* across childhood was
computed as the range (max – min) of the residuals from each individuals’ overall level of over-reactive parenting over time, assessing how far from their mean each individual parent fluctuated over time. Sample descriptive statistics are given in Table 7.2.

**Children’s cortisol.** Morning and evening saliva samples were collected from adopted children for three days around the 4.5 year assessment. Adopted children (through their parents) were instructed to provide samples at 30 minutes after waking in the morning and at bedtime, before brushing teeth on each of 3 days. Samples were returned by 70% of families participating at the 4.5 year assessment ($n = 210$). Samples were stored by participants and then mailed to the primary study site and frozen until all samples for the assessment wave were collected. Then, all samples were sent to the University of Trier Laboratory and frozen at -20° C until being used for cortisol immunoassay (DELFIA procedure; see Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). Samples were assayed in duplicate, with the average of the useable values taken as the level of cortisol in that sample (see Laurent et al., 2013, for further detail). Children’s morning cortisol was, on average, at a level of 0.60 µg/dl ($SD = 0.19$ µg/dl) at 7:38 AM ($SD = 43$ minutes), and evening cortisol was, on average 0.06 µg/dl ($SD = 0.03$ µg/dl) at 8:12 PM ($SD = 51$ minutes).

Values more than 2.5 standard deviations from each assessment (morning or evening) mean were winsorized (replaced with 2.5 standard deviation values, < 4% of values). Next, scores were regressed on a measure of whether the child was using steroid medication to control for medication use. Missing values were then imputed (see below). Then, from the repeated measures of cortisol (assessments within days within child) we extracted a measure of children’s average morning cortisol and of HPA flexibility (the extent to which an individual’s HPA system deviated from its usual diurnal pattern of function, a construct designed to tap into an aspect of stress responsivity, Marceau Ram et al., in resubmission) using individual-level regressions.
Table 7.2.

**Means, Standard Deviations, and Correlations among Study Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>BM Psychopathology Symptoms (lifetime)</th>
<th>Prenatal Risk</th>
<th>Over-reactive Parenting 9.54mo.</th>
<th>Children’s Cortisol 4.5yrs</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>EXT</td>
<td>INT</td>
<td>Substance Use</td>
<td></td>
</tr>
<tr>
<td>BM Psychopathology Symptoms (lifetime)</td>
<td>13.20 (11.08)</td>
<td>.34*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM Psychopathology Symptoms (lifetime)</td>
<td>9.36 (7.06)</td>
<td>.56*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM Psychopathology Symptoms (lifetime)</td>
<td>6.65 (6.60)</td>
<td>.43*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prenatal Risk</td>
<td>4.31 (5.94)</td>
<td>.40*</td>
<td>.37*</td>
<td>.46*</td>
<td></td>
</tr>
<tr>
<td>Prenatal Risk</td>
<td>2.12 (.49)</td>
<td>-.06</td>
<td>-.02</td>
<td>-.04</td>
<td>-.05</td>
</tr>
<tr>
<td>Prenatal Risk</td>
<td>2.11 (.51)</td>
<td>.04</td>
<td>-.13*</td>
<td>-.01</td>
<td>-.04</td>
</tr>
<tr>
<td>Prenatal Risk</td>
<td>.71 (.38)</td>
<td>.01</td>
<td>.07</td>
<td>.04</td>
<td>.03</td>
</tr>
<tr>
<td>Prenatal Risk</td>
<td>.62 (.38)</td>
<td>.13*</td>
<td>.09</td>
<td>.06</td>
<td>.12*</td>
</tr>
<tr>
<td>Over-reactive Parenting 9.54mo.</td>
<td>.03 (.19)</td>
<td>-.01</td>
<td>.19*</td>
<td>.05</td>
<td>.27*</td>
</tr>
<tr>
<td>Over-reactive Parenting 9.54mo.</td>
<td>.11 (.10)</td>
<td>.01</td>
<td>.07</td>
<td>.09</td>
<td>-.05</td>
</tr>
<tr>
<td>Morning Cortisol µg/dl</td>
<td>.11 .11</td>
<td>.05</td>
<td>.13*</td>
<td>-.01</td>
<td>-.01</td>
</tr>
<tr>
<td>HPA Flexibility</td>
<td>.01 .01</td>
<td>.07</td>
<td>.09</td>
<td>.05</td>
<td>.13*</td>
</tr>
<tr>
<td>HPA Flexibility</td>
<td>.07 .07</td>
<td>-.05</td>
<td>.13*</td>
<td>-.01</td>
<td>.02</td>
</tr>
<tr>
<td>Outcomes (6 yrs)</td>
<td>.04 (.85)</td>
<td>-.04</td>
<td>.08</td>
<td>-.02</td>
<td>.01</td>
</tr>
<tr>
<td>Outcomes (6 yrs)</td>
<td>11.24 (5.55)</td>
<td>.21*</td>
<td>.22*</td>
<td>.20*</td>
<td>.12*</td>
</tr>
<tr>
<td>Outcomes (6 yrs)</td>
<td>8.18 (4.78)</td>
<td>.14*</td>
<td>.11*</td>
<td>.02</td>
<td>-.15*</td>
</tr>
</tbody>
</table>
| INT = Internalizing; EXT = Externalizing. * p < .05. N = 361.
Child behavior problems. At the six year assessment adoptive mothers and fathers reported on children’s internalizing and externalizing problems using the broadband internalizing and externalizing subscales on the Child Behavior Checklist (Achenbach, 1991; α’s > .88 for mothers and fathers, n = 253). The maximum score of mothers’ and fathers’ reports on the internalizing and externalizing subscales was used in order to reduce systematic rater bias. Mothers reported higher scores than fathers 44% of the time for internalizing problems and 42% of the time for externalizing problems; mothers and fathers reported equivalent scores 33% of the time for internalizing and externalizing problems; fathers reported higher scores than mothers 24% of the time for internalizing problems and 25% of the time for externalizing problems (r = .37 for internalizing problems, r = .54 for externalizing problems). The sample had symptom levels predominantly in the normal range; 6% of children had sub-clinical levels of internalizing problems and 5% had sub-clinical levels of externalizing problems (T scores between 60 and 63); 5.7% of children had clinical levels of internalizing problems and 3% of children had clinical levels of externalizing problems (T scores > 63). In total, 18% of the sample reached sub-clinical or clinical problems on at least one of the broadband scales.

Control variables. We also included openness/contact in the adoption as a covariate, since openness of the adoption could facilitate associations between birth and adoptive parents (see Ge et al., 1996 for more information). Openness of the adoption was assessed at each assessment via BM and AP report on the extent to which they perceived that the adoption was open on a 7-point scale ranging from 1 (very closed) to 7 (very open). The standardized mean of BM, and adoptive mother and father reports was used at each assessment.

Missing data. Data were not missing completely at random according to Little’s MCAR test, x²(3734) = 4043.1, p < .05. Therefore, missing data were imputed using SAS PROC MI; 40 datasets were imputed and aggregated (Graham, Olchowski, & Gilreath, 2007) to reduce bias. Percentages of missing data from the full sample of 351 at each assessment were as follows: birth mother psychopathology symptoms: 5%, adoptive parent 9-month assessment: 6%, adoptive parent 18-month assessment: 6%, adoptive parent 27-month assessment: 12%, adoptive parent 4.5 year assessment: 27%, saliva samples: 40%, 6-year child internalizing and externalizing problems (outcome): 30%. We tested whether adoptive parents’ age when the child was born and income, birth mothers’ age at the child’s date of birth and income, openness of the adoption, child’s ethnicity and sex, and study variables at prior waves contributed to whether data were missing (yes/no) at each assessment using a series of Kruskal-Wallace one-way analysis of variance tests. Of 151 tests, only nine reached significance at the p < .05 level, and only four reached significance at the p < .01 level, far below
chance levels indicating these would not reach threshold for significance controlling for multiple testing. Therefore, we concluded that demographic and study variables were unrelated to missingness.

**Results**

We controlled for openness of the adoption at the current assessment and prior assessments by regressing each score for all study variables on openness of the adoption variables and using the residual scores in hypothesis testing. Zero-order correlations among study variables are presented in Table 7.2. Children’s internalizing and externalizing problems were highly comorbid ($r = 0.58, p < 0.05$). Generally, correlations supported genetic, prenatal, cortisol, and parenting influences on children’s internalizing and/or externalizing problems, and the pattern of correlations differed slightly for internalizing vs. externalizing problems.

A structural equation model was conducted using Mplus (Muthen & Muthen, 2004) in order to simultaneously test the contributions of genetic (birth mother psychopathology symptoms), prenatal, parenting (mothers’ and fathers’ overall and inconsistency of over-reactive parenting), and cortisol functioning (morning cortisol and HPA flexibility) to children’s internalizing and externalizing problems. Children’s internalizing and externalizing problems were entered into the model simultaneously in order to control on the other type of problem, and test for specificity of influences on each type of problem. First, the a priori model (see Figure 7.1) was fit to the data based on the hypotheses drawn, testing pathways from birth mother characteristics to prenatal risk, HPA activity, and child outcome variables, pathways from prenatal risk to HPA activity and child outcome variables, pathways from parenting variables to HPA activity and child outcome variables, and concurrent associations among variables assessing the same general influence (i.e. among birth mother characteristics, among parenting variables, between morning cortisol and HPA flexibility, and between child internalizing and externalizing problems). This model fit the data well, $\chi^2 (16) = 23.14, p = .11$, CFI = .98, TLI= .95, RMSEA= .04, SRMR = .03 (Figure 7.1). Standardized parameter estimates are presented in Table 7.3; only significant results are presented in the text.

Birth mothers’ substance use, internalizing, and externalizing symptoms were all positively associated, $\psi > .34, SE < .05, p < .05$. Birth mothers’ substance use, internalizing, and externalizing symptoms predicted prenatal risk (i.e., drug use and internalizing symptoms during pregnancy), $B > .18, SE = .05, p < .05$. Birth mothers’ substance use symptoms predicted lower morning cortisol in children at age 4.5 years, $B = -.15, SE = .06, p < .05$. Birth mothers’ internalizing symptoms predicted higher morning cortisol in children, $B = .19, SE = .06, p < .05$. Birth mothers’ substance use symptoms predicted more HPA flexibility in children at age 4.5 years, $B = .13, SE = .05, p < .05$. Experiencing more prenatal risk predicted higher morning cortisol in children at age 4.5 years, $B = .31, SE = .06, p < .05$, and less HPA flexibility in children at 4.5 years $B = -.13, SE = .06, p < .05$. 
Birth mothers’ internalizing symptoms predicted more child externalizing problems at age 6 years, $B = .14$, $SE = .04$, $p < .05$, and birth mothers’ externalizing symptoms predicted fewer child internalizing problems, $B = -.21$, $SE = .06$, $p < .05$. 
Figure 7.1. Model Fitting Results. Assessments from which the data were drawn are labeled across the top. BM psychopathology symptoms represent genetic risk. OVR = over-reactive parenting. Significant (p < .05) paths are depicted in black. Solid lines indicate positive associations; hashed lines indicate negative associations. Paths that were included model but did not reach significance at p < .05 are depicted in gray. Very thick lines depict significant indirect pathways.
Mothers’ and fathers’ overall and inconsistency in over-reactive parenting were on the whole all positively associated, $\psi > .12$, $SE = .05$, $p < .05$, except that the association between fathers’ overall over-reactive parenting and mothers’ inconsistency in over-reactive parenting was not significant. Mothers’ overall over-reactive parenting and fathers’ inconsistent over-reactive parenting predicted higher morning cortisol in children at age 4.5 years, $Bs = .13$, $SEs = .05$, $p < .05$, and mothers’ inconsistent over-reactive parenting predicted lower morning cortisol in children at age 4.5 years, $B = -.13$, $SE = .05$, $p < .05$. Mothers’ overall over-reactive parenting predicted increased HPA flexibility in children at age 4.5 years, $B = .14$, $SE = .05$, $p < .05$. Mothers’ and fathers’ overall over-reactive parenting both predicted more externalizing problems in children at age 6 years, as did mothers’ inconsistent over-reactive parenting, $B > .11$, $SE < .05$, $p < .05$. Fathers’ overall over-reactive parenting predicted more child internalizing problems, $B = .12$, $SE = .05$, $p < .05$. Higher morning cortisol was associated with less HPA flexibility in children at age 4.5 years, $\psi = -.21$, $SE = .05$, $p < .05$. Higher morning cortisol predicted fewer child internalizing problems at age 6 years, $B = -.22$, $SE = .06$, $p < .05$. More HPA flexibility predicted fewer child internalizing problems at age 6 years, $B = -.10$, $SE = .05$, $p < .05$. Child internalizing and externalizing problems were positively associated, $\psi = -.15$, $SE = .06$, $p < .05$.

**Indirect effects.** All pathways to child externalizing problems were direct, and there were no possible indirect effects predicting child externalizing problems. The indirect path from birth mothers’ internalizing symptoms via prenatal risk and child morning cortisol to child internalizing problems was significant, $B = -.042$, $SE = .016$, $p = .01$. The indirect path from birth mothers’ internalizing symptoms via only child morning cortisol to child internalizing problems was also significant, $B = -.013$, $SE = .005$, $p = .02$. There was also a significant indirect effect from birth mothers’ drug use symptoms via prenatal risk and child morning cortisol to child internalizing problems, $B = -.019$, $SE = .007$, $p < .01$. There were significant indirect effects from birth mothers’ externalizing symptoms to child internalizing problems via child morning cortisol alone, $B = .033$, $SE = .016$, $p = .04$, and via prenatal risk and child morning cortisol, $B = -.012$, $SE = .005$, $p = .02$. There were significant indirect effects from adoptive mothers’ and fathers’ overall over-reactive parenting to child internalizing problems via child morning cortisol, $B = -.029$, $SE = .014$, $p = .03$, mothers; $B = -.029$, $SE = .014$, $p = .04$, fathers, and for adoptive mothers’ inconsistent over-reactive parenting to child internalizing problems via child morning cortisol, $B = .028$, $SE = .013$, $p = .03$. Finally, there was a significant indirect effect from prenatal risk to child internalizing problems via child morning cortisol, $B = -.069$, $SE = .022$, $p < .01$. 
<table>
<thead>
<tr>
<th>Variable</th>
<th>BM Psychopathology Symptoms (lifetime)</th>
<th>Prenatal Risk</th>
<th>Over-reactive Parenting (9-54mo.)</th>
<th>Children’s Cortisol (4.5yrs)</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R² EXT INT Substance Use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM Psychopathology Symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Externalizing</td>
<td>NE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internalizing</td>
<td>NE</td>
<td>.34* (.05)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance Use</td>
<td>NE</td>
<td>.56* (.04)</td>
<td>.43* (.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prenatal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prenatal Risk</td>
<td>.27* (.06)</td>
<td>.18* (.05)</td>
<td>.19* (.06)</td>
<td>.28* (.06)</td>
<td></td>
</tr>
<tr>
<td>Mothers’ average</td>
<td>NE</td>
<td></td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Fathers’ average</td>
<td>NE</td>
<td></td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Mothers’ inconsistency</td>
<td>NE</td>
<td></td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Fathers’ inconsistency</td>
<td>NE</td>
<td></td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Over-reactive Parenting (9-54mo.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning Cortisol (µg/dl)</td>
<td>.18* (.06)</td>
<td>-.15* (.06)</td>
<td>.19* (.06)</td>
<td>.09 (.06)</td>
<td>-.09</td>
</tr>
<tr>
<td>HPA Flexibility</td>
<td>.04* (.07)</td>
<td>-.04 (.06)</td>
<td>.07 (.06)</td>
<td>.13* (.05)</td>
<td>.13*</td>
</tr>
<tr>
<td>Outcomes (6 yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Externalizing Problems</td>
<td>.10* (.06)</td>
<td>-.21* (.07)</td>
<td>.08 (.07)</td>
<td>.06 (.07)</td>
<td>-.08</td>
</tr>
<tr>
<td>Internalizing Problems</td>
<td>.13* (.06)</td>
<td>-.06 (.06)</td>
<td>.14* (.07)</td>
<td>-.05 (.07)</td>
<td>.14*</td>
</tr>
</tbody>
</table>

Note. NE = not estimated. * p < .05. N = 361. Standardized beta-weights presented with standard errors in parentheses.
Discussion

The present study tested whether prenatal risk and cortisol functioning mediated genetic influences on children’s internalizing and externalizing problems, and whether cortisol functioning mediated parenting influences on children’s internalizing and externalizing problems at age 6 years, using a prospective adoption design. Results suggested that there were genetic and environmental influences on children’s internalizing and externalizing problems. However, the ways in which these influences operated differed based on the type of problems. For children’s externalizing problems, there were direct effects of genetic and parenting influences, and prenatal risk and child morning cortisol were not implicated. However, for children’s internalizing problems, prenatal risk and child morning cortisol mediated effects of genetic and parenting influences, although there was also some evidence of direct effects of genes and parenting on children’s internalizing problems.

Child HPA Functioning and Behavior Problems

Our findings show different developmental pathways leading to internalizing vs. externalizing problems that are observable even in childhood. Our null mediation findings for externalizing problems were very consistent with the two previous studies testing whether cortisol mediated associations between prenatal risk and child externalizing problems (Marsman et al., 2009; Ryan et al., 2012). Often, there is no association between cortisol and externalizing problems reported in the literature (Alink et al., 2008), and there is evidence that the parenting environment moderates associations between cortisol functioning and externalizing problems (Laurent et al., 2013; Marceau, Shirtcliff et al., under review). For example, children with high evening cortisol and also experienced higher levels parental depression showed the greatest increases in externalizing problems (Laurent et al., 2013). Also, blunted cortisol responsivity predicted externalizing problems in adolescent boys who experienced very high levels conflict with fathers, but heightened cortisol responsivity predicted externalizing problems in boys who experienced low levels of conflict with fathers (Marceau, Shirtcliff et al., under review). This evidence points to a unique role of cortisol in the development of externalizing problems particularly in less-than-optimal environmental contexts, instead of cortisol mediating genetic, prenatal, or parenting influences on externalizing problems.

For internalizing problems, our findings support the hypotheses that cortisol may be a mechanism of genetic, prenatal, and parenting influences on child internalizing problems. Our findings indicate that higher morning cortisol predicted fewer internalizing problems, which is consistent with some (DeBellis et al., 1996; Granger et al., 1998; Laurent et al., 2012), but not all (e.g., Feder et al., 2004; Ruttle et al., 2011) studies of cortisol and internalizing problems. The present sample has a higher average morning cortisol than other ‘normative’ studies (e.g., McCarthy et al., 2009), and is consistent with other at-risk samples, including other adoption designs and children in
foster care (e.g., Dozier et al., 2006; Gunnar & Donzella, 2002). This heightened morning cortisol in part reflects the prenatal risks and genetic influences imparted by birth mothers. Surprisingly, higher morning cortisol appears beneficial in this sample, as it was associated with fewer internalizing problems. This finding may support emerging evolutionary biosocial theories (e.g., DelGuidice et al., 2011; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011) suggesting that genetic and prenatal risks organize children’s HPA functioning to be more sensitive to the environment. That is, because the overall environment that children who are adopted receive is generally positive, it may be that these children who are sensitive to the environment are able to take advantage of the positive aspects of the environment and exhibit fewer internalizing problems. However, this speculative explanation is an empirical question necessitating moderation analyses including measures of positive parenting, and will be an interesting avenue for future research.

**Prenatal Risk and Child Behavior Problems**

Consistent with other findings from the EGDS (Marceau et al., in press; Natsuaki et al., 2010; Neiderhiser et al., 2007; Pemberton et al., 2011), our findings suggest that genetic influences and prenatal influences on child behavior are associated. However, our findings do not show evidence of direct influences of prenatal risk on child behavior problems. Instead the findings support that prenatal risk exerts influence on children’s behavior through organizational changes in physiology – in this case cortisol. The prenatal environment has organizing effects on other systems implicated in child outcomes, including the immune system (e.g., Coussons-Read, 2012), and so genetic and prenatal risk may further impact child behavior through unmeasured aspects of children’s physiology. Although there were associations between birth mother’s substance use, internalizing, and externalizing symptoms and experiencing substance use and internalizing symptoms during pregnancy, there were also some unique, direct effects of birth mother internalizing and externalizing symptoms on children’s externalizing and internalizing problems. Finding direct genetic influences in addition to prenatal influences (operating via children’s morning cortisol) corroborates evidence suggesting that not all genetic influences for behavior are transmitted through the prenatal environment (e.g., Knopik, 2009). Importantly, this evidence suggests that the prenatal environment may differentially transmit genetic risk for internalizing rather than externalizing problems.

**Genetic and Parenting Influences on Child Behavior Problems**

Our findings suggest that birth mothers’ psychopathology symptoms represent genetic influences on children’s externalizing problems. Birth mothers’ internalizing symptoms on child externalizing problems, and birth mothers’ externalizing symptoms on fewer internalizing problems in 6-year olds. While interesting, and not exactly expected, finding that birth mothers’ internalizing problems predicted child externalizing problems supports other evidence that genetic risk for
behavior problems in children in non-specific (Lahey et al., 2011). However, finding that birth mothers’ externalizing problems predicted fewer internalizing problems was surprising. More research on the specificity of genetic risk in studies of the intergenerational transmission of internalizing, externalizing, and substance use problems is needed.

Finally, the present findings suggest unique roles of overall levels of mothers’ and fathers’ over-reactive parenting across childhood, as well as the added role of inconsistency in mothers’ over-reactive parenting. It appears that fathers’ overall over-reactive parenting but not mothers’ over-reactive parenting is more salient to children in terms of direct effects on internalizing problems. However, mothers’ and fathers’ overall over-reactive parenting and inconsistency in mothers’ over-reactive parenting were each directly associated with children’s externalizing problems, as opposed to indirectly associated with children’s internalizing problems via children’s morning cortisol at age 4.5 years. Thus, our results suggest that generally, both mothers’ and fathers’ parenting are associated with children’s behavior problems, and further highlight different patterns of direct and indirect effects of over-reactive parenting on children’s internalizing and externalizing problems.

Limitations and Future Directions

Several limitations of the present study are important to consider when interpreting the results. First, the children in our sample were primarily Caucasian (70%), and participants were limited to US domestic infant adoptions. Therefore, results only generalize to infants adopted domestically in the US. Future studies are needed to determine if these results hold for other types of adoption samples, and to replicate this pattern of results using other types genetically informed samples, including twin and sibling studies.

Second, there were several limitations with regard to measurement. First, cortisol was measured only in the morning and evening on three days. We extracted a parameter meant to tap into HPA flexibility as has been previously used (Marceau et al., under review), but this measure confounds biological flexibility and potential changes in the environment across days. In the future, cortisol responsivity to measured environmental stimuli should also be assessed as a potential mechanism of genetic and environmental influences on child behavior problems. Further, other physiological indices of the stress response system will be important to include in future studies, including measures of the sympathetic nervous system and measures of other hormones related to the stress response system (i.e., dehydroepiandrosterone). Additionally, hormones related to pubertal maturation (i.e., testosterone and estrogens) have been implicated in the development of behavior problems and have been shown to be influenced by genes and family environments (e.g., Romeo, 2005; Marceau et al., under review). Therefore, in the future studies should examine multiple hormones as mechanisms of genetic and environmental influences on child behavior problems.
Finally, there were limitations regarding the analytic methods used in this study. We conducted careful data preparation models in order to extract theoretically meaningful parameters. However, taking this approach, some parameters likely include more measurement error (i.e., over-reactive parenting inconsistency; HPA flexibility) than others (i.e., overall over-reactive parenting; morning cortisol). Further, latent approaches may be better for some aspects of the model. For example, the three birth mother lifetime diagnoses may load onto one factor representing genetic risk for behavior problems, and both the general and specific indexes of genetic risk may be associated with child behavior problems.

While null findings for externalizing problems have been replicated, the present findings for internalizing still need to be replicated, and extended into adolescence, when changes in hormone functioning have been implicated in the development of behavior problems. Further, given evidence that the family context moderates hormone influences on externalizing problems, parenting should be considered as a moderator of the mediation pathways hypothesized for genetic influences on externalizing problems in the future.

**Conclusions**

Even considering these limitations, the current study takes an important initial step toward understanding the mechanisms of genetic and environmental risk for the development of behavior problems. Our findings suggest that prenatal risk and HPA functioning may be mechanisms of genetic and environmental influences for internalizing but not for externalizing problems. These findings potentially have clinical implications, as they provide preliminary evidence that hormone functioning and prenatal risk profiles may eventually serve as markers that could help to identify which types of problems children are likely to develop, given the presence of genetic and environmental risk factors. In the future, studies should continue to combine genetic, prenatal, endocrine, and family environmental influences together in order to investigate the disparate pathways of development of internalizing vs. externalizing problems suggested here.
CHAPTER 8: SUMMARY AND FUTURE DIRECTIONS

Each section of this dissertation has pulled from different theories and hypotheses, all under the umbrella of biosocial theory. However, the hypotheses referenced here are complementary, and can be combined to inform a more comprehensive model of the development of risk for internalizing and externalizing behavior and later substance use. The model outlined in the first chapter (Figure 1.1) combines aspects of the multiple theories and hypotheses outlined thus far into a comprehensive developmental model, and the empirical evidence provided in Chapters 5-7 generally support several aspects of the conceptual framework.

The first tenant of the conceptual model is that there are bidirectional associations between parenting and internalizing and externalizing behavior across childhood (path a, Figure 1.1), as predicted via family systems theory (Minuchin, 1985). In addition to a vast literature (reviewed above) supporting bidirectional associations between parenting and internalizing and externalizing behavior across childhood, the current findings provide some supplementary support for this path, despite not explicitly testing for path a. Bidirectional influences over time were indirectly exemplified in the first (Chapter 5) and third (Chapter 7) papers, as there were environmental influences of parental negativity on 6 year olds’ internalizing and externalizing behavior in the third paper, but in a different adolescent sample parental negativity was a response to adolescents’ genetically influenced externalizing behavior in the first paper (Marceau, Horwitz et al., in press). Thus, the combination of the first and third studies provides evidence for path (a) of Figure 1.1.

Genetic influences were also explicit in the conceptual framework and empirical work here. Findings from Chapter 5 corroborated evidence of genetic influences on adolescent externalizing behavior. Findings from Chapter 7 corroborated evidence of genetic influences on internalizing and externalizing behavior in middle childhood. Thus evidence from Chapters 5 and 7 support the inclusion of path b in the conceptual model (Figure 1.1). Further, genetic influences of parents and adolescents on mothers’ and fathers’ negativity, and the role of evocative rGE for associations between mothers’ and fathers’ negativity and adolescent externalizing behavior found in Chapter 5 are consistent with past literature and support the inclusion of path c in the conceptual model. Further, there are genetic influences on aspects of the family context, including parenting (e.g., Neiderhiser, Marceau, & Reiss, 2013). While explicitly testing path d was out of the scope of the current dissertation, there is evidence that children’s genes contribute to cross-lagged associations between parenting and child behavior over time (Larsson et al., 2008; Burt et al., 2005; Neiderhiser et al., 1999), and that gene-environment interaction is also implicated in associations between parenting and adolescent outcomes (see Horwitz et al., 2011; Marceau & Neiderhiser, in press for reviews).
Next, hormone functioning was hypothesized to be a potential mechanism of genetic influences (path e), based on evidence that hormones can relay genetic information and affect gene expression (Griffin & Ojeda, 1996). Indeed, there was evidence of indirect effects of genetic influences on 6 year-olds’ internalizing behavior via cortisol at 4.5 years in Chapter 7. However, this pattern of effects was not found for externalizing behavior. Further, the prenatal environment was also implicated in the transmission of genetic influences through morning cortisol, supporting paths f, g, and i of the conceptual model, Figure 1.1. Finally, there was also evidence that morning cortisol in part transmitted environmental, parenting influences on internalizing behavior, suggesting that hormone functioning is not only a mechanism of genetic influences on behavioral and emotional development. This last finding is reminiscent of behavioral genetic studies of associations between puberty and internalizing behavior suggesting that pubertal development is linked with internalizing behavior via shared environmental influences. Therefore, the complex findings in Chapter 7 support part of path e, as there were genetic influences on cortisol functioning, part of path f, as lower morning cortisol and reduced cortisol variability were associated with internalizing behavior, path g, as prenatal risk was associated with higher morning cortisol and reduced cortisol variability, path i, as genetic influences and prenatal risk were associated, and part of path j, as there were associations between measures of over-reactive parenting and increased morning cortisol and cortisol variability.

The current findings did not support path h, however, as there were no direct effects of the prenatal environment on internalizing and externalizing behavior in childhood. Instead all effects of the prenatal environment were transmitted through cortisol functioning in the third study. There is evidence of direct effects of the prenatal environment on toddler behavior (Marceau, Hajal et al., in press), and there is also some evidence of prenatal influences on behavior in childhood (reviewed in Chapter 7). However, these studies generally do not include measures of hormone functioning. More work is needed, therefore, to replicate findings from Chapter 7 and to disentangle whether prenatal influences are consistently transmitted via endocrine functioning.

Finally, Chapter 6 provides some support for path m, suggesting that father-adolescent conflict may moderate associations between HPA hormone responsivity and externalizing behavior, but not internalizing behavior in boys. There was also very tentative evidence for maternal negativity as a moderator of HPA hormone responsivity and externalizing behavior in girls, and this area is in need of more studies to discern whether this effect exists. It was out of the scope of the current dissertation to test paths k and l, in order to understand whether genetic and prenatal influences also have the potential to moderate hormone-behavior associations, and is therefore another avenue of future research.
As a whole, the work presented in this dissertation provides support for several aspects of the conceptual framework. Further, the findings offer intriguing possibilities for disentangling mechanisms of development leading to internalizing vs. externalizing behavior. In Chapter 1 several hypotheses were proposed for how genetic and environmental factors may influence behavioral development. The first hypothesis, that prenatal and endocrine influences may transmit genetic and parenting influences on the development of internalizing and externalizing behavior, was supported in Chapter 7, but only for internalizing behavior. The second hypothesis, that parenting influences may moderate genetic and hormone influences on the development of internalizing and externalizing behavior, was in part supported in Chapter 6, though only for boys’ externalizing behavior. Together, Chapters 6 and 7 hint at the possibility that mediation models combining genetic, hormone, and parenting influences may better characterize the development of internalizing behavior, whereas moderation models combining genetic, hormone, and parenting influences may better characterize the development of externalizing behavior. That is, although genetic, hormone, prenatal and parenting influences are important for the development of both types of behavior, the ways in which these influences develop and operate together across childhood and adolescence could help to distinguish which types of behavior youth are likely to exhibit. However, this dissertation provides only a glimpse at the potential underlying mechanisms of genetic, hormone, prenatal, and family environmental influences on the development of internalizing vs. externalizing behavior, and certainly calls for more work testing this hypothesis.

Notably, parts of the conceptual model were not tested in the current dissertation. The third major hypothesis, that genetic influences may moderate later hormone and parenting influences on the development of internalizing and externalizing behavior, was not tested in the current dissertation. Further, paths d, l, and k were not tested, though evidence from existing literature was provided for each of these hypothesized paths. These provide further areas of future research.

Conclusions

The current dissertation suggests that there are transactional bidirectional influences of parenting and the development of psychopathology. Behavioral genetic research (including findings from Chapters 5) shows that gene-environment correlation is an important mechanism for how these transactional influences occur, but there is still more work to be done in understanding exactly how genetic and environmental influences, and gene-environment correlation can translate into behavior and relationship outcomes. Findings from Chapter 5 suggest that adolescent externalizing behaviors are a mechanism of how children’s genetic influences can impact the parenting they receive. The subsequent empirical work focused on mechanisms of the development of internalizing and externalizing behavior, namely, the parenting environment as a moderator of associations between
hormone functioning and internalizing and externalizing behavior (Chapter 6), as well as hormone functioning, and to some extent the prenatal environment as potential mechanisms of genetic and environmental influences on behavior (Chapter 7). Findings from these studies begin to hint that the types of hormones and hormone changes in combination with family context may help us to understand which flavor of risk for psychopathology symptoms (i.e., internalizing vs. externalizing) youth are likely to exhibit.

The current dissertation provides an example of a conceptual framework combining genetic, hormone, prenatal, and family environmental influences to better understand the mechanisms of the development of internalizing and externalizing behavior. By explicitly separating genetic, prenatal, hormone, and family environmental influences on development, transactions among these factors can be examined, and multiple mechanisms of development can be simultaneously examined. In this way, the current conceptual framework moves beyond its component parts to better simulate the complexities of human development. Ultimately, the evidence provided here is just a step on the long path working towards better integration of behavioral genetic and behavioral endocrinology approaches to understand mechanisms of genetic and environmental influences on the development of psychopathology. Findings from these studies inform expectations for effectiveness of targeted interventions on parenting vs. parent-child relationships vs. adolescent behavior. For example, findings from the first study suggest that interventions targeting adolescents to reduce externalizing behavior or parents’ responses to problematic behaviors may be more effective than targeting a reduction in parents’ negativity. Findings from the second and third studies suggest different underlying mechanisms for internalizing and externalizing behavior. Understanding which mechanisms lead to which types of problems will ultimately be informative of prevention efforts, aiding in targeting interventions for youth based upon their biological profiles in conjunction with their family context.
REFERENCES


Azar, R., Paquette, D., Zoccolillo, M., Baltzer, F., & Tremblay, R. E. (2007). The association of major depression, conduct disorder, and maternal overcontrol with a failure to show a cortisol


salivary cortisol and dehydroepiandrosterone (DHEA) at presentation. Psychological Medicine, 26(2), 245-256.


epiphyseal fusion and bone turnover: Lesdaughters from mutations in the genes for aromatase and the estrogen receptor. *Hormone Research, 49*(1), 2–8.


Predicting treatment response for oppositional defiant disorder and conduct disorder using 

Shirtcliff, E.A., Granger, D.A., Booth, A., Johnson, D., 2005. Low salivary cortisol levels and 

Shirtcliff, E. A., & Ruttle, P., 2010. Immunological and neuroendocrine dysregulation following 
early deprivation and stress. In K. H. Brisch (Ed.), Attachment and Early Disorders of 

dehydropiandrosterone responsiveness to social challenge in adolescents with internalizing 

Silberg, J. L., Maes, H., & Eaves, L. J. (2010). Genetic and environmental influences on the 
transmission of parental depression to children’s depression and conduct disturbance: an 
extended Children of Twins study. Journal of Child Psychology and Psychiatry, 51(6), 734-
744. doi: 10.1111/j.1469-7610.2010.02205.x

transmission of parental antisocial behavior to children’s conduct disturbance, depression and 
hyperactivity. Journal of Child Psychology and Psychiatry, 53(6), 668-677. doi:
10.1111/j.1469-7610.2011.02494.x

to childhood behavioural and emotional problems: A model for the children of twins. 
Psychological Medicine, 34, 347-356.

Slutske, W., Heath, A.C., Dinwiddie, S.H., Madden, P.A.F., Bucholz, K.K., Dunne, M.P. et al. 
(1997). Modeling genetic and environmental influences in the etiology of conduct disorder: A 

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 Prospective Study. Child Development, 73(1), 75-92. doi: 10.1111/1467-
8624.00393

responsivity in children with externalizing behavior disorders. Development and 
Psychopathology, 16(2), 389-406.


Kristine Marceau  
Vita  
Kpm170@psu.edu

EDUCATION
2013 Ph.D., Developmental Psychology, The Pennsylvania State University
2011 M.S., Developmental Psychology, The Pennsylvania State University
2006 B.A., Psychology and Philosophy, University of Wisconsin-Madison

SELECTED HONORS/AWARDS
2012-2013 NIDA Ruth H. Kirschstein National Research Service Award Predoctoral Fellowship F31DA033737-01.
2010 Social Science Research Institute grant “Methodological Innovations in Modeling Timing and Tempo of Puberty: A Multiple Cohort 50 Year Approach.” (co-I)
2011-2012 Linda Brodsky Strumpf Liberal Arts Centennial Graduate Endowment: Strumpf Scholar Award

PUBLICATIONS


