Spring 5-31-2021

The Complexities of Crankiness and Cortisol: Exploring the Association Between Irritability, Cortisol Reactivity, and Psychopathology

Rachel Kaplan
rmkapla1@uno.edu

The Complexities of Crankiness and Cortisol: Exploring the Association Between Irritability, Cortisol Reactivity, and Psychopathology

A Thesis

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Master of Science
in
Psychology

by

Rachel M. Kaplan

B.S. Salem State University, 2016
B.A. Salem State University, 2016

May, 2021

Acknowledgement

I first want to thank my advisor, Dr. Sarah R. Black. Even during a global pandemic and new motherhood, she always managed to guide me with patience, humor, and critical but constructive feedback that I needed to hear. Her mentorship has been invaluable and her warmth instrumental in my personal and professional development.

Next, I want to thank my committee members, Drs. Yuliya Kotelnikova and Chris Harshaw, for their insights, tolerance, and support. Their incisive feedback during this process was invaluable and I only wish I had taken more advantage of their expertise.

Finally, I want to thank my astute and loving partner, DRB, for his encouragement and support. He pulled late nights to assist me in making tables (so many tables!), helped me organize and talk through my ideas, made many a dinner, and was an incomparable cat dad.

Table of Contents

List of Figures

List of Tables

Abstract

Irritability is an indicator and predictor of psychopathology, as well as a sign of acute and chronic stress. Cortisol reactivity (CR), a physiological index of psychological stress, is bidirectionally associated with and predictive of psychopathology. Research addressing irritability and CR together is limited. Participants were 156 children enrolled in a longitudinal study. At age three, saliva was collected in relation to a stressor task and parents reported on child psychopathology. Psychopathology reports were also completed for ages six, nine, and 12. Results showed CR to have a moderating effect on the association between irritability and psychopathology symptoms when sex was included as an additional moderator, indicating that this moderating effect occurred differently for males and females. These findings underscore the importance of considering both biological and psychological variables, as well as sex differences, in understanding future risk for psychopathology.

Keywords*:* irritability, cortisol reactivity, ADHD, depression, psychopathology, sex, puberty

Introduction

Psychological disorders emerge from transactions between environmental (e.g., familial, social), biological (e.g., genetic, hormonal), and other individual (e.g., emotion regulation, cognitive style) factors (Goldberg, 2010; Papadimitriou, 2017), as well as dysregulated stress reactivity (Hankin et al., 2015). According to the Centers for Disease Control and Prevention (CDC), 17.4% of American children aged two to eight years old have a diagnosed psychological disorder (CDC, 2020). Research indicates that early psychopathology is associated with poor outcomes during childhood and into adulthood (Finsaas et al., 2020; Loth et al., 2015; Obradović et al., 2010). Psychopathology in childhood is also associated with academic impairments (e.g., low grades and disciplinary actions; Essau et al., 2017), social struggles (e.g., bullying, maintaining friendships; van Lier & Koot, 2010), family-life challenges (e.g., rebelliousness, adjustment issues; Pihlakoski et al., 2006), and in some cases, legal consequences (e.g., disciplinary measures due to theft, truancy; Murray et al., 2010). Early psychopathology also predicts problems in the professional, social, familial, and public realms of adulthood (Heijmens Visser et al., 2000).

For decades, the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), now in its fifth edition, has been considered the authority on determining the presence of psychopathology using the categorical method (i.e., an individual either meets criteria for a disorder or does not; APA, 2013). However, emerging research has shown that dimensional approaches (e.g., The Research Domain of Criteria [RDoC], The Hierarchical Taxonomy of Psychopathology [HiTOP]), in which symptoms are considered along a continuum within multiple domains or along symptom levels; Beauchaine & Hinshaw, 2017; Kotov et al., 2017) avoid the issues observed in categorical approaches (e.g., arbitrary boundaries and cutoffs, not meeting criteria but still having impairment due to severity of symptoms, covarying symptoms between disorders, and a failure to address the heterogeneity within disorders and homogeneity between disorders; Brown & Barlow, 2005; Haslam, 2003). Hence, examining psychological disorders at the symptom level, as opposed to dichotomously, may offer more insight into how psychopathology develops and why some individuals experience more and/or different impairment than others.

Emotion and mood dysregulation (i.e., irritability) have been observed in numerous individuals with psychological impairment, including disorders related to anxiety, depression, attention-deficit/hyperactivity disorder (ADHD), and oppositional defiant disorder (ODD; Roy et al., 2019). Similarly, research has also shown associations between these disorders and dysregulated stress responsivity (i.e., atypical cortisol reactivity; Sapolsky et al., 1986; Zorn et al., 2017). Of particular interest are depression and ADHD, which are among the most commonly diagnosed and impactful disorders in youth (CDC, 2020). Irritability is a symptom of depression in children and is part of the phenomenology of depression in adults and individuals with ADHD (APA, 2013; Roy et al., 2019). Atypical cortisol reactivity has been observed in cases of anxiety, depression, ADHD, and ODD. However, the literature related to depression and ADHD are especially mixed, indicating that some individuals with depression or ADHD symptoms have one type of dysregulated cortisol reactivity, whereas other individuals have another type (e.g., elevated vs. blunted reactivity; Kamradt et al., 2018; Lopez-Duran et al., 2009; Zorn et al., 2017).

Understanding how psychopathology develops is crucial for prevention and treatment, but differences such as these makes psychopathology research complex. Considering how factors such as irritability and cortisol reactivity interact may shine a light on how psychopathology, and ADHD and depression in particular, develops and for whom.

Irritability

Irritability is an apt example of this heterogeneity since it is a transdiagnostic symptom associated with numerous mood and behavioral disorders (American Psychological Association [APA], 2013). Atypical irritability is a common symptom found in both internalizing (i.e., mood, anxiety disorders) and externalizing (i.e., impulse-control, behavioral disorders) disorders, as well as being implicated in poor mood, emotion dysregulation, and extreme behaviors (e.g., destructive tantrums; Eyre et al., 2017; Wiggens et al., 2018). Irritability is often understood as (1) a verbal and/or nonverbal behavior, and (2) a negative emotion or mood (Barata et al., 2016). In the *DSM-5* (APA, 2013), irritability is cited in the diagnostic criteria for multiple disorders, including depressive, bipolar, anxiety, trauma, personality,

oppositional, substance-use, and neurodevelopmental disorders (Toohey & DiGiuseppe, 2017). Additionally, the *DSM-5* has included a relatively new disorder for children, housed within the mood section, called disruptive mood dysregulation disorder (DMDD; APA, 2013; Evans et al., 2021b). DMDD was designed to capture the growing number of severe irritability cases that were being (potentially) misattributed to child mania/bipolar disorder; however, further research has shown that DMDD lacks validity, especially when compared to ODD (Evans et al., 2021b). As Evans et al. (2021b) remark, "the vast majority of youth with DMDD would already (empirically, if not by definition) receive a diagnosis of ODD, and the DMDD diagnosis shows no incremental validity or utility beyond ODD." It is important to note that ODD always includes irritability as a symptom, but irritability may also be distinctly associated with other disorders, such as depression (Stringaris et al., 2018).

In early childhood, irritability is typically a response to frustrations and undeveloped effortful control, but it may also be a sign of stress and poor mental health, especially when chronic or severe (Brotman et al., 2017; Copeland et al., 2015; Leibenluft et al., 2006; Toohey & DiGiuseppe, 2017). Enduring and/or extreme bouts of irritability are key indicators of current mood dysregulation and psychopathology, especially in children and adolescents (APA, 2013; Brotman et al, 2017; Leibenluft et al., 2006).

Previous research also demonstrates longitudinal associations between atypical irritability in early childhood and later psychopathology (Brotman et al., 2006; Dougherty et al., 2015; Keefe et al., 1996; Stringaris et al., 2009; Vogel et al., 2019). For example, Dougherty et al. (2015) reported that increased irritability at age three predicted impaired functioning, increased anxiety, and disruptive behaviors at age nine. Similar results were found by Vogel et al. (2019), in which mood disorders and ADHD in adolescence were predicted by irritability and excitability (i.e., positive emotion dysregulation) during preschool.

Within disorders, irritability also presents differently among individuals (Mick et al., 2005; Jha et al., 2019, Pine, 2019). When considered as a component of emotion dysregulation, irritability is a common yet heterogeneous symptom of ADHD (Shaw et al., 2014). For example, Mick et al. (2005)

found that children with ADHD and no comorbid disorders were more likely to present with ODD-type irritability (e.g., temper tantrums, anger, resentment, easily annoyed). On the contrary, children with ADHD and a comorbid mood disorder were more likely to have irritability that fell within the depressive-type (i.e., mad/cranky). Irritability is also a common symptom of depression in adults and part of the criteria for diagnosis in children (APA, 2013). Jha et al. (2019) found that irritability severity predicted treatment outcomes in adults with major depressive disorder (MDD), with reduced irritability from early treatments (i.e., psychopharmacological therapy) showing increased likelihood for remission. In children with depression, irritability may present as increased susceptibility to anger, without reaching a tipping point into behavioral demonstrations, such as temper outbursts or aggressive displays (e.g., irritability that drives the individual to withdraw instead of lash out; Stringaris et al., 2018). Interestingly, Evans et al. (2021a) found that child self-reported irritability was moderately associated with internalizing disorders such as depression, whereas parent-report on child irritability had little to no association with these disorders.

In addition to comorbid disorders as a potential reason for differences, it may also be that external factors, such as family dynamic and parenting style are contributing to the difference between children with depression and more apparent irritability vs. children with depression and more withdrawn-type irritability (Stringaris et al., 2018). This variability may contribute to a better understanding of differences in psychopathology development and course. That is, why some individuals develop affective and behavioral disorders while others do not. As such, it is important to investigate third variables that may influence the relation between irritability and psychopathology. Specifically, biological factors, such as stress reactivity, may partially explain how irritability is associated with different types of psychopathology in different individuals (Leibenluft & Stoddard, 2013).

Cortisol Reactivity

Cortisol reactivity (CR), a biological measure of acute psychological stress, may help to clarify how irritability and psychopathology are associated (Mikita et al., 2015; Morris et al., 2017). CR originates in the hypothalamic-pituitary-adrenal (HPA) axis, a stress response network consisting of

feedback interactions between the central nervous system (i.e., hypothalamus) and endocrine system (i.e., pituitary and adrenal glands; Nelson, 2011; Sapolsky et al., 1986). When the individual senses a salient stressor, a hormone cascade commences simultaneously with the immediate stress reactivity of the sympathetic nervous system (SNS; Nelson, 2011; Sapolsky et al., 1986). Beginning in the hypothalamus, corticotrophin releasing hormone (CRH) is secreted in response to increased epinephrine and norepinephrine levels (from the SNS). A sudden surge of CRH in the bloodstream stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH), which then signals to the adrenal glands to excrete cortisol (Nelson, 2011; Sapolsky et al., 1986).

Cortisol is one of the measures by which HPA activity is indexed and dysregulation is measured (Nelson, 2011; Sapolsky et al., 1986). Cortisol secretion follows a 24-hour circadian rhythm (Nelson, 2011). An individual's cortisol levels rapidly rise upon waking (called the cortisol awakening response [CAR]) from an already high level, peak about 30 minutes after waking, and then slowly decline and plateau until bedtime. Throughout the day, spikes can be partly attributed to stress responsivity from physical and psychosocial threats (Hankin et al., 2015).

The HPA axis is especially responsive to stressors that include psychosocial threats, such as those that jeopardize perceptions of self-image, and particularly those that do so in novel and unpredictable ways (Dickerson & Kemeny, 2004; Koss & Gunnar, 2018). Cortisol reactivity, in particular, is a physiological marker often used to measure acute social and psychological stress (Dimitrov et al., 2018). Atypically elevated CR, however, indicates heightened arousal and is potentially due to increased anxiety and emotional reactivity (Bagner et al., 2010; McEwen, 1998). Significantly blunted CR – meaning the stress exposure did not incite a normative increase in cortisol – suggests a lack of responsivity caused by chronic hormone secretion over time, often due to repeated exposure to stressful stimuli, mechanistic problems in the HPA axis, or heightened responsivity to innocuous conditions (Lupien et al., 2009; McEwen, 1998).

Like irritability, previous research on atypical CR has shown it to be associated with and predictive of psychopathology (Barrios et al., 2017; Doom & Gunnar, 2013; Grimm et al., 2017; Gunnar

& Vazquez, 2006; Morris et al., 2012; Nelson, 2011; Pariante, 2003; Sapolsky et al., 1986; Slavich & Irwin, 2014; Zorn et al., 2017). This association is observed in children, adolescents, and adults (Doom & Gunnar, 2013; Spies et al., 2011; Steeger et al., 2017; Zorn et al., 2017) and may have origins in early childhood (Barrios et al., 2017; Shonkoff et al., 2009). Significantly elevated CR has typically been associated with internalizing disorders, while atypically blunted CR is often associated with externalizing disorders (Bagner et al., 2010; Ruttle et al., 2011; Zorn et al., 2017). Studies examining specific disorders have observed anxiety and depression to be associated with atypically elevated CR (Bagner et al., 2010; Zorn et al., 2017), while disruptive behavior disorders (e.g., ADHD and ODD) were associated with blunted CR (McBurnett et al., 2000; Ouellet-Morin et al., 2011; Zorn et al., 2017).

However, the literature also reflects inconsistencies in studies of CR to stress and psychopathology, especially with regard to ADHD and childhood depression (Alink et al., 2008; Gunnar et al., 2009b; Harkness et al., 2011; Jaffee et al., 2015; Lopez-Duran et al., 2009; Mazurka et al., 2016; Melham et al., 2016; Ruttle et al., 2011; Suzuki et al., 2013). While some research has demonstrated the expected blunted CR response in children and adults with ADHD (Alink et al., 2008; Hong et al., 2003; King et al, 1998), other studies have shown an elevated CR effect (Corominas-Roso, 2015; Raz & Leykin, 2015; van West et al., 2009). Still other findings have shown no significant differences when compared with healthy controls (Alink et al., 2008; Hirvikoski et al., 2009; Snoek et al., 2004).

Some explanations for these inconsistencies revolve around methodological issues/discrepancies between studies (Kamradt et al., 2018). For example, the stressor task used by researchers may be deficient for eliciting CR, especially in children (Gunnar et al., 2009a). Time of day in which saliva samples are collected may impact the cortisol curve used to detect mean CR, especially if the samples are produced too early in the day (i.e., too close to the CAR; Koh & Koh, 2007). Intra and inter-assay covariance issues leading to measurement errors, especially in non-established or under-qualified labs (Calvi et al., 2017) and not accounting for cortisol disrupting over-the-counter and/or prescription medications are other methodological issues that have may have contributed to the inconsistencies in CR literature (Granger et al., 2009).

Some research has attempted to address these inconsistencies by including moderators, such as traits and disorder subtypes, to their models (Maldonado et al., 2009; Northover et al., 2016; Stadler et al., 20011; van West et al., 2009). In a sample of eight to 14-year-old males, Stadler et al. (2011) found that the association between CR and ADHD was moderated by callous-unemotional (CU) personality traits. Specifically, higher levels of CU traits were associated with blunted CR in males with ADHD (Stadler et al., 2011). Northover et al. (2016) showed similar results, finding that 10 – 17-year-old males with ADHD were more likely to have blunted CR if they had a comorbid diagnosis of conduct disorder. In a meta-analytic review of the relation between CR and ADHD, Kamradt et al. (2018) emphasized the methodological heterogeneity in the literature and found there to be no overall effect between ADHD and CR. Further, they noted that moderators may better explain these findings (Kamradt et al., 2018). However, a limited sample size ($K = 12$) may be the reason for non-significant findings on the association between CR and ADHD. In a study of five to eight-year-olds, Maldonado et al. (2009) observed that children with higher levels of hyperactivity and impulsivity tended to have blunted CR when compared with healthy controls and other ADHD subtypes (i.e., inattentive). In a similar study of six to 12-year-olds with ADHD, van West et al. (2009) found that elevated CR was associated with the inattentive ADHD subtype, whereas blunted CR was associated with the combined subtype (inattentive and hyperactive). On the contrary, Pesonen et al. (2011) found blunted CR to be associated with the inattentive subtype in a large-scale study of eight-year-olds in Finland.

Similar inconsistencies exist for children and adolescents with depression. A meta-analysis on HPA dysregulation in children and adolescents found larger between-subjects CR variability within the depressed samples when compared to healthy controls (Lopez-Duran et al., 2009). These findings indicate potential methodological issues across studies, but also highlight the heterogeneity and variability of CR in children with depression. Luby et al. (2003) found that preschoolers with depression had elevated CR after a psychosocial stressor when compared to both healthy controls and, interestingly, children with ADHD or ODD. However, Suzuki et al. (2013) observed blunted CR following a stress task in preschoolers with either full or subclinical major depressive disorder (MDD). When confronted with

similar follow-up stress tasks in subsequent visits occurring 12 and 24 months after the first visit (i.e., longitudinal waves two and three), children with any history of MDD showed blunted CR and a failure to adjust (Suzuki et al., 2013). Harkness et al. (2011) found depression to be associated with blunted CR in a sample of 12 – 21-year-olds. In a cross-sectional study of preschoolers, third, sixth, and ninth graders, Hankin et al. (2010) observed blunted CR in children (i.e., preschool to sixth grade age range) at risk for depression and elevated CR in ninth graders in the at-risk group, when compared to healthy controls.

Some research suggests that comorbidity may partially explain these inconsistencies (Hastings et al., 2009; Yoon & Joormann, 2012; Young et al., 2004). For example, a sample of six – 11-year-old males with ADHD and either comorbid anxiety or a disruptive behavior disorder showed significantly different CR responses to a stressor (i.e., venipuncture) than children without a comorbid disorder (Hastings et al., 2009). Specifically, males with ADHD and comorbid anxiety had elevated CR, whereas males with ADHD and comorbid disruptive disorders had blunted CR (Hastings et al., 2009). Relatedly, in a study of social anxiety disorder (SAD) and major depressive disorder (MDD), Yoon and Joormann (2012) found that adults with SAD showed elevated CR, but comorbid MDD had a dampening effect on CR.

Like the CR and ADHD subtype findings, research on depression and CR has indicated differences may be related to symptom type (Luby et al., 2003). Specifically, several studies have shown that individuals with melancholic depressive traits tend to have significantly elevated CR when compared to other depressive traits and healthy controls (Luby et al., 2003; Morris et al., 2017; Stetler & Miller, 2011). These effects have been observed in child, adolescent, and adult samples. A 2011 meta-analysis examining 40 years of HPA and depression research noted CR differences in depression form (i.e., atypical, melancholic, psychotic subtypes from the DSM-IV), such that individuals with atypical depression (i.e., symptoms of hypersomnia, fatigue, weight gain, and increased interpersonal emotional reactivity) showed relatively blunted CR when compared with individuals in the melancholic depression group (i.e., symptoms of anhedonia, insomnia, diminished appetite, mood instability, feelings of excessive guilt and worthlessness; Stetler & Miller, 2011). In a sample of preschoolers, Luby et al. (2003) found that children in a melancholic depression subgroup had significantly higher CR than children in all

other comparison groups (i.e., healthy controls, ADHD and ODD group, non-melancholic subgroup) after a stress task.

Irritability and Cortisol Reactivity

The minimal literature on irritability and CR together suggests that irritability may be more associated with blunted CR (Mikita et al., 2015; Morris et al., 2017). Mikita et al. (2015), for example, found that boys with autism spectrum disorder (ASD) and high irritability scores showed blunted CR post lab stressor. Notably, ASD is a neurodevelopmental disorder exhibiting both internalizing (i.e., anxiety and depression) and externalizing (i.e., aggression and impulsivity) symptoms (APA, 2013; Bauminger et al., 2010). Morris et al. (2017) found that irritability had a stronger association with blunted CR when specific symptoms (e.g., insomnia, hypersomnia, fatigue, appetite problems) were present in a sample of adolescents with major depressive disorder (MDD) or at high risk for depressive disorders. That significant results have been found implies more research should be done to better examine how irritability and CR interact.

Sex and Puberty

In addition to irritability and CR, sex and puberty have also shown to be associated with depression and ADHD outcomes. Numerous studies have shown sex to be related to mental disorder prevalence and manifestation. In early and middle childhood, externalizing disorders (such as ADHD and ODD) are diagnosed more frequently in males, while internalizing disorders (e.g., anxiety and depression) show fewer sex differences in prevalence (APA, 2013; Beauchaine & Hinshaw, 2017). However, sex discrepancies in disorder prevalence may be partially attributed to different symptom manifestations (e.g., physical aggressiveness in males and verbal aggressiveness in females with disruptive disorders) and rates of comorbidities (e.g., more anxiety comorbidity in females; Chaplin & Aldao, 2013; Hartung & Lefler, 2019; Skogli et al., 2013). Throughout childhood and adolescence, emotion dysregulation also manifests differently in males and females (Chaplin & Aldao, 2013). Specifically, males in early and middle childhood expressed more externalizing emotions (i.e., anger) than their female counterparts, who tended to show more internalizing emotions (i.e., sadness; Chaplin & Aldao, 2013). However, these findings

changed in adolescence, with females exhibiting more externalizing emotions than their male counterparts (Chaplin & Aldao, 2013). Pubertal timing may also play a role in psychopathology, as many psychological disorders emerge in conjunction with the biological and environmental changes that occur during puberty (Ge & Natsuaki, 2009; Pomerantz et al., 2017; Shelton & van den Bree, 2010; Sontag et al., 2011; Sontag-Padilla et al., 2012; Ullsperger & Nikolas, 2017).

Additionally, research has shown sex and puberty to be associated with irritability, though results are inconsistent. In a sample of nine to 16-year-olds with depression, Stringaris et al. (2013) observed that males had significantly higher irritability scores than their female counterparts. Stewart et al. (2020) observed that adolescent females within the youth justice system scored higher on aggression and irritability measures than their male counterparts and both sexes in the general population, especially if they were diagnosed with ADHD and/or ODD. In investigating the relationship between irritability and future psychiatric diagnoses, Dougherty et al. (2015) found that irritability at age three predicted ADHD in males, but not females, at age nine. The same study observed age three irritability to be predictive of lifetime anxiety disorder at age nine for females, but not males (Dougherty et al., 2015). Pubertal onset, along with other biological and social changes, may also influence irritability symptomatology (Mendle, 2014). Specifically, a review of puberty and psychopathology found that depression during childhood reflects more somatic irritability symptoms (e.g., agitation), whereas depression in pubescence includes internalizing irritability (e.g., social withdrawal and hopelessness; Mendle, 2014).

Associations between CR and sex have also been researched, though much of the published research has been on adults and adolescents (Kelly et al., 2008; Liu et al., 2017). In healthy adults, males tend to have higher CR post stress tasks than females (Kirschbaum et al., 1993; Kudielka & Kirschbaum, 2005; Liu et al., 2017; Zimmer et al., 2003). In adults with psychopathology, studies reflect sex differences in CR as well. For example, Powers et al. (2016) found that females with depression had blunted CR post stressor, whereas males with depression and females with anxiety had elevated CR post stressor. Similar findings showed blunted CR in females with MDD and elevated CR in their male counterparts (Zorn et al., 2017). Several studies on CR in adolescents have been comparable to adult

studies, finding sex to moderate associations between CR and psychopathology (Colich et al., 2015; Klimes-Dougan et al., 2001; Mazurka et al., 2017). A study on stress reactivity and depression in 12 – 18-year-olds found that females with depression had blunted CR when compared to their male counterparts and other females without depression (Mazurka et al., 2017). That is, the social, morphological, environmental, and hormonal changes that females experience in pubertal development may increase the likelihood of encountering stressors and developing psychopathology (Graber, 2013; Hyde et al., 2008; Natsuaki et al., 2011). Colich et al. (2015) found CR patterns similar to Mazurka et al. (2017) in early adolescent males and females.

There is minimal research looking at sex differences in CR and psychopathology in children. In a study examining sex, CR, and psychopathology symptoms over time, Daoust et al. (2018) found that CR at age three was predictive of internalizing and externalizing symptoms at age five. Specifically, three-year-old males and females with elevated CR were more likely to have fewer externalizing symptoms (specifically ODD) when they were re-assessed at age five. However, males with blunted CR at age three did not have these same outcomes. Further, females with blunted CR at age three tended to have fewer symptoms of depression at age five (Daoust et al., 2018). It is also possible that sex differences related to early CR and symptomology emerge during early adolescence, especially if pubertal onset is a moderator. There is ample research suggesting the role of hormonal changes (i.e., CR changes) in psychopathology during puberty (Colich et al., 2015; Ge & Natsuaki, 2009 Gunnar et al., 2009b; Hankin et al., 2010); however, this research is examining CR and puberty concurrently, as opposed to early childhood CR interacting with pubertal development.

The Present Study

Numerous studies have examined the predictive qualities of early irritability and CR on psychopathology separately (irritability: Dougherty et al., 2017; Stringaris et al., 2009; Wiggins et al., 2018; CR: Barrios et al., 2017; Hankin, 2015; Morris et al., 2012). However, the existing research addressing irritability and CR together is sparse. Hence, the present study aimed to investigate how the

interaction between irritability and cortisol reactivity is related to psychopathology, and further, how sex and puberty may influence that interaction.

The present study specifically examined ADHD and depression symptoms in the context of irritability, CR, and sex/puberty, for two main reasons. First, irritability is a heterogeneous yet commonly reported symptom in both ADHD and depression. As such, biological factors, such as CR and sex/puberty, may clarify how psychopathology develops in individuals with high irritability. (i.e., why irritability is associated with depression in some individuals but ADHD in others.) Second, and as noted earlier, there is inconsistency in the literature on CR and depression and ADHD, with previous studies demonstrating both elevated and blunted CR to be associated with both depression and ADHD (Hankin et al., 2010; Harkness et al., 2011; Luby et al., 2003; Maldonado et al. 2009; Pesonen et al., 2011; van West et al., 2009; Zorn et al., 2017). These inconsistencies in the literature merit further exploration of these associations. While both irritability and atypical CR have shown to be associated with anxiety and ODD, these disorders were not of particular interest for the present study. One reason for this is because previous research on CR and psychopathology has not shown the same inconsistencies that have been found for ADHD and depression. Another reason to exclude ODD as an outcome of interest is because it is often too highly correlated with irritability, suggesting the constructs may not be distinct enough to test in such a way. Indeed, severe irritability is often a symptom of oppositionality (Evans et al., 2021b; Stringaris et al., 2018).

The present study used dimensional, symptom-based scores to examine ADHD and depression for several reasons. First, ADHD and depression are heterogeneous disorders with diverse symptom presentations (Fried, 2017; Luo et al., 2019; Lynch et al., 2020; Mick et al., 2005; Pine, 2019). As such, categorical methods make it more difficult to discern heterogeneity (Güleç et al., 2014; Krueger & Bezdjian, 2009). Additionally, using a more dimensional method may clarify results since the majority of CR and psychopathology literature relies on the *DSM* and other categorical approaches. Further, a dimensional scale addresses all levels of impairment, as may be observed in a non-clinical sample (Bjelland et al., 2009). Second, a dimensional score allows irritability to be removed from the

psychopathology symptom scores, thus accounting for construct overlap (Dougherty et al., 2015). Third, there are numerous developmental differences in symptom manifestation between ages three and 12 years, as such a dimensional approach is especially appropriate when examining psychopathology in children and adolescents (Barch et al., 2019; Beauchaine & Hinshaw, 2017). Finally, the dimensional approach allows for a more comprehensive understanding of child behaviors, as both children within and outside of psychopathological criteria may be included in assessment (Andrews et al., 2001; Kamphaus et al., 1999). When the entire spectrum is represented, fewer children fall through the proverbial cracks.

Aims and Hypotheses

The current study aimed to explore the complex associations between childhood irritability, cortisol reactivity, and ADHD and depression symptoms in the context of sex and puberty. To do so, we tested four hypotheses.

Hypothesis One. Irritability at ages three, six and 12 would be significantly associated with or predictive of ADHD and depression symptom scores at ages three, six, nine, and 12. It was expected that these results would support the literature on irritability and psychopathology. Specifically, higher irritability levels would be associated with more depression symptoms. The direction of the association between irritability and ADHD scores was unspecified due to mixed literature on irritability in ADHD (Mick et al., 2005).

Hypothesis Two. Cortisol reactivity at age three would moderate the association between irritability (ages three, six, and 12) and ADHD and depression symptoms (ages three, six, nine, and 12). The direction of this moderated effect was unspecified due to the dearth of existing literature reflecting the relation between irritability and cortisol reactivity.

Hypothesis Three. Cortisol reactivity at age three would moderate the association between irritability (ages three, six, and 12) and ADHD and depression symptoms (ages three, six, nine, and 12) differentially by sex. The direction of this three-way interaction was exploratory and therefore the direction was unspecified.

Hypothesis Four. In another three-way interaction, CR at age three would moderate the association between irritability (ages three, six, and 12) and ADHD and depression symptoms (age 12) differentially by pubertal stage (age 12). Although this was also an exploratory analysis, it was hypothesized that earlier pubertal onset, in conjunction with high irritability and atypical CR (age three), would be associated with higher symptom scores, especially for depression. However, the direction of the CR (i.e., elevated or blunted) was unspecified. Age nine pubertal scores were considered for inclusion in the model, pending further preliminary analyses.

Methods

Participants

The current study used data obtained from a larger longitudinal study exploring the relation between temperament and psychopathology (Klein & Finsaas, 2017). Families with a child between three and four-years old were recruited using a commercial mailing list in the Stony Brook, NY area (Olino, Klein, Dyson, Rose, & Durbin, 2010). Eligible child participants were free of significant medical and developmental afflictions (Olino et al., 2010). At least one parent participant spoke English and was biologically related to the child participant (Olino et al., 2010). Of the 559 children who participated in the age 3 assessment, 160 were randomly chosen to participate in cortisol reactivity collection during one of the laboratory tasks (Barrios et al., 2017). Four participants were excluded from analysis due to saliva sample values (see section on cortisol collection procedure), leaving a sample of 156 three-year old children (Barrios et al., 2017). Of those 156 children, 149 had complete psychopathology data from the first wave. Of the 149 three-year-old participants, 88% returned for the age six visit, 84% at age nine, and 81% at age 12. The final sample for analysis consisted of 149 participants at age three, 130 at age six, 119 at age nine, and 115 at age 12. The mean age of the final analysis sample at the first visit was 3.63 years (SD = .24), at the second visit was 6.28 years (SD = .31), at the third visit was 9.31 years (SD = .38), and at the fourth visit was 12.91 years (SD = .32). Participants were 51% (n = 80) female, and predominantly Caucasian (87%, n = 136); the remaining 12.8% (n = 20) identified as non-white and/or Hispanic/Latino. 67% (n = 105) of participants had parents that both graduated from college. Sensitivity analyses were

performed to compare the 156 cortisol reactivity participants with the rest of the sample (N = 440). When compared to the rest of the sample, children in the cortisol reactivity subsample had lower anxiety scores at ages three, $t(539) = -3.0$, $p < .01$, and nine, $t(486) = -2.2$, $p = .02$. They also had lower ADHD, $t(539) = -2.2$, $p = .03$, and ODD scores at age three, $t(539) = -3.2$, $p = .001$, but higher ODD scores at age 12, $t(474) = 2.0$, $p = .04$. Children in the CR subsample were also more likely to identify as White and non-Hispanic, $t(607) = 2.7$, $p < .01$. Pubertal scores at age nine were highly skewed to the left, indicating significantly low rates of pubertal onset, and kurtotic, suggesting higher variation among those lower scores. Descriptive statistics for age, pubertal status, and psychopathology are in Table 1.

Table 1

Descriptive Statistics of Study Participant Age, and Raw Scores for Psychopathology, Irritability, Cortisol Reactivity, and Puberty

Variable	N	Range	Mean	SD	Skew	Skew *SE*	Kurtosis	Kurtosis *SE*
Age at Visit								
Visit 1 (age 3)	156.00	1.08	3.63	0.24	-0.38	0.19	-0.46	0.39
Visit 2 (age 6)	126.00	1.96	6.28	0.31	1.33	0.22	2.51	0.43
Visit 3 (age 9)	116.00	2.17	9.31	0.38	1.98	0.22	4.22	0.45
Visit 4 (age 12)	121.00	2.08	12.91	0.32	1.42	0.22	4.03	0.44
Pubertal Score								
Age 9	123.00	9.00	7.15	1.81	1.52	0.22	3.43	0.43
Age 12	120.00	12.00	11.39	3.23	-0.39	0.22	-0.85	0.44
CR age 3	156.00	592.77	31.29	148.25	-0.45	0.19	-0.01	0.39
Age 3 Scores								
Irritability		3.83	-0.06	0.92	2.06	0.20	3.40	0.39
ADHD		5.00	0.49	1.39	2.73	0.20	5.91	0.39
Depression	149.00	9.24	1.71	1.79	1.56	0.20	3.15	0.39
Anxiety		31.36	6.29	5.26	1.30	0.20	3.13	0.39
ODD		11.00	1.52	2.90	1.75	0.20	1.69	0.39
Age 6 Scores								
Irritability		4.50	0.07	1.02	1.56	0.21	1.73	0.42
ADHD		12.40	2.80	3.50	1.50	0.21	1.34	0.42
Depression	131.00	7.18	2.92	2.40	0.95	0.21	0.91	0.42
Anxiety		42.24	10.61	11.77	1.38	0.21	2.09	0.42
ODD		7.00	1.33	2.19	1.10	0.21	0.71	0.42
Age 9 Scores								
ADHD		31.00	5.45	9.04	1.58	0.22	1.06	0.43
Depression	125.00	3.00	0.30	0.79	2.76	0.22	6.49	0.43
Anxiety		22.00	2.82	3.95	2.40	0.22	7.27	0.43
ODD		14.00	1.43	3.14	2.46	0.22	5.48	0.43
Age 12 Scores								
Irritability		9.00	1.38	2.29	1.83	0.22	2.49	0.44
ADHD		33.00	4.32	7.92	1.79	0.22	2.22	0.44
Depression	121.00	3.00	0.29	0.78	2.77	0.22	6.58	0.44
Anxiety		14.90	2.63	4.05	1.99	0.22	3.08	0.44
ODD		18.00	1.38	3.47	2.97	0.22	8.73	0.44

Note: ADHD = Attention-deficit/hyperactivity disorder; ODD = Oppositional defiant disorder; CR = Cortisol reactivity; SD = standard deviation. As noted in the text, several variables were highly skewed and/or kurtotic. CR had an initial skew of -1.70 and a kurtosis statistic of 25.58. Age three ADHD had a skew of 5.49 and kurtosis of 34.44. Age six ADHD had a skew of 3.58 and kurtosis of 19.36. Age nine depression had a skew of 4.11 and kurtosis of 18.53. Age twelve depression had a skew of 4.32 and kurtosis of 19.68. Finally, age twelve anxiety had a skew of 3.24 and kurtosis of 12.38.

Measures and Procedures

Stress Task

During the initial visit, three-year-old children were administered 12 tasks from the Laboratory Temperament Assessment Battery: Preschool Version (Lab-TAB; Goldsmith, Reilly, Lemery, Longley, & Prescott, 1995) over a two-hour session. The selected tasks were used to produce a variety of emotions and behaviors from the three-year-old participants (detailed in Dougherty et al., 2011). During the Lab-TAB session, the Stranger Approach task was used to elicit a stress response in children. The Stranger Approach task entails briefly leaving the child alone in a room and then having an adult male confederate enter and speak to the child while gradually walking toward the child. After each Lab-TAB session, participants were given a two-minute play break (Barrios et al., 2017).

Child Cortisol Collection Procedure

The quantity and timepoint increments for salivary collection were derived from previous research suggesting that changes in salivary cortisol concentrations are associated with stress felt in the preceding 20 – 40 minutes of collection (Dickerson & Kemeny, 2004). Saliva samples were collected from three-year old participants four times. The first sample was collected after a habituation period (e.g., directly following informed consent) of approximately 20 minutes post arrival (baseline, Time one; Barrios et al., 2017). The second sample was taken 30 minutes upon completing the Stanger Approach task and is the expected peak of cortisol reactivity (baseline + 60 minutes, Time two). The third sample was collected 30 minutes after the second sample (baseline + 90 minutes, Time three). The final sample was collected 20 minutes after all Lab-TAB tasks were completed (baseline + 130 minutes, Time four). At each collection, participants were instructed to dip a cotton dental roll into .025g of powdered cherry Kool-Aid® and then to chew on it until sufficiently soaked with saliva. Participants took between one – two minutes to produce each sample. Upon collection of each sample, the saliva was extracted from the dental roll, transferred to a microtube, and then stored at -20°C until assayed. A time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA) was used to assay all samples in duplicate. Four different participants were excluded from the sample due to saliva values that exceeded

44 nanomoles per liter (nmol/L). The inter-assay coefficient of variation was between 7.1% – 9.0% and reflects adequate consistency between plates at <15% (calculated using the control high and low values from calculated concentrations). The intra-assay coefficient was between 4.0% – 6.7% and indicates acceptable consistency within the plates and between duplicate samples at <10%.

Saliva Analysis

Positive skew is often observed in the cortisol literature (Hankin et al., 2015). In this sample, cortisol reactivity measures were non-normally distributed, with skewness of -1.70 (*SE* = .19) and kurtosis of 25.58 (*SE* = .39). To protect against a skewed cortisol distribution, raw cortisol scores are typically log10 transformed. To measure cortisol reactivity, log10 transformed values of each timepoint were calculated using two formulae for area under the curve (AUC) as described in Pruessner et al. (2003). Area under the curve (AUC) with respect to ground (AUCg) is the measure of total cortisol output from zero (Pruessner et al., 2003). Area under the curve (AUC) with respect to increase (AUCi) considers the relative increase across time, starting at the initial concentration or baseline, as opposed to starting at zero (Pruessner et al., 2003). AUCi is calculated by subtracting the baseline mean value from AUCg (Pruessner et al., 2003). AUCi scores represented cortisol reactivity in all statistical models. High CR levels (+1SD) are described here as *elevated* and low CR levels (-1SD) as *blunted*.

Child Dimensional Psychopathology Symptoms

Ages Three and Six Psychopathology Symptoms. At ages three and six, parents were interviewed using the Preschool Age Psychiatric Assessment (PAPA; Eggers & Angold, 2004). The PAPA is a structured diagnostic interview intended to evaluate DSM-IV psychiatric disorders, such as anxiety, depression, ADHD, and ODD in preschool-aged children and is appropriate for children aged two – to – eight – years (described in Dougherty et al., 2011). By speaking with the parent, the interviewer decided if the individual criteria for the disorders are present or not. If present, the interviewer also coded for intensity and duration of symptoms. Dimensional scores were calculated by summing the items and excluding symptoms that overlapped with the PAPA derived irritability scores (see Dougherty et al., 2015). The

Cronbach's alpha coefficient for dimensional scores had good internal consistency (ADHD: $\alpha = .88$, depression: $\alpha = .74$, anxiety $\alpha = .85$, ODD: $\alpha = .79$).

Ages Nine and Twelve Psychopathology Symptoms. At ages nine and 12, both parents and children were individually interviewed using the Kiddie Schedule for Affective Disorders (K-SADS- PL; Kaufman et al., 1997). The KSADS is a semi-structured interview designed to measure past and current symptoms related to psychiatric disorders, such as those associated with ADHD, anxiety, depression, and ODD. Dimensional scores were derived from summing the symptoms for each disorder. As with the dimensional scores from the PAPA, the KSADS dimensional scores excluded irritability items (see Dougherty et al., 2015).

Both the PAPA and the KSADS measure current (i.e., within the last 12 months) and lifetime psychiatric symptoms. This study only used dimensional scores derived from current symptoms as reported by the parent. The Cronbach's alpha coefficients for dimensional psychopathology showed moderate to good internal consistency at age nine (ADHD: $\alpha = .86$, depression: $\alpha = .84$, anxiety $\alpha = .85$, ODD: $\alpha = .72$), and strong internal consistency at age 12 (ADHD: $\alpha = .99$, depression: $\alpha = .98$, anxiety $\alpha = .96$, ODD: $\alpha = .96$).

Child Irritability Scores

Ages Three and Six Irritability Scores. Ages three and six irritability scores were calculated using six items from the PAPA that corresponded to the age 12 measure for irritability. The questions were derived from the depression and ODD sections of the PAPA. Final scores were summed for ages three and six, respectively. Standardized irritability scores for this sample ranged from -.53 to 3.3 ($M = -.06$, $SD = .91$) at age three, and -.6 to 4 ($M = .07$, $SD = 1.1$) at age six. See Dougherty et al. (2013, 2015) for a more in-depth description of how this measure was calculated from the PAPA. The Cronbach's alpha coefficient for ages three and six irritability scores had good internal consistency ($\alpha = .73$).

Age Twelve Irritability Scores. The Affective Reactivity Index (ARI; Stringaris et al., 2012), is a well-validated measure of chronic irritability in children and adolescents. The ARI consists of seven items that

are each given a score of 0 (“Not true”), 1 (“Somewhat true”), or 2 (“Certainly true”). To obtain a final score, items one through six are summed. Item seven assesses the impairment of the irritability. The highest possible score is 18. Participants in this sample scored between 0 and 9 (M = 1.4, SD = 2.3) or, when standardized between -.6 and 4 (M = .12, SD = 1.2). ARI scores were parent-report and had good internal consistency for both mother and father report (α = .85, α = .83, respectively).

Pubertal Development

To evaluate pubertal stage at ages nine and 12, the Pubertal Development Scale (PDS; Petersen et al., 1988) was administered. Males and females answered questions on a four-point scale (1 = not yet started puberty, 2 = barely started, 3 = definitely started, and 4 = seems complete) regarding body and facial hair, skin changes, voice changes (males only), breast development (females only), and menarche (females only). Pubertal development scores were derived by summing and averaging the scores. PDS scores were from parent report.

Data Analysis Plan

Analyses were run using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA) and PROCESS version 3.4 (Hayes, 2018). Except for sex and race, all variables were standardized (z-scored) for analysis in order to account for scale differences between waves. Preliminary bivariate correlations were run on dimensional scores for ADHD, anxiety, depression, and ODD (ages three, six, nine, and 12), irritability scores (ages three, six, and 12), cortisol reactivity (CR) AUCi (age three), child sex, race, and pubertal scores for ages nine and 12 (see Table 2).

Table 2

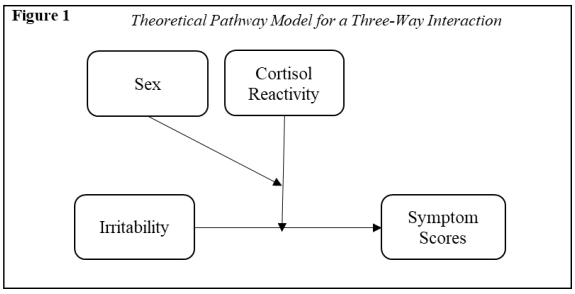
Bivariate Correlations for Variables of Interest and Covariates

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Anxiety age 3	_																		
2. Depression age 3	**0.40**	_																	
3. ADHD age 3	0.05	**0.34**	_																
4. ODD age 3	0.13	**0.58**	**0.47**	_															
5. Irritability age 3	0.16	**0.65**	**0.25**	**0.80**	_														
6. CR age 3	0.00	0.02	0.14	0.09	0.05	_													
7. Anxiety age 6	**0.47**	0.16	0.04	0.18	0.18	0.03	_												
8. Depression age 6	**0.33**	**0.43**	**0.24**	**0.44**	**0.38**	0.12	**0.50**	_											
9. ADHD age 6	0.10	**0.27**	**0.46**	**0.37**	0.19	0.06	0.14	**0.45**	_										
10. ODD age 6	**0.27**	**0.43**	**0.31**	**0.51**	**0.39**	0.07	**0.30**	**0.59**	**0.41**	_									
11. Irritability age 6	**0.21**	**0.36**	0.18	**0.50**	**0.42**	0.08	0.16	**0.50**	**0.32**	**0.66**	_								
12. Anxiety age 9	**0.23**	**0.20**	0.01	0.09	0.04	0.02	**0.48**	**0.33**	0.17	0.16	0.09	_							
13. Depression age 9	0.11	0.15	-0.04	0.10	0.13	0.07	0.11	**0.29**	0.07	**0.26**	**0.28**	0.12	_						
14. ADHD age 9	0.17	**0.23**	**0.35**	**0.28**	0.11	**0.25**	**0.21**	**0.32**	**0.60**	**0.30**	**0.20**	0.18	0.17	_					
15. ODD age 9	**0.23**	**0.59**	**0.20**	**0.53**	**0.55**	0.13	0.15	**0.39**	**0.25**	**0.47**	**0.45**	0.13	**0.40**	**0.24**	_				
16. Anxiety age 12	0.03	0.09	-0.04	0.07	0.06	-0.07	**0.24**	0.15	-0.02	0.14	0.16	**0.46**	0.07	0.09	0.16	_			
17. Depression age 12	0.04	0.10	-0.04	0.02	0.02	0.08	0.09	0.14	0.01	0.07	0.10	**0.22**	0.11	0.07	**0.24**	**0.40**	_		
18. ADHD age 12	**0.20**	**0.27**	**0.31**	**0.24**	0.09	**0.22**	**0.25**	**0.44**	**0.57**	**0.39**	**0.25**	**0.22**	**0.21**	**0.88**	**0.28**	0.17	**0.22**	_	
19. ODD age 12	0.08	**0.31**	0.10	**0.27**	**0.26**	0.13	0.09	**0.36**	**0.25**	**0.47**	**0.36**	0.08	**0.28**	**0.36**	**0.45**	0.19	**0.32**	**0.54**	_
20. Irritability age 12	**0.21**	**0.45**	0.02	**0.28**	**0.33**	0.08	0.05	**0.27**	0.10	**0.33**	**0.38**	0.13	**0.32**	**0.21**	**0.56**	**0.26**	**0.37**	**0.33**	**0.70**

Note: ADHD = Attention-deficit/hyperactivity disorder; ODD = Oppositional defiant disorder; CR = Cortisol reactivity. All correlations with $r \geq .2$ or were significant at < .05 level and/or are bolded. Sex was correlated with race (r = -.18, p < .05), age 9 ADHD symptoms (r = -.18, p < .05), age nine puberty (r = .20, p < .05), age 12 puberty (r = .44, p < .01), and age 12 anxiety (r = .19, p < .05). Race was correlated with age 6 ADHD symptoms (r = -.21, p < .05). Age 9 puberty was correlated with age 6 ADHD symptoms (r = .22, p < .05) and age 12 puberty (r = .47, p < .01).

Multiple linear regression models were used to test the hypotheses that the associations between irritability and psychopathology symptoms were moderated by sex and levels of CR. Hence, cortisol reactivity at age three and child sex served as moderating variables. Irritability at ages three, six, and 12 served as predictor variables. ADHD and depression dimensional symptom scores at ages three, six, nine, and 12 served as outcome or dependent variables (see Figure 1). ADHD and depression, in particular, were chosen as outcome variables because of the inconsistencies in the CR and psychopathology literature. Additionally, ODD was not included in the models as a covariate or outcome variable due to the disorder's high association with irritability (Evans et al., 2021b). Pubertal development scores at age 12 were used as a moderator variable in additional analyses with age 12 psychopathology scores serving as outcome variables. Moderation models were run using the PROCESS macro (Hayes, 2018).

Figure 1 *Theoretical Pathway Model for a Three-Way Interaction*



Covariates. Variables were initially selected as covariates if they were significantly associated with the outcome variables of interest (see Table 2). However, ADHD, anxiety, depression, and ODD tend to be significantly associated with irritability and each other (Evans et al., 2021b; Roy et al., 2019). To reduce multicollinearity issues, the symptom scores at each corresponding age were not included as covariates, nor were irritability scores from other waves (Cohen et al., 2015). Symptom scores from earlier ages served as covariates of the outcome variable in order to index *change* in symptoms over time (e.g., ADHD at age three was a covariate for ADHD at age six).

Results

Descriptive statistics, such as means and standard deviations, are described earlier in the Methods section and in Table 1. Several variables were extremely skewed and kurtotic; to account for this in the regression analyses, these variables were winsorized. Specifically, datapoints below the 5th and above the 95th percentile were changed to reflect the fifth and 95th percentile values, respectively (Tukey, 1962). The number of winsorized datapoints ranged from 5 to 8 depending on the variable in question. The untransformed values are reported in the note for Table 1.

Preliminary Analyses

Bivariate correlations were run between all variables of interest and potential covariates (see Table 2). As illustrated in Table 2, irritability was weakly correlated with anxiety, moderately to strongly correlated with depression and ODD, and weakly to moderately correlated with ADHD. Only ADHD symptom scores at ages nine and 12 were significantly associated with the CR variable (see Table 2).

To better estimate variable differences between males and females, independent samples t-tests were run for pubertal scores, CR, and all ages of psychopathology and irritability. Results from the t-tests suggested that mean sex differences only occurred with regard to age nine ADHD scores, age 12 anxiety scores, and pubertal scores for ages nine and 12. T-test results showed that the frequency of age nine ADHD symptoms was higher in males (M = .29, SD = 1.17) than in females (M = -.11, SD = .96), t(123) = 2.05, p = .04). At age 12, the frequency of anxiety symptoms was higher in females (M = .19, SD = 1.16) than in males (M = -.19, SD = .76), t(119) = -2.12, p = 04. T-test results showed that females (age 9: M = .21, SD = 1.04; age 12: M = .48, SD = .85) were more pubertally advanced (i.e., higher pubertal scores) than males (age 9: M = -.17, SD = .90; age 12: M = -.39, SD = .96), at ages nine t(121) = -2.20, p = .03, and 12, t(118) = -5.23, p < .01, which is consistent with the literature on sex differences in age of pubertal onset (Fechner, 2003). Because puberty scores at age nine were significantly skewed to the left and any variation was likely due to the sex differences within, age nine pubertal scores were not included in any of the hypothesis four models.

Regression Analyses

Multiple linear regression analyses were run using irritability as a predictor, CR and sex as moderators, and ADHD and depression symptoms as respective outcome variables (see Figure 1). In another set of regression analyses, puberty (age 12) was included as a moderator in place of sex, along with CR, for ADHD and depression symptoms at age 12.

Age Three Symptoms as Outcome

Using age three ADHD symptom scores as an outcome variable, a multiple linear regression was run using irritability as a predictor, and CR and sex as moderators (see Table 3), a model that explained 16% of the variance, F(141) = 4.017, p < .01.

Table 3

Summary of Multiple Linear Regression Analysis for Variables Associated with ADHD at Age Three

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	0.19	0.11	1.68	0.10	[-.03, .41]
Cortisol Reactivity (age three)	0.03	0.11	0.27	0.79	[-.18, .24]
Interaction 1: Irritability x CR	-0.08	0.12	-0.69	0.49	[-.31, .15]
Sex	-0.11	0.16	-0.70	0.48	[-.42, .20]
Interaction 2: Irritability x Sex	0.11	0.17	0.61	0.55	[-.24, .45]
Interaction 3: CR x Sex	0.23	0.15	1.47	0.14	[-.08, .53]
Interaction 4: Irritability x CR x Sex	0.51	0.17	2.98	< 0.01	[.17, .86]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

The three-way interaction between irritability, CR, and sex was significant, $R^2 = .05$, $F(141) = 8.865$, $p < .01$. As depicted in Table 4 and Figure 2, the test of simple slopes indicated that females with increased irritability and elevated or mean CR levels tended to have higher ADHD symptom scores. No significant effects were found for males, $p > .05$.

Table 4

Simple Slopes for a Three-Way Interaction Between Irritability, Sex, and CR on ADHD at Age Three

Variable		*B*	*SE*	*t*	*p*	95% CI
Male	Blunted CR	0.27	0.17	1.55	0.12	[-.08, .61]
	Mean CR	0.19	0.11	1.68	0.10	[-.03, .41]
	Elevated CR	0.11	0.15	0.72	0.48	[-.19, .40]
Female	Blunted CR	-0.15	0.20	-0.75	0.45	[-.56, .25]
	Mean CR	0.29	0.13	2.15	0.03	[.02, .55]
	Elevated CR	0.73	0.17	4.35	< 0.01	[.40, 1.06]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

Figure 2

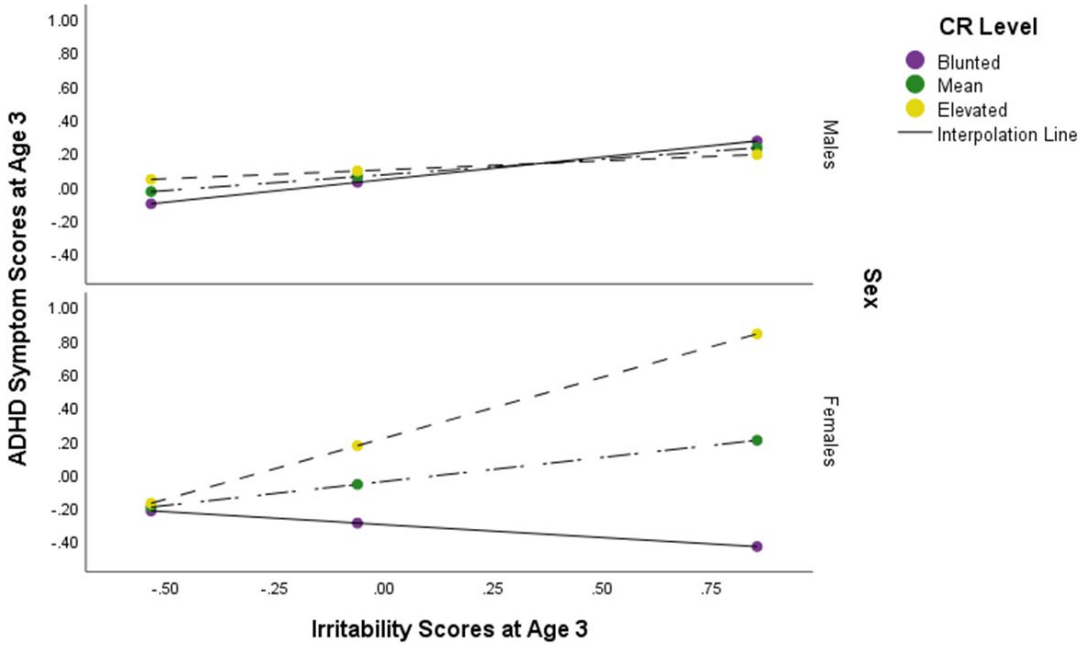


Figure 2. Sex and cortisol reactivity (CR) moderate the association between age three irritability and ADHD symptoms. Females with high irritability and elevated CR (B = .73) or mean levels of CR (B = .29) tended to have increased ADHD symptoms.

Using age three depression symptom scores as an outcome variable, another multiple linear regression was run using irritability as a predictor, and CR and sex as moderators (see Table 5), a model that explained 46% of the variance, $F(141) = 17.03$, $p < .01$.

Table 5

Summary of Multiple Linear Regression Analysis for Variables Associated with Depression at Age Three

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	0.65	0.07	8.80	<.01	[.50, .80]
Cortisol Reactivity (age three)	-0.08	0.07	-1.10	0.27	[-.22, .06]
Interaction 1: Irritability x CR	-0.11	0.08	-1.44	0.15	[-.26, .04]
Sex	-0.03	0.10	-0.24	0.81	[-.23, .18]
Interaction 2: Irritability x Sex	-0.16	0.12	-1.41	0.16	[-.39, .07]
Interaction 3: CR x Sex	0.15	0.10	1.45	0.15	[-.05, .35]
Interaction 4: Irritability x CR x Sex	0.27	0.11	2.35	0.02	[.04, .49]

Note: CR = Cortisol reactivity; CI = confidence interval.

The three-way interaction between irritability, CR, and sex was significant, $R^2 = .02$, $F(141) = 5.51$, $p = .02$. As depicted in Table 6, the test of simple slopes indicated that effects were significant, $p < .01$, for males and females, regardless of CR level. An examination of Figure 3, as well as the effect sizes

in Table 6, shows that males with blunted CR had the largest number of depression symptoms when compared to other males, whereas females with elevated CR had the highest reported depression symptoms.

Table 6

Simple Slopes for a Three-Way Interaction Between Irritability, Sex, and CR on Depression at Age Three

Variable		*B*	*SE*	*t*	*p*	95% CI
Male	Blunted CR	0.76	0.12	6.62	<.01	[.53, .99]
	Mean CR	0.65	0.07	8.80	<.01	[.50, .80]
	Elevated CR	0.54	0.10	5.47	<.01	[.34, .73]
Female	Blunted CR	0.33	0.13	2.41	0.02	[.06, .59]
	Mean CR	0.49	0.09	5.50	<.01	[.31, .66]
	Elevated CR	0.65	0.11	5.84	<.01	[.43, .86]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

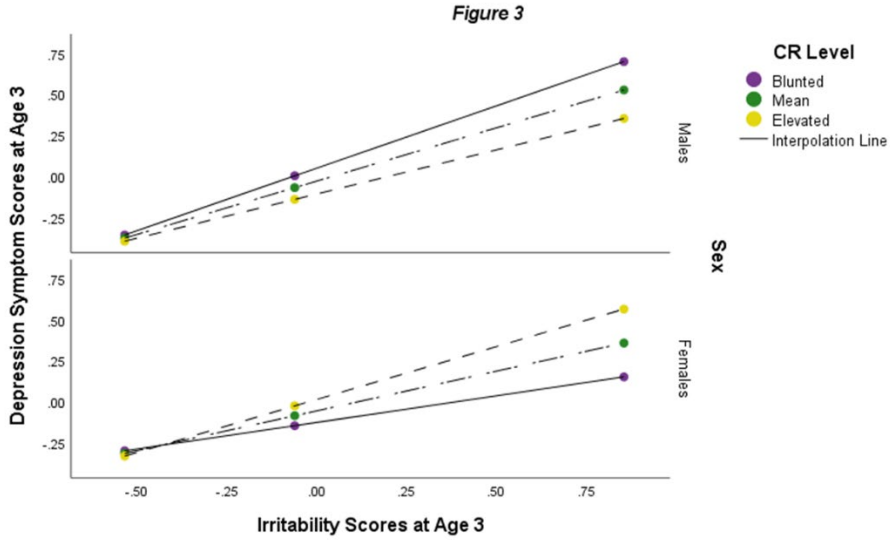


Figure 3. Sex and cortisol reactivity (CR) moderate the association between age three irritability and Depression symptoms. Among males, blunted CR and high irritability were associated with increased depression symptoms. For females, elevated CR and high irritability was associated with the highest depression symptom scores. See Table 6 for effects.

Age Six Symptoms as Outcome

Using age six ADHD symptom scores as an outcome variable, a multiple linear regression was run using concurrent irritability as a predictor, and CR and sex as moderators (see Table 7), a model that explained 31% of the variance, $F(120) = 6.71$, $p < .01$. Interactions were not significant, $p > .05$.

Table 7

Summary of Multiple Linear Regression Analysis for Variables Associated with ADHD at Age Six

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	0.31	0.10	3.12	<.01	[.11, .51]
Cortisol Reactivity (age three)	-0.01	0.10	-0.12	0.90	[-.21, .19]
Interaction 1: Irritability x CR	-0.11	0.09	-1.24	0.22	[-.28, .07]
Sex	-0.25	0.15	-1.63	0.11	[-.55, .05]
Interaction 2: Irritability x Sex	-0.14	0.15	-0.94	0.35	[-.45, .16]
Interaction 3: CR x Sex	-0.01	0.15	-0.03	0.98	[-.30, .29]
Interaction 4: Irritability x CR x Sex	0.24	0.15	1.6	0.11	[-.06, .54]
ADHD (age three)	0.37	0.08	4.82	<.01	[.22, .53]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

To examine any longitudinal effects, a multiple linear regression was run using age three irritability as a predictor, CR and sex as moderators, age six ADHD symptom scores as an outcome variable, and age three ADHD symptom scores as a covariate (see Table 8), a model that explained 54% of the variance, $F(121) = 17.42$, $p < .01$. Interactions were not significant, $p > .05$.

Table 8

Summary of Multiple Linear Regression Analysis for Variables Predicting ADHD at Age Six

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	-0.03	0.11	-0.30	0.76	[-.25, .18]
Cortisol Reactivity (age three)	0.02	0.10	0.16	0.87	[-.19, .22]
Interaction 1: Irritability x CR	0.02	0.11	0.21	0.83	[-.20, .24]
Sex	-0.25	0.16	-1.55	0.12	[-.56, .07]
Interaction 2: Irritability x Sex	0.26	0.18	1.46	0.15	[-.09, .61]
Interaction 3: CR x Sex	-0.03	0.15	-0.20	0.84	[-.33, .27]
Interaction 4: Irritability x CR x Sex	0.14	0.17	0.83	0.41	[-.20, .48]
ADHD (age three)	0.37	0.08	4.59	<.01	[.21, .54]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

Using age six depression symptom scores as an outcome variable, a multiple linear regression was run using concurrent irritability as a predictor, and CR and sex as moderators (see Table 9), a model that explained 37% of the variance, $F(121) = 8.90$, $p < .01$.

Table 9

Summary of Multiple Linear Regression Analysis for Variables Associated with Depression at Age Six

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	0.22	0.09	2.44	0.02	[.04, .39]
Cortisol Reactivity (age three)	0.10	0.09	1.09	0.28	[-.08, .27]
Interaction 1: Irritability x CR	-0.01	0.08	-0.11	0.92	[-.16, .14]
Sex	0.14	0.13	1.09	0.28	[-.12, .40]
Interaction 2: Irritability x Sex	0.32	0.13	2.44	0.02	[.06, .58]
Interaction 3: CR x Sex	-0.04	0.13	-0.30	0.77	[-.29, .21]
Interaction 4: Irritability x CR x Sex	0.13	0.13	1.05	0.3	[-.12, .38]
Depression (age three)	0.28	0.09	3.35	<.01	[.12, .45]

Note: CR = Cortisol reactivity; CI = confidence interval.

A lower-order interaction between irritability and sex was significant, $R^2 = .03$, $F(125) = 5.82$, $p = .02$. A test of simple slopes indicated that females with high irritability tended to have higher depression symptoms than their male counterparts, (for females: $B = .54$, $SE = .10$, $t(125) = 5.17$, $p < .01$, 95% CI [.33, .74]; for males: $B = .22$, $SE = .09$, $t(125) = 2.59$, $p < .01$, 95% CI [.05, .39]), as depicted in Figure 4. All other interactions were not significant, $p > .05$.

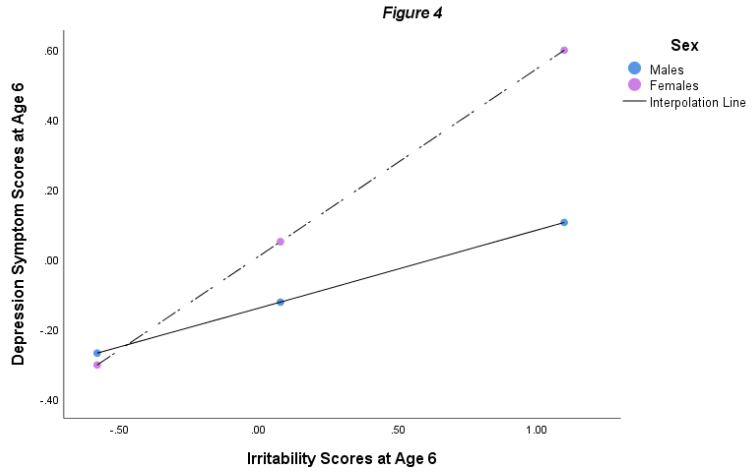


Figure 4. Sex moderates the association between irritability and depression symptoms at age six. Females (B = .54) with high irritability tended to have more depression symptoms than their male counterparts (B=.22).

To examine any longitudinal effects, a multiple linear regression was run using age three irritability as a predictor, CR and sex as moderators, age six depression symptom scores as an outcome variable, and age three depression symptom scores as a covariate (see Table 10), a model that explained 27% of the variance, $F(121) = 5.58$, $p < .01$.

Table 10

Summary of Multiple Linear Regression Analysis for Variables Predicting Depression at Age Six

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	-0.02	0.12	-0.13	0.90	[-.26, .23]
Cortisol Reactivity (age three)	0.13	0.09	1.39	0.17	[-.06, .32]
Interaction 1: Irritability x CR	0.09	0.10	0.91	0.37	[-.11, .29]
Sex	0.21	0.14	1.51	0.14	[-.07, .49]
Interaction 2: Irritability x Sex	0.42	0.16	2.60	0.01	[.10, .73]
Interaction 3: CR x Sex	-0.09	0.14	-0.64	0.52	[-.36, .18]
Interaction 4: Irritability x CR x Sex	-0.24	0.15	-1.56	0.12	[-.54, .06]
Depression (age three)	0.40	0.11	3.50	<.01	[.17, .63]

Note: CR = Cortisol reactivity; CI = confidence interval.

Similar to the results for concurrent irritability and depression at age six, a lower-order interaction between age three irritability and sex was significant, $R^2 = .03$, $F(125) = 5.64$, $p = .02$. As illustrated in Figure 5, a test of simple slopes indicated that females with high irritability tended to have higher depression symptoms overall, (for females: $B = .40$, $SE = .14$, $t(125) = 2.91$, $p < .01$, 95% CI [.13, .68]; for males: $B = .03$, $SE = .12$, $t(125) = .23$, $p = .82$, 95% CI [-.21, .26]). All other interactions were not significant, $p > .05$.

Figure 5

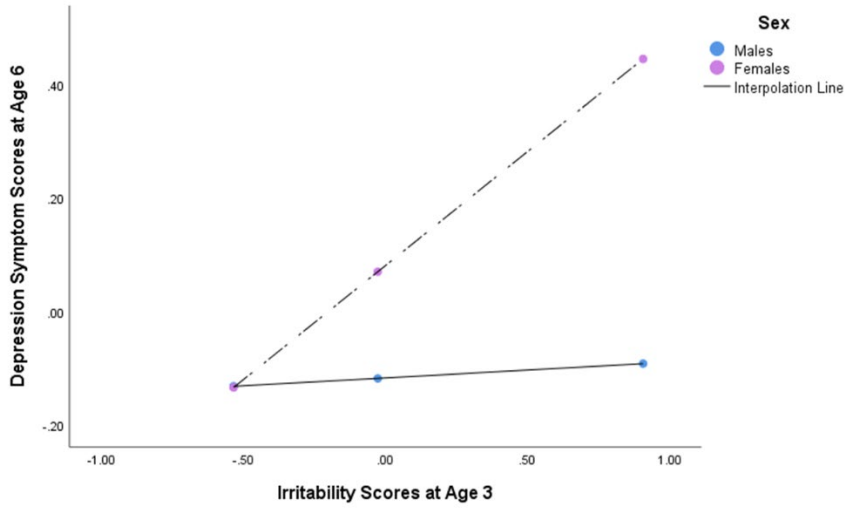


Figure 5. Sex moderates the association between age three irritability and age six depression symptoms. Females (B = .40) with high irritability tended to have more depression symptoms than their male counterparts (B=.03).

Age Nine Symptoms as Outcome

To examine any longitudinal effects, a multiple linear regression was run using age three irritability as a predictor, CR and sex as moderators, age nine ADHD symptom scores as an outcome variable, and ages three and six ADHD symptom scores as covariates (see Table 11), a model that explained 41% of the variance, $F(109) = 8.51$, $p < .01$. Interactions were not significant, $p > .05$.

Table 11

Summary of Multiple Linear Regression Analysis for Variables Associated with ADHD at Age Nine

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	-0.06	0.12	-0.51	0.62	[-.29, .18]
Cortisol Reactivity (age three)	0.13	0.11	1.11	0.27	[-.10, .35]
Interaction 1: Irritability x CR	0.02	0.16	0.12	0.90	[-.30, .34]
Sex	-0.27	0.16	-1.66	0.10	[-.59, .05]
Interaction 2: Irritability x Sex	0.05	0.19	0.25	0.81	[-.32, .41]
Interaction 3: CR x Sex	0.18	0.16	1.11	0.27	[-.14, .50]
Interaction 4: Irritability x CR x Sex	0.01	0.21	0.06	0.95	[-.40, .42]
ADHD (age three)	0.06	0.09	0.66	0.51	[-.12, .25]
ADHD (age six)	0.58	0.09	6.37	<.01	[.40, .76]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

Another multiple linear regression was run using age six irritability as a predictor, CR and sex as moderators, age nine ADHD symptom scores as an outcome variable, and ages three and six ADHD symptom scores as covariates (see Table 12), a model that explained 46% variance, $F(109) = 10.23$, $p < .01$. As shown in Table 12, the lower order two-way interaction between irritability and CR was significant. However, further analysis and a test of simple slopes did not yield significance for any of the variables ($p > .05$).

Table 12

Summary of Multiple Linear Regression Analysis for Variables Associated with ADHD at Age Nine

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	0.01	0.11	0.14	0.89	[-.20, .23]
Cortisol Reactivity (age three)	0.08	0.11	0.77	0.44	[-.13, .29]
Interaction 1: Irritability x CR	0.23	0.09	2.58	0.01	[.05, .41]
Sex	-0.22	0.16	-1.38	0.17	[-.53, .10]
Interaction 2: Irritability x Sex	-0.05	0.16	-0.33	0.74	[-.36, .26]
Interaction 3: CR x Sex	0.22	0.15	1.44	0.15	[-.08, .52]
Interaction 4: Irritability x CR x Sex	-0.28	0.15	-1.8	0.07	[-.58, .03]
ADHD (age three)	0.07	0.09	0.76	0.45	[-.11, .25]
ADHD (age six)	0.60	0.09	6.64	<.01	[.42, .78]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

To examine any longitudinal effects, a multiple linear regression was run using age three irritability as a predictor, CR and sex as moderators, age nine depression symptom scores as an outcome variable, and ages three and six depression symptom scores as covariates (see Table 13), $R^2 = .40$, $F(109) = 2.30$, $p = .02$.

Table 13

Summary of Multiple Linear Regression Analysis for Variables Predicting Depression at Age Nine

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	-0.12	0.16	-0.71	0.48	[-.44, .21]
Cortisol Reactivity (age three)	0.06	0.13	0.46	0.65	[-.20, .31]
Interaction 1: Irritability x CR	0.20	0.18	1.12	0.27	[-.16, .56]
Sex	-0.04	0.18	-0.24	0.81	[-.41, .32]
Interaction 2: Irritability x Sex	0.29	0.22	1.37	0.17	[-.13, .72]
Interaction 3: CR x Sex	-0.02	0.18	-0.11	0.92	[-.38, .34]
Interaction 4: Irritability x CR x Sex	-0.60	0.23	-2.55	0.01	[-1.06, -.13]
Depression (age three)	0.16	0.15	1.06	0.29	[-.14, .46]
Depression (age six)	0.23	0.12	1.85	0.07	[-.02, .47]

Note: CR = Cortisol reactivity; CI = confidence interval.

The three-way interaction between irritability, CR, and sex was significant, $R^2 = .05$, $F(109) = 6.52$, $p = .01$. As depicted in Table 14, the test of simple slopes indicated that effects were significant, p = .02, for females with blunted CR level. An examination of Figure 6 shows that high irritability at age three predicted higher depression scores in females with blunted CR.

Table 14

Simple Slopes for a Three-Way Interaction Between Age Three Irritability, Sex, and CR on Depression at Age Nine

Variable		*B*	*SE*	*t*	*p*	95% CI
Male	Blunted CR	-0.32	0.23	-1.41	0.16	[-.77, .13]
	Mean CR	-0.11	0.64	-0.68	0.50	[-.44, .21]
	Elevated CR	0.10	0.27	0.36	0.72	[-.43, .63]
Female	Blunted CR	0.57	0.25	2.33	0.02	[.09, 1.06]
	Mean CR	0.17	0.18	0.93	0.35	[-.19, .53]
	Elevated CR	-0.23	0.22	-1.05	0.30	[-.67, .21]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

Figure 6

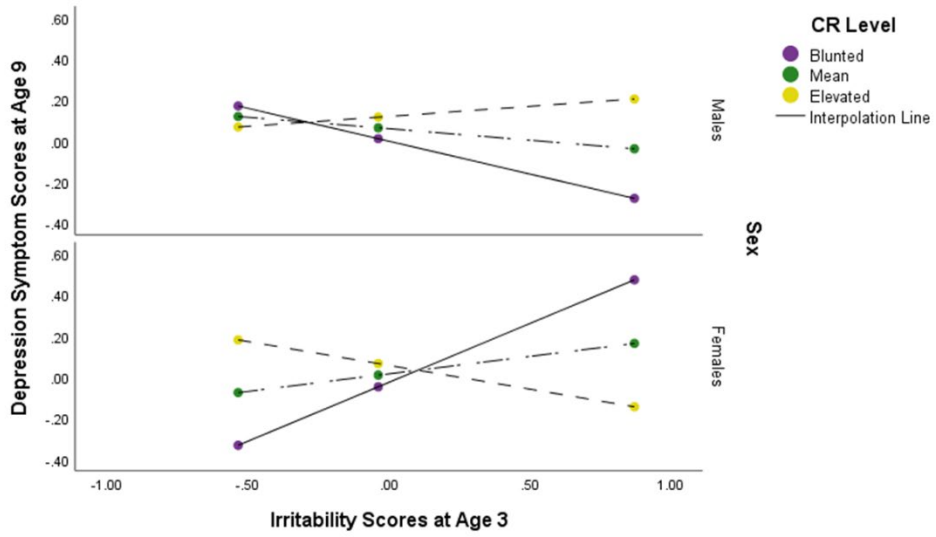


Figure 6. Sex and cortisol reactivity (CR) moderate the association between age three irritability and Depression symptoms. Among females (B=.57), blunted CR and high irritability were associated with increased depression symptoms.

An additional linear regression was run using age six irritability as a predictor, CR and sex as moderators, age nine depression symptom scores as an outcome variable, and ages three and six depression symptom scores as covariates (see Table 15), $R^2 = .42$, $F(109) = 2.58$, $p = .01$.

Table 15

Summary of Multiple Linear Regression Analysis for Variables Predicting Depression at Age Nine

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	0.05	0.13	0.42	0.67	[-.20, .30]
Cortisol Reactivity (age three)	0.01	0.12	0.07	0.95	[-.24, .25]
Interaction 1: Irritability x CR	0.03	0.10	0.29	0.77	[-.17, .23]
Sex	-0.09	0.18	-0.51	0.61	[-.45, .27]
Interaction 2: Irritability x Sex	0.28	0.18	1.53	0.13	[-.08, .64]
Interaction 3: CR x Sex	0.06	0.18	0.36	0.72	[-.29, .41]
Interaction 4: Irritability x CR x Sex	-0.37	0.17	-2.19	0.03	[-.71, - .03]
Depression (age three)	0.01	0.12	0.11	0.91	[-.22, .25]
Depression (age six)	0.22	0.13	1.68	0.10	[-.04, .48]

Note: CR = Cortisol reactivity; CI = confidence interval.

Like the earlier model with age three irritability, the three-way interaction between age six irritability, CR, and sex was significant, $R^2 = .04$, $F(109) = 4.78$, $p = .03$. As depicted in Table 16, the test of simple slopes indicated that effects were significant for females with blunted CR level. Figure 7 illustrates that high irritability at age six predicted higher depression scores in females with blunted or mean levels of CR.

Table 16

Simple Slopes for a Three-Way Interaction Between Age Six Irritability, Sex, and CR on Depression at Age Nine

Variable		*B*	*SE*	*t*	*p*	95% CI
Male	Blunted CR	0.02	0.18	0.13	0.90	[-.33, .37]
	Mean CR	0.05	0.13	0.43	0.67	[-.19, .30]
	Elevated CR	0.08	0.15	0.57	0.57	[-.21, .38]
Female	Blunted CR	0.67	0.20	3.40	<.01	[.28, 1.07]
	Mean CR	0.32	0.16	2.09	0.04	[.02, .63]
	Elevated CR	-0.03	0.22	-0.12	0.91	[-.46, .40]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

Figure 7

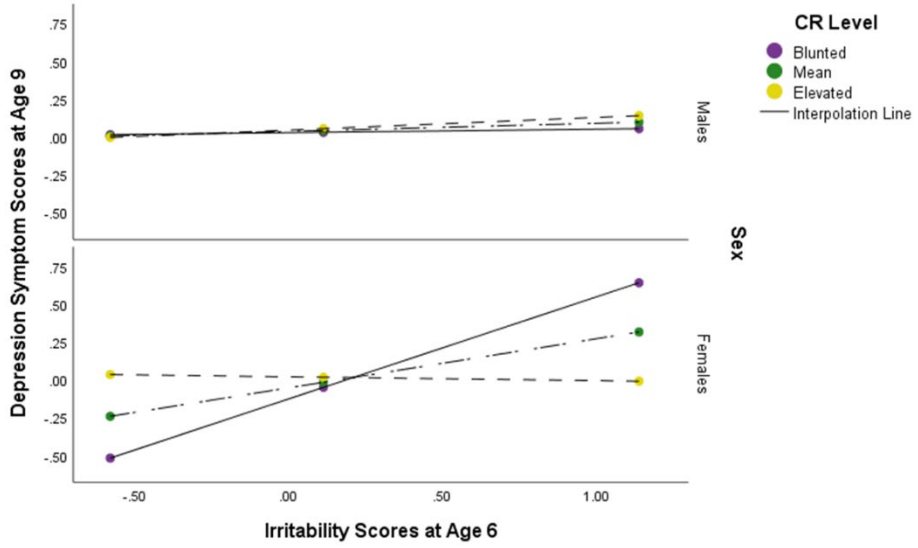


Figure 7. Sex and cortisol reactivity (CR) moderate the association between age six irritability and depression symptoms. Among females blunted (B = .67) or mean (B = .32) CR levels and high irritability were associated with increased depression symptoms.

Age Twelve Symptoms

Using age 12 ADHD symptom scores as an outcome variable, a multiple linear regression was run using concurrent irritability as a predictor, CR and sex as moderators, and age three, six, and nine ADHD scores as covariates (see Table 17), a model that explained 80% of the variance, $F(99) = 38.75$, $p < .01$. Interactions were not significant, $p > .05$.

Table 17

Summary of Multiple Linear Regression Analysis for Variables Associated with ADHD at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age twelve)	0.17	0.06	2.83	<.01	[.05, .29]
Cortisol Reactivity (age three)	-0.04	0.07	-0.59	0.56	[-.18, .10]
Interaction 1: Irritability x CR	0.07	0.08	0.93	0.36	[-.08, .23]
Sex	0.1	0.1	0.94	0.35	[-.11, .30]
Interaction 2: Irritability x Sex	-0.08	0.09	-0.93	0.35	[-.26, .10]
Interaction 3: CR x Sex	0.15	0.1	1.51	0.13	[-.05, .34]
Interaction 4: Irritability x CR x Sex	0.02	0.1	0.2	0.84	[-.18, .22]
ADHD (age three)	0.02	0.06	0.31	0.76	[-.09, .13]
ADHD (age six)	0.09	0.07	1.29	0.2	[-.05, .24]
ADHD (age nine)	0.75	0.07	10.98	<.01	[.61, .89]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

To examine any longitudinal effects, a multiple linear regression was run using age three irritability as a predictor, CR and sex as moderators, age 12 ADHD symptom scores as an outcome variable, and ages three, six, and nine ADHD symptom scores as covariates (see Table 18), $R^2 = .78$, $F(100) = 35.10$, $p < .01$. Interactions were not significant, $p > .05$.

Table 18

Summary of Multiple Linear Regression Analysis for Variables Predicting ADHD at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	0.03	0.08	0.37	0.71	[-.12, .18]
Cortisol Reactivity (age three)	0.00	0.08	0.03	0.97	[-.15, .15]
Interaction 1: Irritability x CR	0.03	0.11	0.27	0.79	[-.18, .24]
Sex	0.09	0.11	0.82	0.42	[-.13, .31]
Interaction 2: Irritability x Sex	0.05	0.12	0.44	0.66	[-.19, .30]
Interaction 3: CR x Sex	0.06	0.11	0.57	0.57	[-.15, .27]
Interaction 4: Irritability x CR x Sex	-0.14	0.14	-1.05	0.3	[-.41, .13]
ADHD (age three)	0.03	0.06	0.41	0.68	[-.10, .15]
ADHD (age six)	0.05	0.07	0.72	0.47	[-.09, .20]
ADHD (age nine)	0.85	0.06	13.24	<.01	[.72, .98]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

Additional linear regression analyses were run using age six irritability as a predictor, CR and sex as moderators, age 12 ADHD symptom scores as an outcome variable, and ages three, six, and nine ADHD symptom scores as covariates (see Table 19), $R^2 = .78$, $F(100) = 35.84$, $p < .01$. Interactions were not significant, $p > .05$.

Table 19

Summary of Multiple Linear Regression Analysis for Variables Predicting ADHD at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	0.01	0.07	0.12	0.91	[-.14, .15]
Cortisol Reactivity (age three)	0.00	0.07	-0.05	0.96	[-.15, .14]
Interaction 1: Irritability x CR	-0.02	0.06	-0.39	0.70	[-.15, .10]
Sex	0.06	0.11	0.52	0.61	[-.16, .27]
Interaction 2: Irritability x Sex	0.13	0.11	1.22	0.23	[-.08, .35]
Interaction 3: CR x Sex	0.08	0.10	0.74	0.46	[-.13, .28]
Interaction 4: Irritability x CR x Sex	0.12	0.11	1.11	0.27	[-.09, .33]
ADHD (age three)	-0.02	0.06	-0.3	0.77	[-.14, .10]
ADHD (age six)	0.03	0.08	0.35	0.73	[-.13, .18]
ADHD (age nine)	0.86	0.07	12.95	<.01	[.73, .99]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval.

Additional analyses were run to explore pubertal development as a moderator. Using age 12 ADHD symptom scores as an outcome variable, a multiple linear regression was run using irritability at ages three, six, and 12, pubertal development at age 12, and age three, six, and 12 ADHD scores as covariates. Interactions were not significant, $p > .05$, when ages three, six, or 12 irritability scores were used as the predictor variable (see Table 20, 21, and 22).

Table 20

Summary of Multiple Linear Regression Analysis for Variables Predicting ADHD at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	0.05	0.06	0.78	0.44	[-.07, .17]
Cortisol Reactivity (age three)	0.03	0.06	0.50	0.62	[-.08, .14]
Interaction 1: Irritability x CR	-0.06	0.07	-0.82	0.42	[-.19, .08]
PD (age twelve)	-0.09	0.06	-1.42	0.16	[-.21, .03]
Interaction 2: Irritability x PD	-0.01	0.06	-0.21	0.84	[-.14, .11]
Interaction 3: CR x PD	0.00	0.06	-0.05	0.96	[-.12, .11]
Interaction 4: Irritability x CR x PD	0.01	0.07	0.16	0.87	[-.12, .14]
Sex	0.16	0.12	1.30	0.20	[-.08, .40]
ADHD (age three)	0.02	0.06	0.33	0.74	[-.10, .14]
ADHD (age six)	0.06	0.08	0.80	0.42	[-.09, .21]
ADHD (age nine)	0.85	0.07	13.05	<.01	[.72, .98]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval; PD = pubertal development.

Model summary: $R^2 = .78, F(98) = 31.28, p < .01$

Table 21

Summary of Multiple Linear Regression Analysis for Variables Predicting ADHD at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	0.06	0.06	0.96	0.34	[-.06, .17]
Cortisol Reactivity (age three)	0.03	0.06	0.56	0.58	[-.08, .14]
Interaction 1: Irritability x CR	0.01	0.05	0.14	0.89	[-.10, .11]
PD (age twelve)	-0.08	0.06	-1.29	0.20	[-.20, .04]
Interaction 2: Irritability x PD	0.00	0.06	-0.06	0.96	[-.11, .11]
Interaction 3: CR x PD	-0.01	0.06	-0.14	0.89	[-.13, .11]
Interaction 4: Irritability x CR x PD	0.01	0.05	0.10	0.92	[-.10, .11]
Sex	0.13	0.12	1.12	0.97	[-.10, .37]
ADHD (age three)	0.02	0.06	0.37	0.71	[-.10, .14]
ADHD (age six)	0.04	0.08	0.56	0.58	[-.11, .19]
ADHD (age nine)	0.84	0.07	12.74	<.01	[.71, .97]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval; PD = pubertal development.

Model summary: $R^2 = .77, F(98) = 31.13, p < .01$

Table 22

Summary of Multiple Linear Regression Analysis for Variables Associated with ADHD at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age twelve)	0.17	0.05	3.29	<.01	[.07, .27]
Cortisol Reactivity (age three)	0.05	0.05	1.00	0.32	[-.05, .16]
Interaction 1: Irritability x CR	0.12	0.07	1.89	0.06	[-.01, .25]
PD (age twelve)	-0.05	0.06	-0.79	0.43	[-.16, .07]
Interaction 2: Irritability x PD	-0.02	0.05	-0.37	0.72	[-.12, .08]
Interaction 3: CR x PD	0.08	0.06	1.41	0.16	[-.03, .20]
Interaction 4: Irritability x CR x PD	0.09	0.07	1.41	0.16	[-.04, .23]
Sex	0.16	0.12	1.37	0.18	[-.07, .39]
ADHD (age three)	0.04	0.06	0.67	0.51	[-.07, .15]
ADHD (age six)	0.10	0.07	1.37	0.17	[-.04, .24]
ADHD (age nine)	0.75	0.07	10.89	<.01	[.61, .88]

Note: ADHD = Attention-deficit/hyperactivity disorder; CR = Cortisol reactivity; CI = confidence interval; PD = pubertal development.

Model summary: $R^2 = .80, F(98) = 34.70, p < .01$

Using age 12 depression symptom scores as an outcome variable, a multiple linear regression was run using concurrent irritability as a predictor, CR and sex as moderators, and age three, six, and nine depression scores as covariates (see Table 23), $R^2 = .18$, $F(99) = 2.18$, $p < .05$. Interactions were not significant, $p > .05$.

Table 23

Summary of Multiple Linear Regression Analysis for Variables Associated with Depression at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age twelve)	0.34	0.12	2.77	0.01	[.10, .59]
Cortisol Reactivity (age three)	0.06	0.13	0.45	0.66	[-.20, .32]
Interaction 1: Irritability x CR	0.11	0.14	0.77	0.44	[-.17, .38]
Sex	0.33	0.20	1.69	0.09	[-.06, .72]
Interaction 2: Irritability x Sex	-0.07	0.17	-0.40	0.69	[-.41, .27]
Interaction 3: CR x Sex	-0.02	0.19	-0.12	0.91	[-.39, .35]
Interaction 4: Irritability x CR x Sex	-0.10	0.19	-0.56	0.58	[-.47, .26]
Depression (age three)	-0.07	0.13	-0.49	0.63	[-.33, .21]
Depression (age six)	0.07	0.13	0.51	0.61	[-.19, .32]
Depression (age nine)	-0.01	0.11	-0.07	0.94	[-.22, .20]

Note: CR = Cortisol reactivity; CI = confidence interval;

To examine any longitudinal effects, a multiple linear regression was run using age three irritability as a predictor, CR and sex as moderators, age 12 depression symptom scores as an outcome variable, and ages three, six, and nine depression symptom scores as covariates (see Table 24), $R^2 = .07$, $F(100) = .77$, $p > .05$. Interactions were not significant, $p > .05$.

Table 24

Summary of Multiple Linear Regression Analysis for Variables Predicting Depression at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	-0.04	0.18	-0.24	0.81	[-.40, .32]
Cortisol Reactivity (age three)	0.16	0.14	1.13	0.26	[-.12, .45]
Interaction 1: Irritability x CR	0.15	0.20	0.76	0.45	[-.24, .55]
Sex	0.29	0.21	1.39	0.17	[-.12, .70]
Interaction 2: Irritability x Sex	-0.08	0.24	-0.34	0.74	[-.55, .39]
Interaction 3: CR x Sex	-0.14	0.20	-0.71	0.48	[-.53, .25]
Interaction 4: Irritability x CR x Sex	-0.22	0.26	-0.81	0.42	[-.73, .31]
Depression (age three)	0.20	0.17	1.18	0.24	[-.14, .53]
Depression (age six)	0.07	0.14	0.5	0.62	[-.21, .35]
Depression (age nine)	0.08	0.11	0.78	0.44	[-.13, .30]

Note: CR = Cortisol reactivity; CI = confidence interval;

To further examine any longitudinal effects, another multiple linear regression was run using age six irritability as a predictor, CR and sex as moderators, age 12 depression symptom scores as an outcome variable, and ages three, six, and nine depression symptom scores as covariates (see Table 24), $R^2 = .08$, $F(100) = .83$, $p > .05$. Interactions were not significant, $p > .05$.

Table 25

Summary of Multiple Linear Regression Analysis for Variables Predicting Depression at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	0.08	0.14	0.55	0.58	[-.20, .36]
Cortisol Reactivity (age three)	0.13	0.14	0.92	0.36	[-.15, .40]
Interaction 1: Irritability x CR	0.01	0.11	0.06	0.95	[-.21, .23]
Sex	0.33	0.20	1.64	0.11	[-.07, .74]
Interaction 2: Irritability x Sex	-0.16	0.21	-0.78	0.44	[-.57, .25]
Interaction 3: CR x Sex	-0.12	0.19	-0.60	0.55	[-.50, .27]
Interaction 4: Irritability x CR x Sex	0.15	0.19	0.81	0.42	[-.22, .53]
Depression (age three)	0.13	0.13	0.96	0.34	[-.13, .38]
Depression (age six)	0.03	0.15	0.21	0.83	[-.27, .33]
Depression (age nine)	0.13	0.11	1.23	0.22	[-.08, .34]

Note: CR = Cortisol reactivity; CI = confidence interval;

Analyses were also conducted to examine pubertal development as a moderator. Using age 12 depression symptom scores as an outcome variable, a multiple linear regression was run using irritability at ages three, six, and 12, pubertal development at age 12, and age three, six, and nine depression scores

as covariates. In a model that explained 8% of the variance, age three irritability did not yield significant interactions, $F(98) = .76$, $p > .05$ (See Table 26).

Table 26

Summary of Multiple Linear Regression Analysis for Variables Predicting Depression at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age three)	-0.06	0.16	-0.37	0.71	[-.37, .25]
Cortisol Reactivity (age three)	0.08	0.10	0.78	0.44	[-.12, .28]
Interaction 1: Irritability x CR	0.02	0.12	0.19	0.85	[-.22, .27]
PD (age twelve)	0.00	0.11	0.00	0.99	[-.22, .22]
Interaction 2: Irritability x PD	-0.10	0.12	-0.79	0.43	[-.34, .15]
Interaction 3: CR x PD	-0.02	0.11	-0.17	0.87	[-.23, .20]
Interaction 4: Irritability x CR x PD	-0.09	0.12	-0.75	0.46	[-.33, .15]
Sex	0.25	0.23	1.01	0.28	[-.20, .70]
Depression (age three)	0.12	0.17	0.69	0.49	[-.22, .46]
Depression (age six)	0.07	0.14	0.47	0.64	[-.21, .34]
Depression (age nine)	0.12	0.11	0.95	0.35	[-.12, .33]

Note: CR = Cortisol reactivity; CI = confidence interval; PD = pubertal development.

Another model using age six irritability as the predictor variable also did not yield significant interactions, $R^2 = .08$, $F(98) = .82$, $p > .05$ (see Table 27).

Table 27

Summary of Multiple Linear Regression Analysis for Variables Predicting Depression at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age six)	-0.03	0.12	-0.25	0.80	[-.27, .21]
Cortisol Reactivity (age three)	0.06	0.10	0.55	0.58	[-.14, .25]
Interaction 1: Irritability x CR	0.03	0.09	0.32	0.75	[-.16, .22]
PD (age twelve)	0.01	0.11	0.06	0.96	[-.22, .23]
Interaction 2: Irritability x PD	-0.11	0.10	-1.11	0.27	[-.31, .09]
Interaction 3: CR x PD	0.01	0.11	0.11	0.91	[-.21, .23]
Interaction 4: Irritability x CR x PD	-0.07	0.09	-0.78	0.44	[-.26, .11]
Sex	0.29	0.22	1.31	0.20	[-.15, .73]
Depression (age three)	0.11	0.13	0.83	0.41	[-.15, .37]
Depression (age six)	0.06	0.15	0.38	0.71	[-.24, .35]
Depression (age nine)	0.12	0.11	1.04	0.30	[-.11, .34]

Note: CR = Cortisol reactivity; CI = confidence interval; PD = pubertal development.

A final model that included age 12 irritability, and explained 22% of the variance, $F(97) = 2.54$, $p < .01$, showed a significant three-way interaction ($R^2 = .04$, $F(95) = 5.32$, $p = .02$; see Table 28).

Table 28

Summary of Multiple Linear Regression Analysis for Variables Associated with Depression at Age Twelve

Predictor Variable	*B*	*SE*	*t*	*p*	95% CI
Irritability (age twelve)	0.43	0.10	4.16	<.01	[.23, .64]
Cortisol Reactivity (age three)	0.11	0.10	1.08	0.28	[-.09, .30]
Interaction 1: Irritability x CR	0.18	0.11	1.56	0.12	[-.05, .41]
PD (age twelve)	0.00	0.11	0.02	0.99	[-.22, .22]
Interaction 2: Irritability x PD	-0.05	0.10	-0.54	0.59	[-.24, .14]
Interaction 3: CR x PD	0.13	0.11	1.18	0.24	[-.09, .34]
Interaction 4: Irritability x CR x PD	0.29	0.12	2.37	0.02	[.05, .53]
Sex	0.42	0.21	1.94	0.06	[-.01, .84]
Depression (age three)	-0.06	0.13	-0.49	0.63	[-.33, .20]
Depression (age six)	0.08	0.13	0.64	0.52	[-.17, .33]
Depression (age nine)	-0.06	0.12	-0.52	0.61	[-.29, .17]

Note: CR = Cortisol reactivity; CI = confidence interval; PD = pubertal development.

As depicted in Table 29, age 12 irritability and age three CR significantly interacted when pubertal scores were +1 SD from the mean, showing an increase in depression symptoms. An additional examination into the Johnson-Neyman regions of significance support this finding, indicating that the effect is strongest for individuals above mean levels of puberty (M = .06, SD = 1.00, point at which the interaction is significant = .269).

Table 29

Test of Conditional Age Twelve Irritability by CR Interaction at the Value of Pubertal Development

Pubertal Development	*B*	*F*	*p*
Less Developed	-0.11	0.65	0.42
Mean PD	0.18	2.36	0.13
More Developed	0.46	5.81	0.02

Note: CR = Cortisol reactivity; PD = pubertal development. Levels of pubertal development were mean and ± 1SD

A test of the simple slopes showed that individuals with typical or more advanced pubertal development, combined with mean or elevated CR and higher irritability scores, were more likely to have increased depression scores (see Table 30).

Table 30

Simple Slopes for a Three-Way Interaction Between Irritability, Pubertal Development, and CR on Depression at Age Twelve

Variable		*B*	*SE*	*t*	*p*	95% CI
Less Developed	Blunted CR	0.59	0.25	2.38	0.02	[.10, 1.09]
	Mean CR	0.48	0.14	3.42	<.01	[.20, .76]
	Elevated CR	0.37	0.13	2.81	<.01	[.11, .62]
Mean PD	Blunted CR	0.26	0.15	1.67	0.10	[-.05, .56]
	Mean CR	0.44	0.10	4.23	<.01	[.23, .64]
	Elevated CR	0.62	0.16	3.85	<.01	[.30, .94]
More Developed	Blunted CR	-0.08	0.22	-0.36	0.72	[-.51, .35]
	Mean CR	0.40	0.14	2.89	<.01	[.13, .67]
	Elevated CR	0.87	0.26	3.37	<.01	[.35, 1.40]

Note: CR = Cortisol reactivity; CI = confidence interval, PD = pubertal development. Levels of pubertal development were mean and ± 1SD

As illustrated in Figure 8 and 9, high irritability and elevated CR interacted in more pubertally developed individuals to increase depression scores.

Figure 8

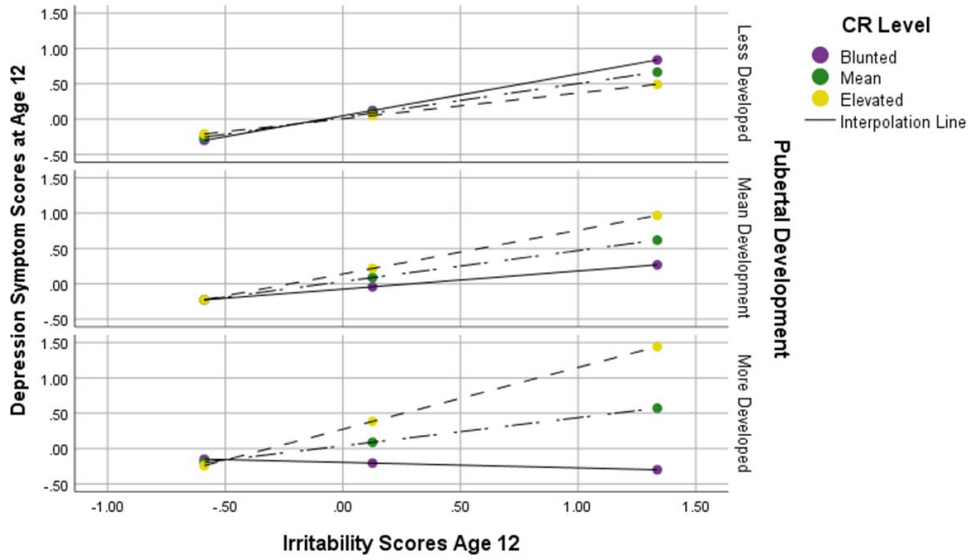


Figure 8. Pubertal development and cortisol reactivity (CR) moderate the association between age twelve irritability and depression symptoms. Twelve-year-olds who were farther along in their puberty, had high concurrent irritability, and either elevated (B=.87) or mean (B = .40) CR tended to have increased depression scores.

Figure 9

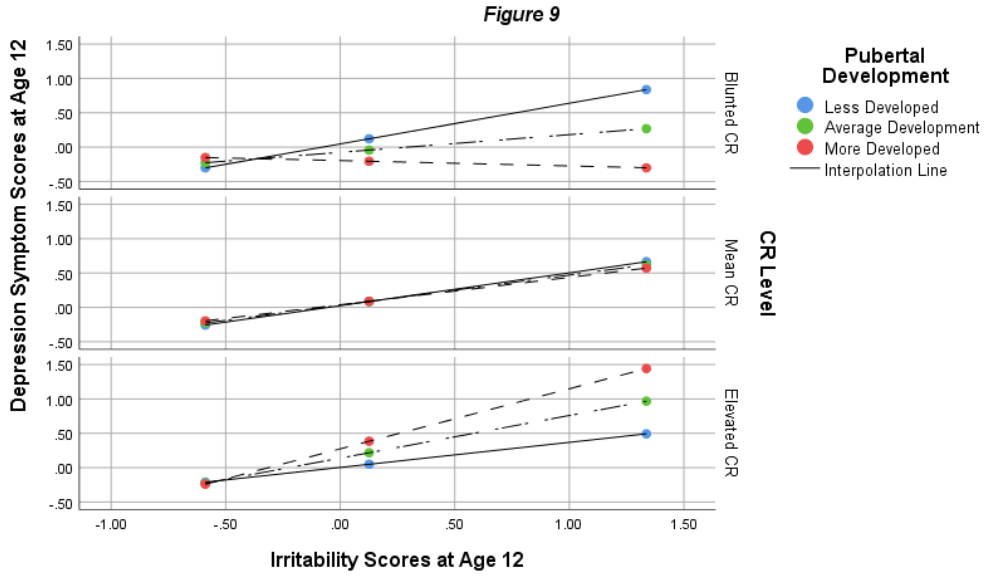


Figure 9. Pubertal development and cortisol reactivity (CR) moderate the association between age twelve irritability and depression symptoms. Twelve-year-olds who were farther along in their puberty, had high concurrent irritability, and either elevated (B=.87) or mean (B = .40) CR in early childhood tended to have increased depression scores.

Discussion

The primary goal of this study was to gain a better understanding of how cortisol reactivity may interact with irritability to influence psychopathology symptoms both concurrently and over time. ADHD and depression symptoms were of particular interest because the existing literature reflects inconsistent findings when examining these disorders in the context of CR (Alink et al., 2008; Bagner et al., 2010; Gunnar et al., 2009b; Harkness et al., 2011; Jaffee et al., 2015; Lopez-Duran et al., 2009; Mazurka et al., 2016; Melham et al., 2016; Ruttle et al., 2011; Suzuki et al., 2013; Zorn et al., 2017). Overall, the findings of this study suggest that CR plays a role in the association between irritability and psychopathology symptoms, especially those related to depression. A secondary purpose of this study was to examine whether sex and puberty further moderated the irritability-CR interaction. These investigations showed sex and puberty to be additional moderators in early childhood and early adolescence, respectively. Our findings add to the minimal published research investigating irritability and CR associations and interactions (Mikita et al., 2015; Morris et al., 2017).

First, we found that CR and irritability interacted in association with ADHD symptoms in preschool, but only in females. That is, three-year-old females with atypically elevated CR and high irritability were more likely to have symptoms of ADHD when compared to other children in the sample. We considered the following possible explanation for this finding. To begin with, we sought to understand what makes elevated CR different from blunted CR in the context of psychopathology. Anxiety is consistently associated with elevated CR (Zorn et al., 2017), and so we surmised that comorbid anxiety symptoms may be related to these findings. Further, research has shown that sex differences occur in anxiety prevalence, even in children as young as preschool age, with females showing higher rates of separation anxiety in particular (Franz et al., 2013). Of interest, the Stranger Approach task, which was administered to our sample, has been used to elicit stress reactivity and anxiety (Kopala-Sibley et al., 2017). Additionally, anxiety in young children often manifests behaviorally as irritability and anger (Roy et al., 2019). It may be that some preschool aged females are experiencing higher levels of anxiety, which

is associated with elevated CR, and is expressed as increased irritability and emotion dysregulation, which may be mistaken for ADHD.

At no other age beyond three years did we find significant interactions when ADHD was the outcome variable. However, in line with findings above, concurrent irritability symptoms were related with ADHD symptoms at both waves where concurrent irritability data were available (ages six and 12). At six- and 12-years-old, children with high irritability tended to have increased ADHD symptoms scores when compared to other children in the sample. That we found a significant association at age three, but not ages six, nine, and twelve, is curious. It appears that CR in early childhood is not a robust predictor of later ADHD symptoms in this sample, suggesting that the relation between irritability and CR, in association with ADHD symptoms, may be specific to concurrent CR (though age nine irritability was not analyzed here).

When examining depression symptoms at age three, we found that irritability interacted with CR at all levels, for both sexes, in association with increased symptoms. However, looking more closely at the effects shown in Table 6 and in Figure 3, the patterns for males and females are opposite. While high irritability was associated with increased depression symptoms in both sexes, this was especially true for males with blunted CR and females with elevated CR. Research indicates that high irritability is associated with depression and is even a symptom of depression in children (APA, 2013; Roy et al., 2019). One reason for the CR and sex interaction here may be related to comorbid symptoms of anxiety and ODD, in females and males, respectively. As was already discussed, anxiety is associated with elevated CR and is more prevalent in females (Roy et al., 2019; Zorn et al., 2017). Similarly, ODD is associated with blunted CR and is more prevalent in males (APA, 2013; Bagner et al., 2010; Ruttle et al., 2011; Zorn et al., 2017). Future studies would be well served to explore these potential comorbidities in this context.

Although CR was not significant for six-year-olds, our results did show that sex and irritability interacted in a meaningful way. For six-year-old females, depression scores tended to be higher when they had high irritability both concurrently and when they were three. Interestingly, the literature on

depression prevalence suggests that sex differences do not emerge until pubertal onset (Beauchaine & Hinshaw, 2017). However, it may be that the sex difference here is reflecting a qualitative difference in the presentation of irritability. Specifically, as noted above, females typically show more withdrawn-type irritability, whereas males tend to display more blatant, even oppositional-type, irritability (Stringaris et al., 2018). In this case, the female-typical irritability manifestation may be more related to (or possibly even conflated with) depression symptoms.

At age nine, irritability significantly interacted with CR and sex in association with depression symptoms. Echoing earlier waves, this effect specifically occurred in females. However, blunted CR (as opposed to elevated), in conjunction with high irritability at ages three and six was found to predict increased depression symptoms at age nine. One explanation for this finding may be related to chronic versus concurrent irritability. That is, chronic irritability may be more associated with chronic depressive symptoms, which are also typically associated with blunted CR. That this interaction was significant for irritability at age three and six, in conjunction with the moderate correlation between irritability at those ages (see Table 2), suggests that age nine children's depressive symptoms may have been more influenced by chronic type irritability. Unfortunately, this is difficult to confirm without a concurrent irritability measure for age nine.

Results for age 12 showed that a three-way interaction including sex was not significant. However, puberty did interact with irritability and CR (while controlling for sex) in association with depression symptoms. In particular, more pubertally developed 12-year-olds had higher symptom scores when they had high concurrent irritability and elevated CR. From the puberty and CR literature, we know that normative hormonal changes occur during pubertal development, with CR changes happening in both healthy and at-risk adolescents (Colich et al., 2015; Ge & Natsuaki, 2009 Gunnar et al., 2009b; Hankin et al., 2010); however, previous research has focused on CR and puberty concurrently, as opposed to early childhood CR interacting with pubertal development. These results suggest that atypical CR in early childhood may be a more robust predictor of psychopathology than previously thought.

Strengths and Limitations

This study had several strengths of note. First, the use of longitudinal data allowed us to examine how factors in early childhood may predict symptoms of psychopathology. The earlier that risk factors are identified, the earlier interventions may be arranged, and trajectories improved. Second, examining psychopathology dimensionally allowed us to capture symptom changes in individuals who would otherwise not meet criteria for a psychiatric diagnosis. This was a particular strength for examining ADHD, because symptoms in females often go undetected or are misattributed to other disorders, such as depression (e.g., social withdrawal is a common symptom of depression and of ADHD in young females; Beauchaine & Hinshaw, 2017; Chaplin & Aldao, 2013; Mendle, 2014). This was also useful because the dimensional scores accounted for construct overlap by removing irritability from the symptom scores. Third, that three-way interactions emerged as significant in this small sample suggests large effects.

There were also several limitations to this study. First, cortisol reactivity was only collected at the first visit (i.e., age three) and only one AUC was calculated for each participant. As a result, the CR levels may be more representative of stress reactivity in that moment, as opposed to average CR at age three. Additionally, the lack of CR data for ages six, nine, and 12, prevented us from assessing how CR may change over time. Although CR may be relatively stable from three to six years, this was not explicitly considered in this study (Hankin et al., 2011). Second, the sample was demographically homogeneous (i.e., primarily Caucasian, middle-class, college-educated parents). This lack of variability may make the sample less generalizable in terms of psychopathology symptoms and stress reactivity in the population. Third, because the CR measure was only collected on 156 participants, the overall sample was relatively small, especially for the statistical models used. Fourth, ADHD symptoms were summed scores (rather than modeling hyperactivity and inattentiveness separately), preventing us from assessing whether subtypes or certain symptoms were more affected by the irritability-CR interaction. Fourth, although our psychopathology measures were created with a dimensional approach in mind, the symptoms were still based on the DSM disorders, thus detracting from the strength of a truly dimensional framework.

Future Research and Implications

Future research should continue to investigate cortisol reactivity as a moderator between irritability and psychopathology. Irritability is an indication of dysregulated emotion and mood that can be measured through behavioral observation and self-report, and CR is a biological measure of sociopsychological stress response. When examined together in the context of psychopathology, findings may provide greater insight into vulnerabilities and protective factors related to mental illness and impairment.

Additionally, our findings are particularly fascinating, because it suggests that early childhood CR may still be valuable in understanding later psychopathology. However, this relation was inconsistent over time. For example, we found that the three-way interaction was significant at ages three and nine, but not age six. Given that the variability in both irritability and depression symptoms at age six are comparable to the respective variabilities at ages three and nine, make interpretation a challenge. Future research should look to elucidate this association further.

Further, research should continue to examine psychopathology at the symptom and subtype level. For example, in a cross-sectional study of adolescents with depressive disorders, Morris et al. (2017) found that differences in psychopathology symptom combinations may account for the heterogeneity in the literature on CR. With respect to ADHD, examining subtype and specific symptoms may help shine a light on which executive functions are implicated and impaired during stressful moments.

Additionally, more research should examine CR longitudinally throughout early and middle childhood. Specifically, does CR change from ages three to six to nine? If so, how does it change? If not, is it still predictive? Learning more about the development of early and middle childhood CR may impact our understanding of psychosocial stress responsivity in prepubertal children at risk for psychopathology.

Moreover, there is little research investigating the association between early CR and other outcomes longitudinally from early childhood to adolescence and adulthood. Existing research has examined CR associations longitudinally in early childhood (i.e., from ages three to six), with findings suggesting CR is a good concurrent predictor of psychopathology (Barrios et al., 2017; Kopala-Sibley et

al., 2017). However, there is no published research investigating whether early CR fades in impact as a predictor throughout childhood. Our results suggest that early CR may still be impactful as a predictor of adolescent psychopathology.

Finally, all future irritability and CR research would benefit from the inclusion of sex as an additional moderator. The relation between cortisol reactivity and irritability was better understood when sex was included in the model in this way. This is especially so in light of the findings that six-year-old males and females showed opposite irritability by CR effects on ADHD symptoms.

Conclusion

In sum, an abundance of research has observed that irritability (an index of emotion and mood dysregulation) and CR (an index of sociopsychological stress responsivity) are respectively associated with and predictive of later psychopathology (Barrios et al., 2017; Brotman et al., 2006; Doom & Gunnar, 2013; Dougherty et al., 2015; Grimm et al., 2017; Gunnar & Vazquez, 2006; Keefe et al., 1996; Morris et al., 2012; Nelson, 2011; Pariante, 2003; Sapolsky et al., 1986; Slavich & Irwin, 2014; Stringaris et al., 2009; Vogel et al., 2019; Zorn et al., 2017). Both atypical irritability and CR have been associated with psychopathology in the context of sex. The present study set out to investigate how these variables were related longitudinally. To date, this is the first study to examine a three-way interaction between irritability, CR, and sex in relation to psychopathology symptoms, and at multiple ages. Significant findings for ADHD and depression suggest that further research on this association should be conducted.

References

Adani, S., & Cepanec, M. (2019). Sex differences in early communication development: behavioral and neurobiological indicators of more vulnerable communication system development in boys. *Croatian medical journal*, *60*(2), 141–149. https://doi.org/10.3325/cmj.2019.60.141

Alink, L. R., van Ijzendoorn, M. H., Bakermans-Kranenburg, M. J., Mesman, J., Juffer, F., & Koot, H. M. (2008). Cortisol and externalizing behavior in children and adolescents: Mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Developmental Psychobiology*, 50(5), 427–450. doi: 10.1002/dev.20300.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: Author.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Andrews, J., Janzen, Henry L, & Saklofske, Donald H. (2001). *Handbook of psychoeducational assessment ability, achievement, and behavior in children* (Educational psychology). San Diego: Academic Press.

Bagner, D. M., Sheinkopf, S. J., Vohr, B. R., & Lester, B. M. (2010). A preliminary study of cortisol reactivity and behavior problems in young children born premature. *Developmental psychobiology*, *52*(6), 574–582. doi:10.1002/dev.20464

Barata, P. C., Holtzman, S., Cunningham, S., O'Connor, B. P., & Stewart, D. E. (2016). Building a definition of irritability from academic definitions and lay descriptions. *Emotion review: journal of the International Society for Research on Emotion*, *8*(2), 164–172. doi:10.1177/1754073915576228

Barch, D. M., Harms, M. P., Tillman, R., Hawkey, E., & Luby, J. L. (2019). Early childhood depression, emotion regulation, episodic memory, and hippocampal development. *Journal of abnormal psychology*, *128*(1), 81–95. https://doi.org/10.1037/abn0000392

Barrios, C. S., Bufferd, S. J., Klein, D. N., & Dougherty, L. R. (2017). The interaction between parenting and children's cortisol reactivity at age 3 predicts increases in children's internalizing and externalizing symptoms at age 6. *Development and psychopathology*, *29*(4), 1319–1331. doi:10.1017/S0954579417000293

Beauchaine, T. P., & Hinshaw, S. P. (2017). *Child and adolescent psychopathology* (3rd ed.). Wiley.

Bjelland, I., Lie, S. A., Dahl, A. A., Mykletun, A., Stordal, E., & Kraemer, H. C. (2009). A dimensional versus a categorical approach to diagnosis: anxiety and depression in the HUNT 2 study. *International journal of methods in psychiatric research*, *18*(2), 128–137. https://doi.org/10.1002/mpr.284

Bowie B. H. (2007). Relational aggression, gender, and the developmental process. *Journal of child and adolescent psychiatric nursing : official publication of the Association of Child and Adolescent Psychiatric Nurses, Inc*, *20*(2), 107–115. https://doi.org/10.1111/j.1744-6171.2007.00092.x

Brotman, M. A., Kircanski, K., Stringaris, A., Pine, D. S., & Leibenluft, E. (2017). Irritability in Youths: A Translational Model. *American Journal of Psychiatry*, *174*(6), 520–532. https://doi.org/10.1176/appi.ajp.2016.16070839

Brotman, M. A., Schmajuk, M., Rich, B. A., Dickstein, D. P., Guyer, A. E., Costello, E. J., … Leibenluft, E. (2006). Prevalence, clinical correlates, and longitudinal course of severe mood dysregulation in children. *Biological Psychiatry*, *60*(9), 991–997. doi:10.1016/j.biopsych.2006.08.042

Brown, T. A., & Barlow, D. H. (2005). Dimensional versus categorical classification of mental disorders in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders and beyond: comment on the special section. *Journal of abnormal psychology*, *114*(4), 551–556. https://doi.org/10.1037/0021-843X.114.4.551

Calvi, J. L., Chen, F. R., Benson, V. B., Brindle, E., Bristow, M., De, A., Entringer, S., Findlay, H., Heim, C., Hodges, E. A., Klawitter, H., Lupien, S., Rus, H. M., Tiemensma, J., Verlezza, S., Walker, C. D., & Granger, D. A. (2017). Measurement of cortisol in saliva: a comparison of measurement

error within and between international academic-research laboratories. *BMC research notes*, *10*(1), 479. https://doi.org/10.1186/s13104-017-2805-4
Centers for Disease Control and Prevention. (2020, June 15). *Data and statistics on children's mental health*. https://www.cdc.gov/childrensmentalhealth/data.html.
Chaplin, T. M., & Aldao, A. (2013). Gender differences in emotion expression in children: a meta-analytic review. *Psychological bulletin*, *139*(4), 735–765. https://doi.org/10.1037/a0030737
Cohen, J., Cohen, P., West, S. G., & Aiken, L. S. (2015). *Applied multiple regression/correlation analysis for the behavioral sciences*. Routledge.
Colich, N. L., Kircanski, K., Foland-Ross, L. C., & Gotlib, I. H. (2015). HPA-axis reactivity interacts with stage of pubertal development to predict the onset of depression. *Psychoneuroendocrinology*, *55*, 94–101. doi:10.1016/j.psyneuen.2015.02.004
Copeland, W. E., Brotman, M. A., & Costello, E. J. (2015). Normative irritability in youth: Developmental findings from the Great Smoky Mountains study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *54*(8), 635–642. doi:10.1016/j.jaac.2015.05.008
Cordier, R., Bundy, A., Hocking, C., & Einfeld, S. (2010). Comparison of the play of children with attention deficit hyperactivity disorder by subtypes. *Australian occupational therapy journal*, *57*(2), 137–145. https://doi.org/10.1111/j.1440-1630.2009.00821.x
Daoust, A. R., Kotelnikova, Y., Kryski, K. R., Sheikh, H. I., Singh, S. M., & Hayden, E. P. (2018). Child sex moderates the relationship between cortisol stress reactivity and symptoms over time. *Comprehensive Psychiatry*, *87*, 161–170. https://doi.org/10.1016/J.COMPPSYCH.2018.10.009
Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin*, *130*(3), 355-391. doi:http://dx.doi.org/10.1037/0033-2909.130.3.355
Dimitrov, A., Demin, K., Fehlner, P., Walter, H., Erk, S., & Veer, I. M. (2018). Differences in neural recovery from acute stress between cortisol responders and non-responders. *Frontiers in psychiatry*, *9*, 631. doi:10.3389/fpsyt.2018.00631
Doom, J. R., & Gunnar, M. R. (2013). Stress physiology and developmental psychopathology: past, present, and future. *Development and psychopathology*, 25(4 Pt 2), 1359–1373. doi:10.1017/S0954579413000667
Dougherty, L. R., Bufferd, S. J., Carlson, G. A., Dyson, M., Olino, T. M., Durbin, C. E., & Klein, D. N. (2011). Preschoolers' observed temperament and psychiatric disorders assessed with a parent diagnostic interview. *Journal of clinical child and adolescent psychology: the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *40*(2), 295–306. https://doi.org/10.1080/15374416.2011.546046
Dougherty, L. R., Smith, V. C., Bufferd, S. J., Kessel, E., Carlson, G. A., & Klein, D. N. (2015). Preschool irritability predicts child psychopathology, functional impairment, and service use at age nine. *Journal of child psychology and psychiatry, and allied disciplines*, *56*(9), 999–1007. doi:10.1111/jcpp.12403
Dougherty, L. R., Smith, V. C., Bufferd, S. J., Stringaris, A., Leibenluft, E., Carlson, G. A., & Klein, D. N. (2013). Preschool irritability: longitudinal associations with psychiatric disorders at age 6 and parental psychopathology. Journal of the American Academy of Child and Adolescent Psychiatry, 52(12), 1304–1313. doi:10.1016/j.jaac.2013.09.007
Egger, H. L., & Angold, A. (2004). The Preschool Age Psychiatric Assessment (PAPA): A structured parent interview for diagnosing psychiatric disorders in preschool children. In R. DelCarmen-Wiggins, & A. Carter (Eds.). *Handbook of infant, toddler, and preschool mental assessment* (pp. 223–243). New York: Oxford University Press.
Essau, C., Leblanc, S., & Ollendick, T. H. (2017). *Emotion regulation and psychopathology in children and adolescents*. Oxford university press.
Evans, S. C., Abel, M. R., Doyle, R. L., Skov, H., & Harmon, S. L. (2021a). Measurement and correlates of irritability in clinically referred youth: Further examination of the Affective Reactivity Index. *Journal of affective disorders*, *283*, 420–429. https://doi.org/10.1016/j.jad.2020.11.002

Evans, S. C., Roberts, M. C., Keeley, J. W., Rebello, T. J., de la Peña, F., Lochman, J. E., Burke, J. D., Fite, P. J., Ezpeleta, L., Matthys, W., Youngstrom, E. A., Matsumoto, C., Andrews, H. F., Elena Medina-Mora, M., Ayuso-Mateos, J. L., Khoury, B., Kulygina, M., Robles, R., Sharan, P., Zhao, M., … Reed, G. M. (2021b). Diagnostic classification of irritability and oppositionality in youth: a global field study comparing ICD-11 with ICD-10 and DSM-5. *Journal of child psychology and psychiatry, and allied disciplines*, *62*(3), 303–312. https://doi.org/10.1111/jcpp.13244

Eyre, O., Langley, K., Stringaris, A., Leibenluft, E., Collishaw, S., & Thapar, A. (2017). Irritability in ADHD: Associations with depression liability. *Journal of affective disorders*, *215*, 281–287. doi:10.1016/j.jad.2017.03.050

Fechner, P. Y. (2003). *The biology of puberty: New developments in sex differences.* In C. Hayward (Ed.), *Gender differences at puberty* (p. 17–28). Cambridge University Press. https://doi.org/10.1017/CBO9780511489716.003

Finsaas, M. C., Kessel, E. M., Dougherty, L. R., Bufferd, S. J., Danzig, A. P., Davila, J., Carlson, G. A., & Klein, D. N. (2020). Early Childhood Psychopathology Prospectively Predicts Social Functioning in Early Adolescence. *Journal of clinical child and adolescent psychology: the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *49*(3), 353–364. https://doi.org/10.1080/15374416.2018.1504298

Franz, L., Angold, A., Copeland, W., Costello, E. J., Towe-Goodman, N., & Egger, H. (2013). Preschool anxiety disorders in pediatric primary care: prevalence and comorbidity. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(12), 1294–1303.e1. https://doi.org/10.1016/j.jaac.2013.09.008

Fried E. I. (2017). The 52 symptoms of major depression: Lack of content overlap among seven common depression scales. *Journal of affective disorders*, *208*, 191–197. https://doi.org/10.1016/j.jad.2016.10.019

Ge, X., Natsuaki, M. N. (2009). In search of explanations for early pubertal timing effects on developmental psychopathology: Pubertal timing and psychopathology. *Current Directions in Psychological Science*, *18*(6), 327–331. doi: 10.1111/j.1467-8721.2009.01661.x

Goldberg, D. (2010). The interplay between biological and psychological factors in determining vulnerability to mental disorders. *Psychoanalytic Psychotherapy*, *23*(3), 236-247

Goldsmith, H. H., Reilly, J., Lemery, K. S., Longley, S., & Prescott, A. (1995). *Laboratory Temperament Assessment Battery*: *Preschool version*. Unpublished manuscript.

Graber, J. A. (2013). Pubertal timing and the development of psychopathology in adolescence and beyond. *Hormones and behavior, 2,* 262–269.

Granger, D. A., Hibel, L. C., Fortunato, C. K., & Kapelewski, C. H. (2009). Medication effects on salivary cortisol: tactics and strategy to minimize impact in behavioral and developmental science. *Psychoneuroendocrinology*, *34*(10), 1437–1448. https://doi.org/10.1016/j.psyneuen.2009.06.017

Grimm, S., Wirth, K., Fan, Y., Weigand, A., Gartner, M., Feeser, M., Dziobek, I., Bajbouj, M., & Aust, S. (2017). The interaction of corticotropin-releasing hormone receptor gene and early life stress on emotional empathy. *Behavioral Brain Research*, *329*, 180-185.

Güleç, H., KarabekİroğIu, A., Yenel, A., Baykaran, M. B., & Keleş Ünal, E. (2014). Effects of dimensional and categorical classification on the clinical manifestation of major depressive disorder. *Noro psikiyatri arsivi*, *51*(3), 233–241. https://doi.org/10.4274/npa.y6834

Gunnar, M. R., & Vazquez, D. (2006). Stress neurobiology and developmental psychopathology. In D. Cicchetti & D. J. Cohen (Eds.), *Developmental psychopathology: Developmental neuroscience* (pp. 533-577). Hoboken, NJ: John Wiley & Sons Inc.

Gunnar, M. R., Talge, N. M., & Herrera, A. (2009a). Stressor paradigms in developmental studies: what does and does not work to produce mean increases in salivary cortisol. *Psychoneuroendocrinology*, *34*(7), 953–967. https://doi.org/10.1016/j.psyneuen.2009.02.010

Gunnar, M. R., Wewerka, S., Frenn, K., Long, J. D., & Griggs, C. (2009b). Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: normative changes and associations with puberty. *Development and psychopathology*, *21*(1), 69–85. doi:10.1017/S0954579409000054

Hamed, A. M., Kauer, A. J., & Stevens, H. E. (2015). Why the diagnosis of attention deficit hyperactivity disorder matters. *Frontiers in psychiatry*, *6*, 168. https://doi.org/10.3389/fpsyt.2015.00168

Hankin, B. L., Badanes, L. S., Abela, J. R., & Watamura, S. E. (2010). Hypothalamic-pituitary-adrenal axis dysregulation in dysphoric children and adolescents: Cortisol reactivity to psychosocial stress from preschool through middle adolescence. *Biological psychiatry*, *68*(5), 484–490. https://doi.org/10.1016/j.biopsych.2010.04.004

Hankin, B. L., Badanes, L. S., Smolen, A., & Young, J. F. (2015). Cortisol reactivity to stress among youth: Stability over time and genetic variants for stress sensitivity. *Journal of abnormal psychology*, *124*(1), 54–67. doi:10.1037/abn0000030

Harkness, K. L., Stewart, J. G., Wynne-Edwards, K. E. (2011). Cortisol reactivity to social stress in adolescents: Role of depression severity and child maltreatment. *Psychoneuroendocrinology*, *36*(2), 173–181. doi: 10.1016/j.psyneuen.2010.07.006

Hartung, C. M., & Lefler, E. K. (2019). Sex and gender in psychopathology: DSM–5 and beyond. *Psychological Bulletin, 145*(4), 390–409. https://doi.org/10.1037/bul0000183

Haslam N. (2003). Categorical versus dimensional models of mental disorder: the taxometric evidence. *The Australian and New Zealand journal of psychiatry*, *37*(6), 696–704. https://doi.org/10.1080/j.1440-1614.2003.01258.x

Hastings, P. D., Fortier, I., Utendale, W. T., Simard, L. R., & Robaey, P. (2009). Adrenocortical functioning in boys with attention-deficit/hyperactivity disorder: examining subtypes of ADHD and associated comorbid conditions. *Journal of abnormal child psychology*, *37*(4), 565–578. https://doi.org/10.1007/s10802-008-9292-y

Hayes, A. F., & Little, T. D. (2018). *Introduction to mediation, moderation, and conditional process analysis: a regression-based approach*. Guilford.

Heijmens Visser, J. H., van der Ende, J., Koot, H. M., & Verhulst, F. C. (2000). Predictors of psychopathology in young adults referred to mental health services in childhood or adolescence. *The British journal of psychiatry : the journal of mental science*, *177*, 59–65. https://doi.org/10.1192/bjp.177.1.59

Hirvikoski, T., Lindholm, T., Nordenström, A., Nordström, A. L., & Lajic, S. (2009). High self-perceived stress and many stressors, but normal diurnal cortisol rhythm, in adults with ADHD (attention-deficit/hyperactivity disorder). *Hormones and behavior*, *55*(3), 418–424. https://doi.org/10.1016/j.yhbeh.2008.12.004

Hong, H. J., Shin, D. W., Lee, E. H., Oh, Y. H., & Noh, K. S. (2003). Hypothalamic-pituitary-adrenal reactivity in boys with attention deficit hyperactivity disorder. *Yonsei medical journal*, *44*(4), 608–614. https://doi.org/10.3349/ymj.2003.44.4.608

Jaffee, S. R., McFarquhar, T., Stevens, S., Ouellet-Morin, I., Melhuish, E., & Belsky, J. (2015). Interactive effects of early and recent exposure to stressful contexts on cortisol reactivity in middle childhood. *Journal of Child Psychology and Psychiatry*, *56*(2), 138–146. doi:10.1111/jcpp.12287

Jha, M. K., Minhajuddin, A., South, C., Rush, A. J., & Trivedi, M. H. (2019). Irritability and Its Clinical Utility in Major Depressive Disorder: Prediction of Individual-Level Acute-Phase Outcomes Using Early Changes in Irritability and Depression Severity. *The American journal of psychiatry*, *176*(5), 358–366. https://doi.org/10.1176/appi.ajp.2018.18030355

Kamphaus, R. W., Petoskey, M. D., Cody, A. H., Rowe, E. W., Huberty, C. J., & Reynolds, C. R. (1999). A typology of parent rated child behavior for a national U.S. sample. *Journal of child psychology and psychiatry, and allied disciplines*, *40*(4), 607–616.

Kamradt, J. M., Momany, A. M., & Nikolas, M. A. (2018). A meta-analytic review of the association between cortisol reactivity in response to a stressor and attention-deficit hyperactivity disorder.

Attention deficit and hyperactivity disorders, *10*(2), 99–111. https://doi.org/10.1007/s12402-017-0238-5

Kaufman, J., Birmaher, B., Brent, D., Rao, U. M. A., Flynn, C., Moreci, P., & Ryan, N. (1997). Schedule for affective disorders and schizophrenia for school-age children present and lifetime version (K-SADS-PL): Initial reliability and validity data. *Journal of the American Academy of Child & Adolescent Psychiatry*, *36*, 980–988.

Keefe, M. R., Kotzer, A. M., Froese-Fretz, A., & Curtin, M. (1996). A longitudinal comparison of irritable and nonirritable infants. *Nursing Research*, *45*(1), 4–9.

Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., & Carpenter, L. L. (2008). Sex differences in emotional and physiological responses to the Trier Social Stress Test. *Journal of behavior therapy and experimental psychiatry*, *39*(1), 87–98. https://doi.org/10.1016/j.jbtep.2007.02.003

King, J. A., Barkley, R. A., & Barrett, S. (1998). Attention-deficit hyperactivity disorder and the stress response. *Biological psychiatry*, *44*(1), 72–74. https://doi.org/10.1016/s0006-3223(97)00507-6

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1-2), 76–81. https://doi.org/10.1159/000119004

Klein, D.N., & Finsaas, M.C. (2017). The Stony Brook Temperament Study: Early antecedents and pathways to emotional disorders. *Child Development Perspectives, 11,* 257 – 263. doi:10.1111/cdep.12242

Klimes-Dougan, B., Hastings, P. D., Granger, D. A., Usher, B. A., & Zahn-Waxler, C. (2001). Adrenocortical activity in at-risk and normally developing adolescents: individual differences in salivary cortisol basal levels, diurnal variation, and responses to social challenges. *Development and psychopathology*, *13*(3), 695–719. https://doi.org/10.1017/s0954579401003157

Koh, D. S-Q., & Koh, G. C-H. (2007). The use of salivary biomarkers in occupational and environmental medicine. *Occupational and environmental medicine*, *64*(3), 202–210. https://doi.org/10.1136/oem.2006.026567

Kopala-Sibley, D. C., Dougherty, L. R., Dyson, M. W., Laptook, R. S., Olino, T. M., Bufferd, S. J., & Klein, D. N. (2017). Early childhood cortisol reactivity moderates the effects of parent-child relationship quality on the development of children's temperament in early childhood. *Developmental science*, *20*(3), 10.1111/desc.12378. https://doi.org/10.1111/desc.12378

Koss, K. J., & Gunnar, M. R. (2018). Annual Research Review: Early adversity, the hypothalamic-pituitary-adrenocortical axis, and child psychopathology. *Journal of child psychology and psychiatry, and allied disciplines*, *59*(4), 327–346. doi:10.1111/jcpp.12784

Kotov, R., Krueger, R. F., Watson, D., Achenbach, T. M., Althoff, R. R., Bagby, R. M., Brown, T. A., Carpenter, W. T., Caspi, A., Clark, L. A., Eaton, N. R., Forbes, M. K., Forbush, K. T., Goldberg, D., Hasin, D., Hyman, S. E., Ivanova, M. Y., Lynam, D. R., Markon, K., Miller, J. D., … Zimmerman, M. (2017). The Hierarchical Taxonomy of Psychopathology (HiTOP): A dimensional alternative to traditional nosologies. *Journal of abnormal psychology*, *126*(4), 454–477. https://doi.org/10.1037/abn0000258

Krueger, R. F., & Bezdjian, S. (2009). Enhancing research and treatment of mental disorders with dimensional concepts: toward DSM-V and ICD-11. *World psychiatry : official journal of the World Psychiatric Association (WPA)*, *8*(1), 3–6. https://doi.org/10.1002/j.2051-5545.2009.tb00197.x

Leibenluft, E., Cohen, P., Gorrindo, T., Brook, J. S., & Pine, D. S. (2006). Chronic versus episodic irritability in youth: A community-based, longitudinal study of clinical and diagnostic associations. *Journal of child and adolescent psychopharmacology, 4,* 456–466.

Leibenluft, E., & Stoddard, J. (2013). The developmental psychopathology of irritability. *Development and psychopathology*, *25*(4 Pt 2), 1473–1487. https://doi.org/10.1017/S0954579413000722

Li, R. Y. H., & Wong, W. I. (2016). Gender-typed play and social abilities in boys and girls: Are they related? *Sex Roles: A Journal of Research, 74*(9-10), 399–410. *https://doi.org/10.1007/s11199-016-0580-7*

Liu, J., Ein, N., Peck, K., Huang, V., Pruessner, J. C., & Vickers, K. (2017). Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-analysis. *Psychoneuroendocrinology*, *82*, 26–37. https://doi.org/10.1016/j.psyneuen.2017.04.007

Lopez-Duran, N. L., Kovacs, M., & George, C. J. (2009). Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. *Psychoneuroendocrinology*, *34*(9), 1272–1283. https://doi.org/10.1016/j.psyneuen.2009.03.016

Loth, A. K., Drabick, D. A., Leibenluft, E., & Hulvershorn, L. A. (2014). Do childhood externalizing disorders predict adult depression? A meta-analysis. *Journal of abnormal child psychology*, *42*(7), 1103–1113. https://doi.org/10.1007/s10802-014-9867-8

Luby, J. L., Heffelfinger, A., Mrakotsky, C., Brown, K., Hessler, M., & Spitznagel, E. (2003). Alterations in stress cortisol reactivity in depressed preschoolers relative to psychiatric and no-disorder comparison groups. *Archives of general psychiatry*, *60*(12), 1248–1255. https://doi.org/10.1001/archpsyc.60.12.1248

Luo, Y., Weibman, D., Halperin, J. M., & Li, X. (2019). A Review of Heterogeneity in Attention Deficit/Hyperactivity Disorder (ADHD). *Frontiers in human neuroscience*, *13*, 42. https://doi.org/10.3389/fnhum.2019.00042

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature reviews. Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Lynch, C. J., Gunning, F. M., & Liston, C. (2020). Causes and Consequences of Diagnostic Heterogeneity in Depression: Paths to Discovering Novel Biological Depression Subtypes. *Biological psychiatry*, *88*(1), 83–94. https://doi.org/10.1016/j.biopsych.2020.01.012

Maldonado, E. F., Trianes, M. V., Cortés, A., Moreno, E., & Escobar, M. (2009). Salivary cortisol response to a psychosocial stressor on children diagnosed with attention-deficit/hyperactivity disorder: differences between diagnostic subtypes. *The Spanish journal of psychology*, *12*(2), 707–714. https://doi.org/10.1017/s1138741600002079

Mazurka, R., Wynne-Edwards, K. E., & Harkness, K. L. (2016). Stressful Life Events Prior to Depression Onset and the Cortisol Response to Stress in Youth with First Onset Versus Recurrent Depression. *Journal of abnormal child psychology*, *44*(6), 1173–1184. https://doi.org/10.1007/s10802-015-0103-y

McBurnett, K., Lahey, B. B., Rathouz, P. J., & Loeber, R. (2000). Low Salivary Cortisol and Persistent Aggression in Boys Referred for Disruptive Behavior. *Archives of General Psychiatry*, *57*(1), 38. https://doi.org/10.1001/archpsyc.57.1.38

McEwen B. S. (1998). Protective and damaging effects of stress mediators. *The New England journal of medicine*, *338*(3), 171–179. https://doi.org/10.1056/NEJM199801153380307

Melhem, N. M., Keilp, J. G., Porta, G., Oquendo, M. A., Burke, A., Stanley, B., … Brent, D. A. (2016). Blunted HPA axis activity in suicide attempters compared to those at high risk for suicidal behavior. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, *41*(6), 1447–1456. doi:10.1038/npp.2015.309

Mendle, J. (2014). Why puberty matters for psychopathology. *Child Development Perspectives*, *8*(4), 218 – 222. doi: 10.1111/cdep.12092

Mick, E., Spencer, T., Wozniak, J., & Biederman, J. (2005). Heterogeneity of irritability in attention-deficit/hyperactivity disorder subjects with and without mood disorders. *Biological psychiatry*, *58*(7), 576–582. https://doi.org/10.1016/j.biopsych.2005.05.037

Mikita, N., Hollocks, M. J., Papadopoulos, A. S., Aslani, A., Harrison, S., Leibenluft, E., … Stringaris, A. (2015). Irritability in boys with autism spectrum disorders: an investigation of physiological reactivity. *Journal of child psychology and psychiatry, and allied disciplines*, *56*(10), 1118–1126. doi:10.1111/jcpp.12382

Morris, M. C., Kouros, C. D., Mielock, A. S., & Rao, U. (2017). Depressive symptom composites associated with cortisol stress reactivity in adolescents. *Journal of affective disorders*, *210*, 181–188. doi:10.1016/j.jad.2016.12.023

Morris, M. C., Rao, U., & Garber, J. (2012). Cortisol responses to psychosocial stress predict depression trajectories: social-evaluative threat and prior depressive episodes as moderators. *Journal of affective disorders*, *143*(1-3), 223–230. doi:10.1016/j.jad.2012.05.059

Murray, J., Irving, B., Farrington, D. P., Colman, I., & Bloxsom, C. A. (2010). Very early predictors of conduct problems and crime: results from a national cohort study. *Journal of child psychology and psychiatry, and allied disciplines*, *51*(11), 1198–1207. https://doi.org/10.1111/j.1469-7610.2010.02287.x

Natsuaki, M. N., Klimes-Dougan, B., Ge, X., Shirtcliff, E. A., Hastings, P. D., & Zahn-Waxler, C. (2009). Early pubertal maturation and internalizing problems in adolescence: sex differences in the role of cortisol reactivity to interpersonal stress. *Journal of clinical child and adolescent psychology : the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53*, *38*(4), 513–524. https://doi.org/10.1080/15374410902976320

Nelson, R. J. (2011). *An introduction to behavioral endocrinology (4th ed.)*. Sunderland, MA: Sinauer Associates, Inc

Northover, C., Thapar, A., Langley, K., Fairchild, G., & van Goozen, S. (2016). Cortisol levels at baseline and under stress in adolescent males with attention-deficit hyperactivity disorder, with or without comorbid conduct disorder. *Psychiatry research*, *242*, 130–136. https://doi.org/10.1016/j.psychres.2016.05.052

Obradović, J., Bush, N. R., Stamperdahl, J., Adler, N. E., & Boyce, W. T. (2010). Biological sensitivity to context: The interactive effects of stress reactivity and family adversity on socioemotional behavior and school readiness. *Child development*, *81*(1), 270–289. doi:10.1111/j.1467-8624.2009.01394.x

Ouellet-Morin, I., Odgers, C. L., Danese, A., Bowes, L., Shakoor, S., Papadopoulos, A. S., Caspi, A., Moffitt, T. E., & Arseneault, L. (2011). Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children. *Biological psychiatry*, *70*(11), 1016–1023. https://doi.org/10.1016/j.biopsych.2011.06.017

Papadimitriou G. (2017). The "Biopsychosocial Model": 40 years of application in psychiatry. *Psychiatriki*, *28*(2), 107–110. https://doi.org/10.22365/jpsych.2017.282.107

Pariante, C. M. (2003). Depression, stress and the adrenal axis. *Journal of neuroendocrinology, 8,* 811–812.

Pesonen, A. K., Kajantie, E., Jones, A., Pyhälä, R., Lahti, J., Heinonen, K., Eriksson, J. G., Strandberg, T. E., & Räikkönen, K. (2011). Symptoms of attention deficit hyperactivity disorder in children are associated with cortisol responses to psychosocial stress but not with daily cortisol levels. *Journal of psychiatric research*, *45*(11), 1471–1476. https://doi.org/10.1016/j.jpsychires.2011.07.002

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of youth and adolescence*, *17*(2), 117–133. https://doi.org/10.1007/BF01537962

Pihlakoski, L., Sourander, A., Aromaa, M., Rautava, P., Helenius, H., & Sillanpää, M. (2006). The continuity of psychopathology from early childhood to preadolescence: a prospective cohort study of 3-12-year-old children. *European child & adolescent psychiatry*, *15*(7), 409–417. https://doi.org/10.1007/s00787-006-0548-1

Pine D. S. (2019). Heterogeneity in Major Depressive Disorder: Lessons From Developmental Research on Irritability. *The American journal of psychiatry*, *176*(5), 331–332. https://doi.org/10.1176/appi.ajp.2019.19020214

Pomerantz, H., Parent, J., Forehand, R., Breslend, N. L., & Winer, J. P. (2017). Pubertal timing and youth internalizing psychopathology: The role of relational aggression. *Journal of child and family studies*, *26*(2), 416–423. doi:10.1007/s10826-016-0598-z

Ponitz, C. C., McClelland, M. M., Matthews, J. S., & Morrison, F. J. (2009). A structured observation of behavioral self-regulation and its contribution to kindergarten outcomes. *Developmental psychology*, *45*(3), 605–619. https://doi.org/10.1037/a0015365

Powers, S. I., Laurent, H. K., Gunlicks-Stoessel, M., Balaban, S., & Bent, E. (2016). Depression and anxiety predict sex-specific cortisol responses to interpersonal stress. *Psychoneuroendocrinology*, *69*, 172–179. https://doi.org/10.1016/j.psyneuen.2016.04.007

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 7,* 916–931.

Raz, S., & Leykin, D. (2015). Psychological and cortisol reactivity to experimentally induced stress in adults with ADHD. *Psychoneuroendocrinology*, *60*, 7–17. https://doi.org/10.1016/j.psyneuen.2015.05.008

Roy, A. K., Brotman, M. A., & Leibenluft, E. (2019). *Irritability in pediatric psychopathology*. Oxford University Press.

Ruttle, P. L., Shirtcliff, E. A., Serbin, L. A., Fisher, D. B., Stack, D. M., & Schwartzman, A. E. (2011). Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviors in youth: longitudinal and concurrent associations with cortisol. *Hormones and behavior*, *59*(1), 123–132. https://doi.org/10.1016/j.yhbeh.2010.10.015

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrine reviews, 3,* 284–301.

Seymour, K. E., Rosch, K. S., Tiedemann, A., & Mostofsky, S. H. (2020). The Validity of a Frustration Paradigm to Assess the Effect of Frustration on Cognitive Control in School-Age Children. *Behavior therapy*, *51*(2), 268–282. https://doi.org/10.1016/j.beth.2019.06.009

Shaw, P., Stringaris, A., Nigg, J., & Leibenluft, E. (2014). Emotion dysregulation in attention deficit hyperactivity disorder. *The American journal of psychiatry*, *171*(3), 276–293. https://doi.org/10.1176/appi.ajp.2013.13070966

Shelton, K. H., & van den Bree, M. B. (2010). The Moderating Effects of Pubertal Timing on the Longitudinal Associations Between Parent-Child Relationship Quality and Adolescent Substance Use. *Journal of research on adolescence : the official journal of the Society for Research on Adolescence*, *20*(4), 1044–1064. https://doi.org/10.1111/j.1532-7795.2010.00643.x

Shirtcliff, E. A., Dahl, R. E., & Pollak, S. D. (2009). Pubertal development: Correspondence between hormonal and physical development. *Child Development*, *80*(2), 327–337.

Shonkoff, J. P., Boyce, W. T., & McEwen, B. S. (2009). Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention. *JAMA, 21,* 2252–2259.

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive disorder: A social signal transduction theory of depression. *Psychological bulletin*, *140*(3), 774–815. doi:10.1037/a0035302

Skogli, E. W., Teicher, M. H., Andersen, P. N., Hovik, K. T., & Øie, M. (2013). ADHD in girls and boys--gender differences in co-existing symptoms and executive function measures. *BMC psychiatry*, *13*, 298. https://doi.org/10.1186/1471-244X-13-298

Snoek, H., Van Goozen, S. H., Matthys, W., Buitelaar, J. K., & van Engeland, H. (2004). Stress responsivity in children with externalizing behavior disorders. *Development and psychopathology*, *16*(2), 389–406. https://doi.org/10.1017/s0954579404044578

Sontag, L. M., Graber, J. A., & Clemans, K. H. (2010). The role of peer stress and pubertal timing on symptoms of psychopathology during early adolescence. *Journal of youth and adolescence, 10,* 1371–1382.

Sontag-Padilla, L. M., Dorn, L. D., Tissot, A., Susman, E. J., Beers, S. R., & Rose, S. R. (2012). Executive functioning, cortisol reactivity, and symptoms of psychopathology in girls with premature adrenarche. *Development and psychopathology*, *24*(1), 211–223. https://doi.org/10.1017/S0954579411000782

Spies, L. A., Margolin, G., Susman, E. J., & Gordis, E. B. (2011). Adolescents' cortisol reactivity and subjective distress in response to family conflict: the moderating role of internalizing symptoms.

The Journal of adolescent health: official publication of the Society for Adolescent Medicine, *49*(4), 386–392. doi:10.1016/j.jadohealth.2011.01.014

Stadler, C., Kroeger, A., Weyers, P., Grasmann, D., Horschinek, M., Freitag, C., & Clement, H. W. (2011). Cortisol reactivity in boys with attention-deficit/hyperactivity disorder and disruptive behavior problems: the impact of callous unemotional traits. *Psychiatry research*, *187*(1-2), 204–209. https://doi.org/10.1016/j.psychres.2010.05.004

Steeger, C. M., Cook, E. C., & Connell, C. M. (2017). The interactive effects of stressful family life events and cortisol reactivity on adolescent externalizing and internalizing behaviors. *Child psychiatry and human development*, *48*(2), 225–234. doi:10.1007/s10578-016-0635-6

Stetler, C., & Miller, G. E. (2011). Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosomatic medicine*, *73*(2), 114–126. https://doi.org/10.1097/PSY.0b013e31820ad12b

Stewart, S. L., Thornley, E., Lapshina, N., Erickson, P., Vingilis, E., Hamilton, H., & Kolla, N. (2020). Sex differences in youth with mental health problems in inpatient, outpatient and youth justice settings. *BMC psychiatry*, *20*(1), 11. https://doi.org/10.1186/s12888-019-2413-z

Stringaris, A., Cohen, P., Pine, D. S., & Leibenluft, E. (2009). Adult Outcomes of Youth Irritability: A 20-Year Prospective Community-Based Study. *American Journal of Psychiatry*, *166*(9), 1048–1054. https://doi.org/10.1176/appi.ajp.2009.08121849

Stringaris, A., Goodman, R., Ferdinando, S., Razdan, V., Muhrer, E., Leibenluft, E., & Brotman, M. A. (2012). The affective reactivity index: a concise irritability scale for clinical and research settings. *Journal of child psychology and psychiatry, and allied disciplines*, *53*(11), 1109–1117. doi:10.1111/j.1469-7610.2012.02561.x

Stringaris, A., Vidal-Ribas, P., Brotman, M. A., & Leibenluft, E. (2018). Practitioner Review: Definition, recognition, and treatment challenges of irritability in young people. *Journal of child psychology and psychiatry, and allied disciplines*, *59*(7), 721–739. https://doi.org/10.1111/jcpp.12823

Suzuki, H., Belden, A. C., Spitznagel, E., Dietrich, R., & Luby, J. L. (2013). Blunted stress cortisol reactivity and failure to acclimate to familiar stress in depressed and sub-syndromal children. *Psychiatry research*, *210*(2), 575–583. doi:10.1016/j.psychres.2013.06.038

Toohey, M. J., & DiGiuseppe, R. (2017, April 1). Defining and measuring irritability: Construct clarification and differentiation. *Clinical Psychology Review*, Vol. 53, pp. 93–108. https://doi.org/10.1016/j.cpr.2017.01.009

Tukey, J. W. (1962). The future of data analysis. *Annals of Mathematical Statistics*, *33* (1), 1–67. doi:10.1214/aoms/1177704711

Ullsperger, J. M., & Nikolas, M. A. (2017). A meta-analytic review of the association between pubertal timing and psychopathology in adolescence: Are there sex differences in risk? *Psychological Bulletin*, *143*(9), 903 – 938. doi:http://dx.doi.org/10.1037/bul0000106

van Lier, P. A., & Koot, H. M. (2010). Developmental cascades of peer relations and symptoms of externalizing and internalizing problems from kindergarten to fourth-grade elementary school. *Development and psychopathology*, *22*(3), 569–582. https://doi.org/10.1017/S0954579410000283

van West, D., Claes, S., & Deboutte, D. (2009). Differences in hypothalamic-pituitary-adrenal axis functioning among children with ADHD predominantly inattentive and combined types. *European child & adolescent psychiatry*, *18*(9), 543–553. https://doi.org/10.1007/s00787-009-0011-1

Vogel, A. C., Jackson, J. J., Barch, D. M., Tillman, R., & Luby, J. L. (2019). Excitability and irritability in preschoolers predicts later psychopathology: The importance of positive and negative emotion dysregulation. *Development and Psychopathology*, *31*(3), 1067–1083. doi:10.1017/S0954579419000609

Wiggins, J. L., Briggs-Gowan, M. J., Estabrook, R., Brotman, M. A., Pine, D. S., Leibenluft, E., & Wakschlag, L. S. (2018). Identifying Clinically Significant Irritability in Early

Childhood. *Journal of the American Academy of Child and Adolescent Psychiatry*, *57*(3), 191–199.e2. https://doi.org/10.1016/j.jaac.2017.12.008

Yehuda, R., & Seckl, J. (2011). Minireview: Stress-related psychiatric disorders with low cortisol levels: a metabolic hypothesis. *Endocrinology*, *152*(12), 4496–4503. https://doi.org/10.1210/en.2011-1218

Yoon, K. L., & Joormann, J. (2012). Stress reactivity in social anxiety disorder with and without comorbid depression. *Journal of abnormal psychology*, *121*(1), 250–255. https://doi.org/10.1037/a0025079

Young, E. A., Abelson, J. L., & Cameron, O. G. (2004). Effect of comorbid anxiety disorders on the hypothalamic-pituitary-adrenal axis response to a social stressor in major depression. *Biological psychiatry*, *56*(2), 113–120. https://doi.org/10.1016/j.biopsych.2004.03.017

Zakreski, E., Dismukes, A. R., Tountas, A., Phan, J. M., Moody, S. N., & Shirtcliff, E. A. (2018). Developmental trajectories of HPA-HPG dual axes coupling: Implications for social neuroendocrinology. In Schultheiss, O. C., & Mehta, P. H. (Eds.). *Routledge International Handbook of Social Neuroendocrinology*. Abingdon, UK: Routledge.

Zorn, J. V., Schür, R. R., Boks, M. P., Kahn, R. S., Joëls, M., & Vinkers, C. H. (2017). Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *77*, 25–36. doi: 10.1016/j.psyneuen.2016.11.036

Vita

The author was born in Boston, Massachusetts. She obtained dual bachelor's degrees in psychology (B.S.) and philosophy (B.A.) from Salem State University in 2016. In 2018, she joined the University of New Orleans psychology graduate program to pursue a Ph.D. in applied developmental psychology. She joined Dr. Sarah R. Black's Biological and Environmental Risk for Affective Disorders (BERAD) lab in 2019.