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Biological Correlates of Early Life Stress and
Aggression in School Aged Children

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Biological Correlates of Early Life Stress and Aggression in School Aged Children

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An abstract of
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Abstract

Biological Correlates of Early Life Stress and Aggression in School Aged Children
By D. Anne Winiarski

Although a plethora of research exists on the trajectories of aggression and deviancy from school age into adolescence, much less research exists on aggression earlier in childhood, and in particular, on the biological correlates of these behaviors. Even less is known about the role that callous-unemotional traits (characterized by low levels of empathy) and exposure to early life stress play in the emergence and persistence of aggression across childhood. In Study 1 of this dissertation, biosocial risk factors for aggression were examined in a sample of 119 mother-child dyads assessed during both child preschool age (ages 2.5 to 5) and school age (ages 5 to 10)—periods which are considered critical in a child's emotional development. Overall, the results of Study 1 suggested that ELS was related to measures of school age and early childhood persistent aggression, but that stress reactivity did not mediate these relationships. Associations between ELS and stress reactivity were stronger for females, whereas associations between stress reactivity and aggression were generally stronger for males. In addition, the ELS-aggression association was stronger for children with higher levels of callousness. Only physiological stress reactivity for males predicted to school-age aggression above and beyond caregiver reports of preschool aggression. Study 2 focused on a separate community sample of 20 children, ages 7-10 years of age, in which we examined neural (fMRI) correlates of early life stress and aggression. The results of Study 2 were similar to those of Study 1 in that, although ELS was not related to amygdala activity in the sample as a whole, there was a trend for ELS to relate to higher amygdala activity among females. There was also a statistical trend noted for an association between proactive aggression and reduced right amygdala activity in response to emotional stimuli for both males and females in the sample. General themes that emerge across Study 1 and Study 2, as well as clinical and future research implications are discussed.

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General Introduction

Aggressive behavior is viewed as developmentally appropriate and normative in toddlers and preschoolers (ages 2-5 years; Campbell, Shaw, & Gilliom, 2000; Côté, Vaillancourt, LeBlanc, Nagin, & Tremblay, 2006; Tremblay, 2000). By elementary school age (age 6 years and older), rates of aggressive behaviors decline as children learn to more effectively regulate their emotions and develop more appropriate coping strategies for dealing with frustration (Cole et al., 1996). In some cases, however, aggression persists from preschool age to school entry (Tremblay et al., 2005). Persistent childhood aggression is a precursor of educational under-attainment, unemployment, increased mental health problems, and higher rates of physical violence later in life (Fergusson & Horwood, 1998; Nagin & Tremblay, 1999; Stattin & Magnusson, 1989; Tremblay, 2004), and its predictors are therefore worthy of empirical study. Epidemiological data suggest that identification of children at risk for aggression earlier in development could have far-reaching clinical and policy implications, including enhanced early treatment options and reduced financial burden (Kazdin, 1995; Kazdin, 2011).

Early Life Stress and Aggression

There is strong research support for the association between early life stress (ELS) and adolescent behavioral problems, including aggression (Grant et al., 2006). Both diathesis stress and differential susceptibility models have been suggested as explanations for the stronger correlation between stress and psychopathology in high-risk adolescents (Belsky & Pluess, 2009). In addition, vulnerability to stress appears to be moderated by the child's sex, with adolescent females being more likely than males to evidence depression in response to stressful life events (Rudolph & Hammen, 1999). While the stress and psychopathology association has been well researched among adolescent samples, it is less clear how stressful life events might be

associated with emotional functioning in young children at risk for psychopathology, and whether sex might moderate these associations.

Accumulating data from animal and human literatures suggest that exposure to early life stress greatly increases the likelihood of aggression and violence later in life (Dodge, Bates, & Peitit, 1990; Ethier, Lemelin, & Lacharité, 2004; Hildyard & Wolfe, 2002; Loeber & Stouthamer-Loeber, 1998), and early childhood trauma is generally viewed as a "universal" risk factor for aggression problems in adulthood (Veenema, Blume, Niederle, Buwalda, & Neumann, 2006).

Animal studies have examined genetic, hormonal, and neural pathways through which these effects occur, and have highlighted the important role of the hypothalamic pituitary adrenal (HPA) axis, along with limbic and cortical structures, as biological mechanisms through which stress exposure may affect aggressive behavior (Haller, Harold, Sandi, & Neumann, 2014; Veenema, 2009). In animal studies, separation from the mother shortly after birth is often viewed as a proxy for early life stress. In these separation studies, rat pups are found to have an increased incidence of aggression later in life, and the stress-aggression relationship is mediated by hypothalamic arginine vasopressin (AVP) mRNA expression and serotonin (5-HT) immunoreactivity (Veenema et al., 2006). Despite these experimental advances, evidence about the mechanisms by which early life stress shapes the emergence of aggressive behavior problems in human samples remains sparse.

One of the major gaps in the literature is the lack of studies that prospectively assess stress in early childhood, examine the associations among stress exposure and child hormonal and neural processes, and link these potential biological mechanisms to later measures of aggressive behavior. Another neglected area of research is the study of the potential role of personality

factors, particularly callous-unemotional traits, in predicting which children may be more vulnerable to the impacts of early life stress and the development and persistence of aggression. The present study will address both of these limitations by examining early childhood aggression through a longitudinal design that not only includes observational, maternal report, physiological, and neural data, but also explores how callous-unemotional traits influence the prediction of aggressive outcomes.

Early Life Stress and the HPA Axis

Early life stress, particularly when chronic, is often linked to changes in the body's hypothalamic-pituitary adrenal (HPA) axis, a system involved in the mammalian stress response. Exposure to a stressful situation results in a cascade of events, in which the hypothalamus increases the amount of corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) that is released into the anterior pituitary gland, which subsequently releases adrenocorticotropic hormone (ACTH). The end-product of this cascade is cortisol, a glucocorticoid synthesized by the adrenal gland (Watamura, Donzella, Kertes, & Gunnar, 2004) that is capable of crossing the blood-brain barrier (Gunnar & Quevedo, 2007). In the short-term, elevations in cortisol can be adaptive in facilitating the body's "fight-or-flight" response, but chronic elevations (for example, in response to ELS) can be detrimental to physical, immune, and cognitive functioning (Sapolsky, Romero, & Munck, 2000). Previous research has also established a link between HPA axis dysregulation and aggressive and delinquent behaviors (Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011; Popma et al., 2007; Sondeijker et al., 2007), so it is reasonable to postulate that HPA axis dysregulation in early childhood may be a mechanism by which ELS influences later childhood aggression.

Exposure to stress early in life may be especially detrimental to the developing HPA axis because the HPA system is not fully mature at birth. A review of the literature on the effects of social stress (e.g., poor care, child abuse) illustrates the developmental vulnerability of the HPA axis (Gunnar & Donzella, 2002). A normal diurnal cortisol response peaks in the morning and then decreases over the course of the day. In contrast, children reared in adverse environments, such as foster care children exposed to maltreatment, show dysregulated basal cortisol responses (Bruce, Fisher, Pears, & Levine, 2009). The specific kind of dysregulation differs as a function of the type of ELS, such that children with histories of physical neglect show decreased levels of morning cortisol, whereas those with histories of emotional maltreatment show increased levels of morning cortisol.

Much of the research on the effects of ELS on the HPA axis has focused on stress reactivity among adults who were maltreated as children (Tarullo & Gunnar, 2006), and on basal cortisol rather than cortisol reactivity. When combined, these limitations preclude the extrapolation of the adult literature to our current conceptualization of the effects of ELS on physiological stress reactivity in children. The proposed study overcomes these limitations by examining cortisol reactivity in children exposed to early life stress in the context of a prospective longitudinal design.

Early Life Stress and Brain Function

The neurobiological mechanisms by which early life stress contributes to aggression have not been clearly elucidated. As mentioned above, early life stress has been linked to disruptions in the secretion of cortisol. Because there is evidence to suggest that cortisol is able to cross the blood-brain barrier, it seems reasonable to explore brain regions that are especially sensitive to the effects of glucocorticoids. The amygdala, a region of the brain often implicated in fear

conditioning and in the control of fear behaviors, is a limbic brain region that is especially rich in glucocorticoid receptors (Teicher et al., 2003).

Results from a study utilizing animal models shows that exposure to ELS has long-lasting effects on amygdala circuitry and function, and that these functional changes persist even after removal of the stressor (Malter-Cohen et al., 2013). Researchers have also postulated that the impact of ELS on brain development depends on a variety of factors including severity, type of stressors, and age at the time of occurrence. Therefore, brain regions with extended postnatal development, like the amygdala, are particularly vulnerable to the effects of chronic ELS. Moreover, unlike other regions of the brain that show relative plasticity following removal from an adverse environment, impacts of ELS on the amygdala appear to be longstanding. For example, results from a recent study suggest that adults with histories of childhood abuse display more amygdala reactivity to emotional faces than control participants (van Harmelen et al., 2013). Greater amygdala activation when processing emotional faces was also found among youth with histories of child abuse (Maheu et al., 2010), and among adults who retrospectively reported high levels of childhood family stress (Taylor, Eisenberger, Saxbe, Lehman, & Lieberman, 2006).

Many of the studies linking early life stress to amygdala activity examine children and adults who have diagnoses of post-traumatic stress disorder or anxiety (De Bellis et al., 2000), thereby making generalizability of these findings to other populations (e.g., aggressive children) difficult. Moreover, several of these studies focus solely on child abuse and maltreatment, therefore excluding other forms of early life stress (e.g., frequent moving, death of a close family member, etc.). Overcoming these limitations, the present study assesses a variety of exposures to stressful life events in a non-clinical sample of children to examine how processing of emotional stimuli

at the neural level relates to both early life stress and aggressive behavior outcomes in the school age period of development.

Physiological Reactivity and Aggression

There is a well-established literature exploring the links between various measures of physiological reactivity (e.g., cortisol reactivity, respiratory sinus arrhythmia, and heart rate) and aggression in children, adolescents, and adults (e.g., Kalvin, Bierman, & Gatze-Kopp, 2016; Murray-Close et al., 2014; Stadler et al., 2011; Steeger, Cook, & Connell, 2016). In addition, rodent models provide additional support for this HPA axis-aggression relationship. Cortisol reactivity and basal cortisol levels have been shown to influence aggressive behavior in rats (Kruk, Halász, Meelis, & Haller, 2004), such that activation of the HPA axis resulted in noticeable increases in aggressive behavior, which then further activated the HPA axis. Rats exposed to early life stress have higher corticosterone levels (the rodent equivalent of cortisol), and show concurrent increases in aggression toward other rats (Márquez et al., 2013). In humans, the HPA axis has been implicated in aggression among clinical (Marsman et al., 2008; Stadler et al., 2011), as well as nonclinical samples (Böhnke, Bertsch, Kruk, & Naumann, 2010).

A recent meta-analysis on the cortisol-aggression relationship concluded that age moderates the relationship between basal cortisol and aggression, such that aggressive preschool-aged children demonstrate elevated basal cortisol, whereas school-aged children's basal profiles reflect the blunted response typically characteristic of adolescent delinquency (Alink et al., 2008). Furthermore, this meta-analysis determined that many of the research findings relating disruptive behavior to low cortisol are correlational, and are conducted primarily among samples of children who have already begun demonstrating problematic behavior. The present study

explores predictors of aggression in school-aged children based on cortisol data gathered at preschool age, thereby proving a stronger methodology than a correlational design.

Brain Function and Aggression

The brain structures that are presumed to be the most sensitive to the effects of ELS are also often implicated in the development of aggression across the lifespan. Previous studies have linked structural and functional changes within the amygdala to aggression in both children and adults. For example, in a recent longitudinal study of adult males (age 26), those with lower amygdala volume in adulthood had histories of aggression and violence, as well as a higher likelihood of manifesting callous-unemotional traits dating back to first grade (Pardini, Raine, Erickson, & Loeber, 2014). Though retrospective, this was among the first studies to demonstrate that men with reduced amygdala volume have histories of aggression dating back to childhood. Furthermore, the authors suggested that future studies should examine whether amygdala abnormalities may be a useful biomarker for severe and persistent aggression.

The amygdala also plays an important role in several aspects of emotion processing that have important implications for understanding the development of severe aggression. Relatedly, it is important to examine how different types of aggression relate to neural processing. Whereas reactive aggression is characterized by explosive outbursts in response to perceived threat, proactive aggression is goal-oriented and premeditated (Raine et al., 2006). Although the amygdala is implicated in both, it is thought to relate in different ways. Individuals with reactive aggression show exaggerated amygdala activity when viewing emotionally ambiguous and angry facial expressions (Pardini et al., 2014). Conversely, individuals with proactive aggression show blunted amygdala reactivity, and some studies associate proactive aggression with lower gray matter concentrations (Pardini et al., 2014). It is possible that some of the inconsistencies in the

literature (hypo- versus hyper-reactivity of the amygdala) can be at least partially explained by the failure to distinguish between these subtypes of aggression in analyses.

Given that advances in brain imaging technology are fairly recent, fMRI studies examining the role of the amygdala in childhood aggression are lacking. One of the goals of the proposed study is to provide preliminary data on amygdala function and reactive and proactive aggression in a community sample of children who have not been diagnosed with major mental disorders.

Callous-Unemotional Traits and Risk Modification

Callous-unemotional (CU) traits can be closely linked to aggression. In general, CU traits characterize children with blunted affect and who display a general disregard for the affective experiences of others (Chabrol, Goutaudier, Melioli, van Leeuwen, & Gibbs, 2014; Essau, Sasagawa, & Frick, 2006). These traits have shown utility in identifying aggressive adults at-risk for criminal recidivism (Marsee, Silverthorn, & Frick, 2005), thereby suggesting that CU traits may help identify a unique profile of individuals who are especially prone to persistent aggression across the lifespan. Similarly, children who display conduct problems and are also high on CU traits tend to be more thrill-seeking, less sensitive to punishment, and less emotionally and physiologically sensitive to threatening stimuli (Frick & Ellis, 1999). Compared to children without CU traits, those with CU traits generally exhibit a form of severe and chronic antisocial behavior (Frick, Cornell, Barry, Bodin, & Dane, 2000; Frick, Ray, Thornton, & Kahn, 2013; Frick & White, 2008). In a longitudinal study of school-age children, Frick and colleagues (2005) found that youth with comorbid conduct problems and elevated CU traits showed the highest rates of persistent conduct problems and self-reported delinquency, and accounted for a disproportionately higher amount of police contacts in their sample.

Callous-unemotional personality traits appear to be an important and identifiable risk factor for later aggressive outcomes. Their predictive ability is strengthened when combined with other physiological or behavioral assessments (Stadler et al., 2011; von Polier et al., 2013). For example, the data suggest that the worst antisocial outcomes occur in youth with both blunted HPA activity and high levels of CU traits (Hawes, Brennan, & Dadds, 2009). A recent study of youth with comorbid attention and behavior problems also found that CU traits helped explain differences in physiological responding to a laboratory-based stressor task; specifically, youth with ADHD and behavior problems who also had high CU traits demonstrated a blunted cortisol reactivity response in the stressor task. This same effect was not observed for the youth who scored low on CU traits (Stadler et al., 2011). A similar trend was found among males with early-onset conduct disorder. Specifically, boys with early-onset conduct disorder, high CU traits, and impulsivity had decreased diurnal cortisol levels, an effect not observed in healthy controls and also not explained by antisocial behavior in general (von Polier et al., 2013). Collectively, these studies demonstrate that CU traits may be an important moderator in studies of ELS, stress reactivity, and aggressive outcomes.

A noted limitation of the extant literature examining the moderating role of CU traits on aggression is the restrictive developmental time period in which most data are collected. Many studies, including the ones described above, limit their samples to pre-adolescent and adolescent children (Willoughby, Waschbusch, Moore, & Propper, 2011). In the present study, CU traits are explored as a moderator of the relationship between early life stress, stress reactivity, and aggression in *early childhood*. Given that there is little research about how CU traits impact aggression in the transition period from preschool age to school age, the moderating effects of CU traits are examined in an exploratory fashion.

Overlooked Variables: Child Sex and Age

This project proposes to link ELS to stress reactivity in preschool age, as well as to continued aggression problems in school age, thereby including the often-overlooked developmental period of early childhood. One of the simplest explanations for why this time period is overlooked is that many young children show developmentally normative "acting out" or aggressive behaviors, sometimes making it difficult for caregivers to distinguish between normal and atypical levels of aggression. The first study addresses this limitation in the literature by evaluating whether physiological and behavioral proxies of stress reactivity during the preschool period can reliably predict continued aggression in elementary school age above and beyond observable aggressive behaviors noted by caregivers.

On the whole, males tend to demonstrate higher rates of clinically significant physical aggression (Crick, 1997; Krakowski & Czobor, 2004) than females. Since most studies on aggression in children, adolescents, and adults tend to explore biosocial markers among clinical populations, it is no surprise that females are often excluded from analyses, as these studies would be underpowered to assess sex as a moderator. Some scholars have suggested that females may be especially vulnerable to the effects of ELS (Rudolph & Hammen, 1999). In contrast, Dodge & Pettit (2003) theorized that boys tend to be more adversely affected by various forms of ELS, and postulated that male sex may itself be a diathesis that, in conjunction with environmental strains, puts individuals at risk for future conduct problems.

Though rates of conduct disorder diagnoses might differ between the sexes, recent findings suggest that, similarly to males, females exhibit three trajectories of behavior problems in childhood, with the highest group demonstrating increased risk for several maladaptive outcomes in adolescence, including depression and risky sexual behavior (van der Molen et al., 2015).

Although the authors highlight the importance of early intervention efforts, particularly for females on the "high risk" trajectory, more studies are needed to evaluate sex as a potential moderator of relationships between early childhood risk factors and aggressive behavior outcomes. Only when these differences are disentangled can interventions be tailored to address the potentially unique constellation of factors that predispose male and female children to persistent behavioral problems. To address this gap in the literature, and given the relatively even split of males and females across both samples, child sex was explored as a moderator in both of the studies in this dissertation project.

Informing Existing Criminological Theories

As discussed above, one of the principal reasons why it is important to understand the developmental antecedents of aggressive behavior is to intervene at earlier stages so that child behavior problems do not manifest as adolescent delinquency and future criminality. In particular, this research may inform well-established theories of criminal offending, such as Agnew's General Strain Theory of Crime (Agnew, 2005). Briefly, Agnew postulates that criminal behavior is modulated by motivations and constraints, which can be either internal or external. Criminal behavior is more likely to occur when an individual feels "pressured" by negative affective states (Agnew & White, 1992) that result from poor relationships within the five life domains (the self, family, school, peer group, and workplace).

Agnew acknowledges that delinquency is only one possible consequence of exposure to strain, and has suggested that biological factors and individual differences, such as inherited temperamental factors, poor problem-solving, and low self-efficacy, may contribute to interpersonal variations in crime (Agnew, 2005; Rebellon, Barnes, & Agnew, 2015).

Additionally, other authors have made similar recommendations about utilizing psychological and biological variables to bolster existing criminological theories (Wright & Boisvert, 2009).

The current studies focus on physiological and behavioral correlates of stress reactivity and the neural correlates of response to emotional stimuli, respectively. Though these studies do not directly evaluate Agnew's General Strain Theory, they can help inform future iterations of the theory by exploring ways in which biological variables influence motivating and constraining variables, thereby making an individual more susceptible to the "pressures" of strain. Within the context of General Strain Theory, an individual who develops a heightened sense of emotional reactivity in response to social provocation may be more likely to act out against those perceived threats in socially inappropriate, or aggressive, ways. Earlier identification of youth who are more likely to engage in delinquent behaviors as adolescents is critical as this could reduce the societal and psychological burdens of persistent criminal trajectories.

The Current Studies

Because individual levels of reactivity and regulation are moderately stable across childhood and later development (Raffaelli, Crockett, & Shen, 2005), deficits in these areas of functioning may serve as early indicators of persistent behavioral problems across the lifespan. Measuring indicators of reactivity in younger children may therefore allow for an examination of potential mechanisms linking stress and behavior problems before the onset or detection of prolonged aggression, and may provide a powerful platform for preventative measures. As was summarized in the literature review above, many studies linking physiological, behavioral, and neural mechanisms to the development of aggression tend to focus on pre-adolescence, adolescence, and adulthood. Even though so many important developmental changes are occurring in early childhood, it surprisingly remains an understudied period in the aggression literature.

The two studies that form the basis of this dissertation aim to address the gaps in the literature by examining aggression across childhood, beginning with the preschool years. The first of these studies examines distinct behavioral and physiological mechanisms linking early life stress and childhood aggression. The second study seeks to link early life stress to neural responses to emotional stimuli and to subsequent childhood measures of aggression during the school age period of development.

Examining the Mediating Role of Stress Reactivity in the Relationship Between Early Life Stress and Aggression Among Children at High Risk for Psychopathology

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Abstract

Early life stress (ELS) has been implicated in the development of aggression, though the exact mechanisms linking ELS and aggression have not been clearly elucidated. The current study tested associations between early life stress, stress reactivity during the preschool years, and ratings of school-age and persistent early childhood aggression. Child sex and measures of callousness were evaluated as moderators of the aforementioned associations. A longitudinal sample of 119 mother-child dyads completed a lab visit and mothers completed an online follow-up when children were preschool-aged and school-aged, respectively. Children participated in a frustration task during the preschool-age lab visit, at which time both physiological and behavioral measures of stress reactivity were collected. Mothers completed ratings of child behavior, callousness, and ELS. Overall, the results of this study suggested that ELS was related to measures of school-age and persistent early childhood aggression, but that stress reactivity did not mediate these relationships. Child sex significantly moderated the association between ELS and stress reactivity, as well as stress reactivity and aggression. Moreover, measures of callousness significantly moderated the relationship between ELS and aggression. Clinical implications are discussed.

Examining the mediating role of stress reactivity in the relationship between early life stress and aggression among children at high risk for psychopathology

Early life stress (ELS) has been implicated in a variety of emotional and behavioral problems, one of which is aggression. Although there is considerable evidence linking early life stress to adverse behavioral outcomes in adolescence and later adulthood, much less is known about how ELS relates to the development of high stress reactivity in childhood, and how that in turn relates to higher levels of aggression in school age. The present study aims to address this developmental gap in the literature.

Early Life Stress and Aggression

Individuals with histories of ELS (e.g., physical and emotional neglect, abuse, loss of a parent) often show higher levels of aggression later in development (Dodge et al., 1990; Jonson-Reid et al., 2010; Loeber & Stouthamer-Loeber, 1998). Evidence in support of these associations exists in both the human and animal literatures. For example, rat pups separated from their mothers were shown to display greater intermale aggression compared to rats who were not separated (Veenema et al., 2006). Similarly, rats weaned in isolation from their mothers showed higher levels of behavioral agitation and reactive aggression (i.e., the victim had no warning of the attack and was more likely to be attacked in a vulnerable region; Tóth, Halász, Mikics, Barsy, & Haller, 2008) than those weaned by their mothers.

In a recent study of children exposed to maltreatment, Jonson-Reid and colleagues (2010) found that childhood adversity was linked to antisocial outcomes in school age. Specifically, children identified as genetically at-risk (i.e., they had a twin with externalizing behavior problems) who also experienced ELS (in this study, defined as exposure to maltreatment in childhood) showed a 10-25 percent increase in externalizing outcomes as measured by the Child

Behavior Checklist. The authors concluded that early child adversity exhibited causal influences on antisocial outcome, even when controlling for inherited liability. It is also important to note that contextual factors and type of ELS differentially contribute to aggression. For example, it has been hypothesized that physical abuse leads to hypervigilance, which then increases the likelihood of aggression. Neglect, on the other hand, leads to aggression through the development of poor emotion regulatory abilities (Lee & Hoaken, 2007). Although previous research has established a link between ELS and aggression, the mechanisms by which ELS contributes to higher rates of aggression remain less clear.

Linking the HPA Axis with Early Life Stress and Aggression

One of the primary pathways for the stress response is the hypothalamic-pituitary-adrenal (HPA) axis (McEwen & Wingfield, 2003), which has been implicated in the emergence of externalizing behaviors, most notably aggression (Raine, 2013). The HPA axis directs a cascade of events resulting in the release of glucocorticoids, one of which is cortisol (Tarullo & Gunnar, 2006). Previous studies have suggested that dysregulation of the HPA axis is involved in the pathogenesis of child behavior disorders and mood disorders, as well as later adolescent delinquency (Allwood et al., 2011; Popma et al., 2006; Sondeijker et al., 2007; Tyrka et al., 2012). The timing of stress exposure can be especially important for the developing HPA axis, and can set the stage for future outcomes (Gunnar, 2016; Tarullo & Gunnar, 2006).

The detrimental effects of ELS on the developing HPA axis (and subsequent dysregulation of emotions and behavior) have been well elucidated in both the human and animal literature (Lupien, McEwen, Gunnar, & Heim, 2009; Ouellet-Morin et al., 2011; Turner-Cobb, 2005). Furthermore, previous studies have documented the detrimental physical and psychological effects of prolonged cortisol elevations in the body (Hellhammer & Wade, 1993; Sapolsky et al.,

2000). Preschoolers growing up in caregiver-rated high-stress environments exhibit higher basal cortisol levels than their peers from less stressful environments (Essex, Klein, Cho, & Kalin, 2002). Moreover, ELS predicts more emotion regulation problems only if children also demonstrate disrupted HPA axis functioning (von Klitzing et al., 2012), suggesting that the HPA axis plays a critical role in shaping maladaptive behaviors stemming from ELS. Given the relationship between ELS and the developing stress reactivity system, it makes sense to postulate that disruptions in the normal development of the HPA axis caused by early life stress may lead to future emotional and behavioral disturbance across the lifespan.

Contrary to previously-held expectations, stress does not always correlate with hyperactivation of the body's stress-mediating systems, and can sometimes instead manifest as hyporeactivity (Gunnar, 2016). In addition, research findings linking HPA axis dysregulation to aggression among older children and adolescents are conflicting in terms of direction of effect. Some studies of adolescents and school-age children have found that aggression negatively correlates with cortisol levels (Poustka et al., 2010), whereas others have found that this relationship can be better explained by high levels of callous-unemotional (CU) traits rather than antisocial behavior in general (Stadler et al., 2011; von Polier et al., 2013).

Even less is known about the potential of HPA axis reactivity in preschool-age children to predict later aggressive behavior. A meta-analysis on the cortisol-aggression relationship concluded that age moderates the relationship between basal cortisol and aggression, such that aggressive preschool-aged children demonstrate elevated basal cortisol, whereas aggressive school-aged children evidenced the lower basal cortisol levels typically characteristic of adolescent delinquency (Alink et al., 2008). Furthermore, this meta-analysis determined that many of the research findings relating disruptive behavior to low cortisol are correlational, and

are conducted primarily among samples of children who have already begun demonstrating problematic behavior, precluding clear evidence for predictive risk. Alink and colleagues also found that children exposed to high levels of stress early in life are not only more likely to develop externalizing problems earlier, but are also more likely to have elevated basal cortisol levels early in life (Alink et al., 2008). Similar findings have been observed among preschoolers exposed to concurrent maternal stress (Essex et al., 2002).

Extant HPA axis-aggression studies typically focus on basal cortisol levels rather than cortisol reactivity. Disruptions to the HPA axis that result in increased stress reactivity responses are hypothesized to result in increased aggressive behavior problems, and are therefore the focus of the current study. It is also unclear from the existing literature whether HPA axis dysregulation may be a potential mechanism by which early adversity shapes the development of aggression. In a study examining cortisol as a mediator of the relationship between early childhood trauma and aggression among incarcerated males and healthy controls, Cima, Smeets, and Jelicic (2008) found that cortisol did not act as a mediator between trauma and aggression for participants rated high on psychopathy. However, a more recent study found that, among low warmth families, an early intervention program's effects on child aggression were largely mediated by the intervention's effects on changes in cortisol (specifically, increases in cortisol; O'Neal et al., 2010). These discrepant findings highlight the need to more carefully evaluate the mediating effects of cortisol on the relationship between ELS and aggression, which is one of the primary goals of the current study.

Although there is disagreement in the field about the best way to define "physiological dysregulation," there is consistent evidence that cortisol does relate to both ELS and aggression. However, very few studies of stress and aggression have focused on evaluating cortisol as a

mediator. A further limitation is that much of the previously-published research in this area relies on retrospective reporting of adults concerning their early trauma histories, thereby failing to prospectively link physiological dysregulation in early childhood to continued problems at later time points. The longitudinal design of the present study allows for the analysis of stressful life event data that were prospectively collected at preschool age, thereby avoiding biases inherent in retrospective recall of ELS.

Behavioral Indicators of Stress Reactivity and Aggression

Behavioral indicators of stress reactivity have also been linked to later aggression. For example, results from previous studies suggest that children with high negative reactivity and poor behavioral inhibition tend to be at higher risk of continuing along a trajectory that results in behavioral problems (Blair, Denham, Kochanoff, & Whipple, 2004; Cole, Martin, & Dennis, 2004). Several prospective studies link aggressive behavior and early childhood temperament, especially negative emotionality and intense reactive responding, with continued aggression across the lifespan (Burke, Loeber, & Birmaher, 1997; Coie, Lochman, Terry, & Hyman, 1992; Crick, 1996; Kellam, Ling, Merisca, Brown, & Ialongo, 1998). Similarly, Gartstein and colleagues (2012) found that children with high levels of negative emotionality (i.e., sadness and frustration) measured throughout infancy and toddlerhood exhibited higher levels of externalizing behavior problems at age five.

In adults, stress reactivity has been associated with negative emotional reactivity in response to provocation and uncertainty (Venables, Patrick, Hall, & Bernat, 2011). Since emotion regulation is a key component of the development of appropriate regulatory social behaviors (e.g., expressing one's frustration calmly as opposed to acting out physically), one can see how an inability to regulate emotions in response to stressors (i.e., poor stress reactivity) in the early

years of childhood may be linked to the development of aggression (Crick, 1995; Helmsen, Koglin, & Petermann, 2012; Röll, Koglin, & Petermann, 2012; Rubin, Burgess, Dwyer, & Hastings, 2003). Individuals with no regulatory skills in place are much more sensitive to the effects of negative affect brought on by interactions with a stressful environmental context, and may be more likely to act out aggressively (Lee & Hoaken, 2007). Because of the link between poor stress reactivity and deficits in emotion regulatory abilities, this study conceptualizes preschool behavioral stress reactivity as a measure of a child's emerging abilities to effectively regulate emotion. Moreover, given the previously established notion that ELS can influence stress reactivity at both a physiological and behavioral level, the present study will evaluate multiple proxies of stress reactivity as mediators of the ELS-aggression relationship.

Gender and Callous-Unemotional Traits as Moderators

Callous-unemotional traits are seen as a precursor to future psychopathy, and are typically characterized by a lack of guilt and remorse, superficial expression of emotions, and a lack of concern for the feelings of others (Frick, Ray, Thornton, & Kahn, 2013, 2014; Frick & White, 2008). Previous studies demonstrate links between many constructs of early childhood CU traits, such as poor fear conditioning and fearlessness (Gao, Raine, Dawson, & Mednick, 2010; Gao, Raine, Venables, Dawson, & Mednick, 2010; Raine, Reynolds, Venables, Mednick, & Farrington, 1998), and later psychopathology. Considerable evidence also suggests that, among children at risk for clinically significant aggressive behavior, there may be a particularly treatment-resistant and at-risk group of youth who show high levels of aggression, high levels of CU traits, and low levels of physiological arousal (Hawes et al., 2009). Similarly, Frick and White (2008) argue that CU traits are particularly important for distinguishing subgroups of antisocial youth, and that these traits predict a more stable form of aggression that begins earlier

in life. In line with the evidence supporting this subgroup, we generally expect ELS to predict higher stress reactivity and aggression, but we also anticipate that among a subgroup of children with high levels of CU traits in childhood, this relationship will be reversed (i.e., low stress reactivity will relate to higher aggression).

Finally, it is important to explore the effects of sex as a moderator of the relationships between ELS, physiological and behavioral markers of stress reactivity, and aggression. Studies linking the impacts of early life stress on development generally find stronger associations between stress and depression for females (Hankin & Abramson, 2001), but at the same time, the literature tends to show a stronger correlation between stress and aggression for males (Vaillancourt, Miller, Fagbemi, Co, & Tremblay, 2007). The longitudinal cohort in the present study allows for a systematic analysis of how ELS differentially affects male and female stress reactivity, and how stress reactivity then influences the development of aggression into the school-age years.

Current Study

Although previous research has established a link between aggression in school-aged children and later adolescent delinquency (e.g., Frick, Cornell, Barry, Bodin, & Dane, 2000; Moffitt, 1993), as well as future adult criminality (e.g., Olweus, 1979), there is less research identifying the various early childhood physiological and behavioral risk factors that predict both school-age and early childhood persistent aggressive behavior. The proposed study will add to what is known about the development of aggression by utilizing a longitudinal sample of children studied during preschool age and elementary school age to examine physiological and behavioral predictors of aggression across childhood. Since previous research has demonstrated a link between HPA axis dysregulation and both aggression and delinquency, it is reasonable to

explore early-life HPA axis dysregulation as a potential predictor of continued aggression across childhood. A better understanding of how early physiological and behavioral markers of stress reactivity can put children at risk of developing later disruptive behaviors can inform early intervention efforts aimed at reducing persistent aggression. Furthermore, predicting future aggression from preschool measures of aggression has not proven to be very informative given the developmental normalcy of aggression among preschoolers. Therefore, a clearer understanding of specific mechanisms through which ELS impacts the development of aggression might help to better identify children at risk.

We hypothesized that high levels of early life stress would predict to higher behavioral and physiological stress reactivity in preschool age, which would in turn relate to higher levels of caregiver-reported aggression in school age as well as higher levels of early childhood persistent aggression. We also predicted that child sex and callous-unemotional traits would moderate the aforementioned relationships. Specifically, we predicted that the relationship between stress exposure and stress reactivity would be stronger for females than males, whereas the relationship between stress reactivity and aggression would be stronger for males. The relationships between ELS and stress reactivity were predicted to be weaker for children with higher levels of callousness, relative to children with lower levels of callousness. And low stress reactivity was predicted to relate to higher levels of aggression in children who were rated high, but not children who were rated low, on measures of callousness. We also explored sex and callousness as moderators of the relationship between ELS and school age and persistent early childhood aggression. Finally, we predicted that physiological and behavioral stress reactivity measures would incrementally predict children's school-age aggression, above and beyond caregivers' reports of aggression during preschool age.

Method

Participants were drawn from an existing sample of 219 mother-child dyads, 178 of whom were recruited from the Emory Women's Mental Health Program (WMHP) within the Department of Psychiatry at the Emory University School of Medicine. The WMHP serves as a referral program that provides services and care for women suffering from mental illness. The women recruited from WMHP were first evaluated during pregnancy and then seen during multiple visits across pregnancy and postpartum. The remaining 41 women in the sample were recruited from the community at the time of the preschool study visit. These 41 women did not take psychotropic medications during pregnancy, as verified by obstetrical records.

Participants recruited from the WMHP and controls did not differ on any demographics relevant to the current study (mother age, child age, mother/child ethnicity, mother's marital status, education level, number of hours worked per week, number of adults in the household) with the exception of number of children in the home ($p = 0.046$) with controls having more children in the household than participants recruited from WMHP.

This study was approved by the Institutional Review Board of Emory University. Mothers and alternate caregivers provided consent for their participation in the study. Children's mothers also provided consent for their participation in the preschool study. In the preschool study, mothers were financially compensated for their involvement in the study, and children received a toy for participation. In the school-age follow-up, mothers and alternate caregivers were both financially compensated for their involvement in the study.

The proposed study examined associations between early life stress, preschool markers of stress reactivity, and childhood aggression. Prospectively collected preschool data on child aggression, child behavioral responses to a lab-based frustration task, and child cortisol measures

were supplemented by follow-up school-age measures of parent and alternate caregiver ratings of child aggression and callous-unemotional traits.

Demographics of the Sample

During the preschool phase of the study, children's ages ranged from 2.5 to 5.5 years (*M*=3.7, *SD*=0.89) and mother's ages ranged from 21 to 49 years (*M*=36.9, *SD*=5.0). Child sex was evenly split (*N*=110 females). The women in the sample were predominantly Caucasian (82.6%), although other ethnicities were represented as well (9.6% African American, 3.2% Hispanic, 2.3% Asian, 1.4% Biracial). The children in the sample also represented a variety of ethnic groups. Mothers were well educated (2.3% GED, 12.8% completed part of college, 6.8% graduated 2-year college, 32.9% graduated 4-year college, 4.1% completed part of graduate/professional school, 40.2% completed graduate/professional school), and most were married (81.7%). A total of 83.9% of mothers in the sample were diagnosed with one or more DSM-IV Axis I diagnoses across their lifetimes. Furthermore, 59.4% of the mothers were undergoing mental health treatment (e.g., individual therapy, psychiatric services) at the time of the preschool visit.

The mother-child dyads who were followed up at school-age (*N*=159) represent approximately 73% of the participants who were initially recruited for the preschool phase of the study. Those participants lost to follow-up differed significantly from retained participants in terms of mother's age (lost, *M*=35.63, *SD*=5.41; retained, *M*=37.43, *SD*=4.77) and mother's education (lost, *M*=5.93, *SD*=1.56; retained *M*=6.66, *SD*=1.44). During the school-age phase of the study, children's ages ranged from 5 to 10 years (*M*=7.04, *SD*=1.07) and mother's ages ranged from 24 to 53 years (*M*=41.1, *SD*=4.7). Child sex was evenly split (49.7% male, 50.3% female). Mother's employment status varied (27.7% reported being unemployed/not working,

25.8% were employed part-time, 43.4% were employed full-time, and 0.6% were retired). Mothers reported having varying levels of higher education (6.9% High School or Equivalent, 4.4% Two-Year Degree, 38.4% Bachelor's Degree, 30.2% Master's Degree, 6.9% Professional Degree, 11.9% Doctoral Degree). The inclusion criteria for the final sample included having data for the emotion regulation variables and cortisol collected at preschool-age, as well as the callous-unemotional trait data collected at school-age. The final school-age sample consisted of 119 mother-child dyads. The children included in the final sample did not significantly differ from the 40 children not included (due to missing data) on measures of aggression, ELS, or maternal BDI at either time point ($p \geq 0.255$).

Procedure

During the preschool phase of the study, participants visited the BUILD laboratory in the psychology department at Emory University where children completed several measures of behavioral, cognitive, and language functioning. Mothers also completed questionnaires about their current symptoms of depression, as well as stressors they experienced during their child's lifetime. Children's aggressive behavior was rated by an alternate caregiver (e.g., grandmother, father, babysitter, etc.) to supplement our mother-report measures. During this visit, children also participated in a lab-based frustration task and provided two saliva samples: one at the beginning of the lab visit, and one following a 20-minute delay post-frustration task.

In the follow-up school-age study, data were collected via a secure online database called REDCap. Permission to re-contact, along with contact information, was obtained during the preschool phase of the study. Mothers received a direct hyperlink to the online measures. Participants were instructed to click on the link, read consent information thoroughly, and complete the online questionnaires if they agreed to study details. Participants were not required

to complete every questionnaire in one sitting, and were re-contacted if measures were left incomplete for longer than two weeks. Similar to the preschool phase of the study, behavioral questionnaires were also completed by an alternate caregiver using the same REDCap procedures outlined above.

Early Life Stress. To assess early life stress, mothers were asked to complete a 57-item self-report measure called the Life Experiences Survey (Sarason, Johnson, & Siegel, 1978) during their lab visit, endorsing the specific number of events they had experienced in the past 6 months or since their pregnancy with the enrolled child (i.e., child's "lifetime"), as well as subjectively evaluating how positively or negatively those events impacted them. The kinds of stressful life events included on the Life Experiences Survey include characteristics of the family environment, such as parental conflict or divorce, financial difficulties, low maternal social support, and major change in occupation or living conditions (Bronfenbrenner, 1979). The 6-month and lifetime subjective ratings of ELS were used in all analyses.

Behavioral Stress Reactivity. The "Attractive Toy in a Transparent Box" (Clear Box) task from the preschool version Laboratory Temperament Assessment Battery (Lab-TAB; Hill Goldsmith, Reilly, Lemery, Longley, & Prescott, 1999) was used to induce frustration and stress in the preschool sample of children. In this task, children were asked to pick one of two toys (ball or slinky) that was then placed inside a box and locked by one of the research assistants. The assistant then instructed the child to find the correct key on a set of keys and to open the box with that key. Before leaving the room, the experimenter told the child, "most kids do this fast." The child was left alone for two minutes. After two minutes, the research assistant entered the room and apologized to the child, saying that it was her fault that the child could not open the box

because she had the correct key all along. She then helped the child open the box and play with the toy. The task was recorded for later offline behavioral coding.

To code the children's behavioral responses to stress, each video was divided into 10-second epochs. For each epoch, the child's behavior was coded along the following dimensions: intensity of anger expression, presence of bodily anger, peak intensity of frustration, intensity of sadness expression, presence of bodily sadness, and peak intensity of gaze aversion. A trained researcher coded each video, and ten percent of the videos were selected for additional reliability coding. Reliability was high (α>0.8) across all behavioral codes.

Physiological Stress Reactivity. Salivary cortisol samples were collected from the children during the preschool study lab visit at two time points: when they arrived at the lab (baseline) and 20 minutes after the completion of the frustration task. To aid in sample collection, children were asked to chew on a piece of cotton dipped in a very small quantity of Kool-Aid™ (Gunnar & Donzella, 2002). Previous studies have suggested that, when used consistently and in small quantities (Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005), such noninvasive saliva "stimulant" collection methods can be useful, should not distort either within or between subject comparisons, and can yield reliable measures of cortisol (Bruce, Davis, & Gunnar, 2002; Gordon, Peloso, Auker, & Dozier, 2005; Kirschbaum & Hellhammer, 1994; Vining & McGinley, 1987). Furthermore, given the young age of the subjects (2.5-5 years), this method was deemed most appropriate in order to increase compliance.

After chewing the cotton for one minute, a research assistant retrieved the cotton from the child, put it inside the lumen of a 0.5ml syringe, and squeezed out about 1cc of saliva into a small tube. All saliva samples were frozen and stored at -20°C before being transported for assay to the Yerkes National Primate Research Lab at Emory. Upon arrival to Yerkes, saliva samples

were stored at -20°C until the day of assay. On the day of assay, samples were thawed, vortexed, and centrifuged to remove any particulate matter. Salivary cortisol was assayed using an enzyme immunoassay kit (Diagnostic Systems Laboratories; DSL, Webster, TX), catalogue number DSL-10-67100. This assay procedure has an analytical sensitivity of 0.10 mg/dl, using 25 ml of saliva. The intra- and inter-assay coefficient of variation is 4.1% and 7.2%, respectively. Each sample was assayed in duplicate. Duplicate tests with an error of more than 20% were retested. Duplicate test results were averaged and this cortisol value was used in analyses. Cortisol values were heavily skewed at time 1 (initial collection at the start of the lab visit; 6.79) and at time 2 (post-frustration task; 7.40). Therefore, cortisol values were winsorized and time 1 values were regressed on time 2 cortisol values, thereby creating a residualized change score. Standardized residuals were used as indicators of physiological reactivity in all subsequent analyses. Previous studies have advocated using this residualized change score as a more statistically reliable measure of cortisol reactivity than calculating a (pre- and post-stressor task) difference score (Hagan, Roubinov, Mistler, & Luecken, 2014).

Aggression. To assess levels of aggression in preschool children, each child's mother and an alternate caregiver were asked to complete the 100-item Preschool-Age Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2000) which evaluates children's behavior across several domains of functioning. Raters were asked to indicate whether the items listed described the child's behavior over the past two months, and whether the item was "not true" (0), "somewhat or sometimes true" (1), or "very true or often true" (2). The primary subscale of interest in the present study was the aggression subscale. Examples of items that load onto this subscale include "gets in many fights," "hits others," and "physically attacks people."

Aggressive behaviors in the school-age follow-up were measured using the School-Age CBCL completed by the child's mother and an alternate caregiver. The School-Age CBCL (Achenbach, 2001) is very similar to the Preschool-Age form in that there is overlap on the items measuring aggression, except that there are 113 items, and the rater is asked to rate the child's behavior over the course of the last six months. The aggressive behavior subscale was used as the outcome variable in analyses. Internal consistency was high (α>0.8) for aggression subscales across reporters and time points. Given the modest correlations between maternal and alternate-caregiver rated aggression during the preschool assessment (r=0.432, p<0.01) and school-age follow-up (r=0.599, p<0.01), the researchers combined parent and alternate caregiver ratings of children's aggression into overall standardized Preschool Aggression and School-Age Aggression variables. In addition, the researchers created a Persistent Early Childhood Aggression variable which identified the children whose aggression was rated as above the mean on the combined parent and alternative caregiver measure of aggression at both assessment time points (N=27).

Callous-Unemotional Traits. For the school-age follow-up study, mothers completed the Inventory of Callous-Unemotional Traits (ICU; Frick, 2003). They were asked to read a series of 24 statements, and rate their children on a scale of 0 ("not true at all") to 3 ("definitely true"). Items include: "expresses his/her feelings openly"; "seems very cold and uncaring"; "shows no remorse when he/she has done something wrong." Three factors emerge on the ICU (callousness, uncaring, unemotional; Frick, 2003). For the purposes of these analyses, the focus was on the callousness factor. Reliability for this scale was moderate (α=0.666). Given that there are no widely-accepted clinical cut-offs for the ICU (Essau et al., 2006; Ezpeleta et al., 2013), callousness was entered as a continuous variable in all moderation analyses.

Statistical Approach

A Principal Components Analysis (PCA) was conducted on the different behavioral variables of the Clear Box (CB) task. Latent variables were extracted using principal axis factoring and Oblique rotation (Fabrigar, Wegener, MacCallum, & Strahan, 1999). Two factors, Anger and Sadness, were identified using the eigenvalue rule (Kaiser, 1960), which recommends including elements with values greater than 1, and verified through scree plot analysis (Cattell, 1966). Anger expression (0.736) and bodily anger (0.769) loaded onto the Anger factor, whereas sadness expression (0.815) and bodily sadness (0.865) loaded onto the sadness factor. Factor scores were used in all subsequent statistical analyses of behavioral reactivity.

Figure 1 illustrates the overall moderated mediation models that were tested in this study. A series of PROCESS models (Hayes, 2013) were utilized to test the primary hypotheses. In the first set of analyses, simple mediation models (model 4) were used to examine the pathway from early life stress (6-month and lifetime) to school age aggression/persistent childhood aggression through measures of preschool stress reactivity (CB Anger, CB Sadness, and cortisol reactivity). PROCESS uses an ordinary least squares or logistic regression-based path analytic framework to estimate direct and indirect effects, and produces bootstrap standard errors and 95% confidence intervals for the specific indirect effects using 1,000 bootstrap samples. The absence of a zero value within the bootstrap confidence interval is suggestive of an indirect effect.

In the next set of analyses, moderated mediation PROCESS models were tested. Child sex was tested as a moderator of the relationship between ELS and preschool stress reactivity (model 8), as well as stress reactivity and aggression (model 14). In addition, child callousness was tested as a moderator of the relationship between ELS and preschool stress reactivity (model 8), as well as stress reactivity and aggression (model 14). In the final set of analyses, analyses that

had demonstrated significant associations between physiological and behavioral reactivity and school age aggression were re-run, controlling for preschool age aggression, in order to evaluate the predictive utility of stress reactivity variables above and beyond behavioral reports of preschool age aggression.

Results

Descriptive Analyses

Correlations between early life stress, aggression, emotion regulation, and moderating variables are presented in Table 1. All aggression measures were significantly and positively related to one another. Callousness significantly and positively related to each aggression measure, but not to either of the behavioral indices of stress reactivity or to cortisol. Gender did not relate to any stress reactivity or aggression variable. Physiological measures of measures of reactivity were not correlated to either behavioral measure.

Determining Covariates

During the preschool visit, mothers completed a 20-item Child Health Questionnaire which was designed to address several variables that have been shown in the literature to influence cortisol, such as child stimulant or antihistamine use, whether the child had a cold or flu, and the child's time of awakening the morning of his or her lab visit (Granger, Hibel, Fortunato, & Kapelewski, 2009; Hellhammer, Wüst, & Kudielka, 2009; Jessop & Turner-Cobb, 2008; Kudielka & Kirschbaum, 2003). None of the health variables were significantly related to our measure of cortisol reactivity.

Demographic variables previously associated with childhood aggression and callous-unemotional traits, such as socioeconomic status (SES; Dodge, Pettit, & Bates, 1994; Rutter, 1981), ethnicity (Gray, Indurkhya, & McCormick, 2004), and child sex (Coutinho & Oswald,

2005; Loeber & Keenan, 1994; McIntosh, Reinke, Kelm, & Sadler, 2012), were tested as potential covariates. Although there was not a standardized measure of SES available in the present study, maternal education, which previous studies have shown to be related to SES (Hauser, 1994), was tested as a covariate. Given well-established sex differences in child aggression, child sex was also tested as a covariate.

Furthermore, because many of the women in this clinical sample were diagnosed with Axis I disorders during the child's lifetime and many were prescribed psychotropic medications during pregnancy, both maternal psychiatric history and medication exposure were tested as potential covariates in the analyses. Maternal depressive symptomatology was assessed using the 21-item Beck Depression Inventory, 2nd edition (BDI-II). Mothers were asked to report on behavioral, cognitive, and emotional symptoms using a 4-point Likert scale. Total BDI scores for both the preschool and school-age phases of the study were calculated and tested as potential covariates for all analyses. Similarly, mothers' current and lifetime health information was assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders (First, Spitzer, Gibbon, & Williams, 2002). This assessment also obtained information on both prenatal and present maternal psychotropic use. No demographic or maternal mental health covariates were identified for the Preschool, School Age, and Persistent Aggression variables. There was no multicollinearity between moderators and independent variables.

Hypothesis Testing

Our first hypothesis was that high levels of early life stress would predict to higher behavioral and physiological stress reactivity in preschool age, which would in turn relate to higher levels of caregiver-reported aggression in school age as well as higher levels of early childhood persistent aggression. Results for the overall mediation models testing this hypothesis

for both school-age aggression and persistent early childhood aggression outcomes are presented in Table 2.

Results showed significant direct effects of subjective ratings of ELS within the past six months on school-age aggression as well as significant direct effects of both subjective ratings of ELS within the past six months and lifetime ratings of ELS on persistent early childhood aggression. Contrary to our hypothesis, no indirect effects were significant, suggesting that physiological and behavioral reactivity did not mediate these ELS-aggression relationships.

Our second hypothesis was that child sex and child callousness would moderate the above tested associations between stress and reactivity and reactivity and aggression. Results of sex moderation analyses are presented in Table 3, and Table 4 presents results from the child callousness moderation analyses. In cases where sex or callousness significantly moderated the proposed mediation model, additional simple linear regression analyses were conducted to probe the directionality of the relationship, and these results are reported below.

As shown in Table 3, there were a number of significant sex moderations. There was a significant interaction between subjective 6-month ratings of ELS and child sex to predict measures of anger on the behavioral stress reactivity task. Follow-up regression analyses suggest that the relationship between stress exposure in the last six months and preschool ratings of emotion regulation (anger) is stronger for females (β_{female}=-0.326, p=0.009) than males (β_{male}=0.228, n.s.). Similarly, child sex was found to moderate the relationship between all three measures of preschool stress reactivity and school age aggression. Post-hoc regression analyses revealed that child sadness decreased the risk for school age aggression for males (β_{male}=-0.273, p=0.046) but not for females (β_{female}=0.038, n.s.) In contrast, child anger was positively associated with school age aggression for males (β_{male}=0.223, n.s.) but negatively associated for

females (β_{female}=-0.087, n.s.). Lower levels of cortisol reactivity also predicted school-age aggression for males (β_{males}=-0.301, p=0.027) but not females (β_{female}=0.032, n.s.). Lastly, child sex moderated the relationship between sadness and persistent early childhood aggression, such that sadness was associated with decreased aggression for males (β_{male}=-0.212, n.s.) but not for females (β_{female}=0.113, n.s.).

As seen in Table 4, child callousness moderated the relationship between 6-month ratings of ELS and school-age aggression such that ELS only related to aggression for children who were rated as having high levels of callousness by their mothers (β=-0.275, p=0.028). Callousness did not moderate the relationship between 6-month ratings of ELS and persistent aggression. Moreover, child callousness did not moderate the relationship between lifetime ratings of ELS and either persistent or school age aggression.

Our final hypothesis was that measures of emotional reactivity would predict to school-age aggression above and beyond maternal and alternate caregiver behavioral reports of aggression during the preschool period. We tested this hypothesis by re-analyzing associations between stress reactivity measures and school age aggression that were significant (sadness and cortisol, males only), and adding preschool aggression as a statistical control. Controlling for preschool age aggression rendered the association between boys' sadness and school age aggression nonsignificant (β=-0.184, p=n.s.). However, lower cortisol remained a significant predictor of school age aggression for boys, even after preschool age aggression was controlled (β=-0.302, p=0.025, R^2 change=.061).

Discussion

This study contributes to the aggression literature by linking the effects of early life stress to aggression among young children. Results suggest that ELS within the past six months has a

direct effect on school-age measures of aggression as well as persistent measures of early childhood aggression. ELS over the course of a child's lifetime only has a direct effect on persistent aggression, not on school-age aggression. Child sex moderated the relationship between ELS and preschool-age anger. Sex was also found to moderate associations between all three measures of stress reactivity and school-age aggression, as well as preschool-age sadness and persistent aggression. Only lower cortisol remained a significant predictor for school-age aggression for boys even after controlling for preschool levels of aggression. Unexpectedly, callousness only moderated the relationship between 6-month subjective ratings of stress and school-age aggression. Contrary to our hypothesis, reactivity did not act as a mediator between ELS and aggression.

There are several strengths of the present study, including the longitudinal nature of the design as well as multiple raters of child behavior (mothers, and alternate caregivers), stress reactivity (physiological makers and trained coders), and callousness (mothers). There was a high rate of participant follow-up from the preschool to the school-age period, and only minor differences between those families who participated in the follow-up study and those who did not. Also important is the fact that this study examined the development of aggression among a unique sample of children: though primarily higher SES, these children were exposed to high levels of maternal psychopathology both pre- and postnatally. This particular group of children represents an understudied sample in the aggression literature.

Although this study examined several markers of stress reactivity, and while the behavioral indicators were correlated in the expected direction, neither of the behavioral indicators was correlated with cortisol. As such, these measures were conceptualized as separate proxy measures of stress reactivity. This distinction between physiological and behavior indicators of

emotion reactivity is consistent with the work of researchers who argue that response coherence among behavioral and physiological indices is not necessary (Mauss & Robinson, 2009). However, this lack of response coherence does raise intriguing questions about the ways in which stress reactivity is measured in the field. Additional research is needed to further interrogate the best ways to conceptualize this construct.

Though there was a link between ELS and both school age aggression and persistent early childhood aggression, reactivity in the preschool age did not explain this link. It is possible that preschool-aged children manifesting general affective and behavioral dysregulation might be at a greater risk for internalizing rather than externalizing pathology, particularly as they age into adolescence. Given that this is a sample of children born to mothers evidencing high levels of internalizing pathology themselves, this is a hypothesis worthy of exploration in future follow ups.

The results of the present study suggest that ELS affects males and females differently. For girls, ELS is predictive of behavioral stress reactivity (anger) during the preschool period, but contrary to prediction, behavioral reactivity in preschool is not predictive of higher levels of aggression in school-age. Although a recent study suggests that females (like males) display high levels of disruptive behavior in childhood that put them at greater risk for multiple adverse outcomes in adolescence (van der Molen et al., 2015), other studies have noted a later age of onset for aggression in girls relative to boys (Silverthorn & Frick, 1999). Therefore, it may be that emotion dysregulation in the preschool period does increase the risk for aggression for girls, but these effects may not evidence until later in development.

It is also important to note that the 6-month rather than lifetime ratings of ELS were stronger predictors in this study, potentially suggesting a more immediate impact of stressors on

emotional reactivity that may dissipate over time. It is also possible that lifetime ratings of stress may be less accurate given a higher likelihood of maternal memory bias, or that early events (e.g., those occurring when the child was an infant) did not impact the child as much, as the child may not have even been aware of their occurrence.

The findings from this study suggest that the association between physiological and behavioral reactivity in preschool and school aged aggression is rather complex. First, sex does act as a moderator, which suggests that boys and girls with regulation problems are at differential risk for aggression. Second, the direction of the associations between emotional reactivity and aggression are not consistent even for boys. It appears that higher levels of anger and lower cortisol are more predictive of aggression, rather than greater "reactivity" per se. The finding that boys are more likely to be aggressive if they respond to a stressor paradigm with anger is also consistent with Agnew's Strain Theory, which argues that boys externalize anger related to perceived strains in their environment more often than girls (Broidy & Agnew, 1997). The finding that lower cortisol relates to aggression is also supported by existing literature (Shoal, Giancola, & Kirillova, 2003), though further longitudinal work is needed to corroborate whether this observed difference in middle childhood continues to reliably predict which males are at a particularly high risk for future delinquency. Lastly, the finding that child sadness decreased the risk of aggression for boys may be suggestive that these particular youth are at greater risk of internalizing symptoms in response to stress, rather than externalizing behavior problems. However, this sample would need to be followed up again in order to determine whether the current stress reactivity variables have utility in predicting differential trajectories of pathology.

The finding that high callousness interacts with ratings of ELS from the past six months to predict higher levels of school-age aggression is in line with previous literature that suggests that

high levels of CU traits help characterize a unique sample of youth who are more likely to persistently offend (Frick & White, 2008). Further longitudinal follow-up with the present sample is needed to determine whether this is a stable predictor of continued problems into adolescence. However, these results demonstrate that callousness is a useful variable to assess for when evaluating behavioral outcomes in children exposed to high levels of psychosocial stress.

These results may have clinical implications for those treating or developing treatments for children with aggressive behavior. The finding that child sex moderated the relationship between all three measures of stress reactivity and school-age aggression in the present study reinforces the importance of tailoring treatments differently for males and females, thereby reinforcing the work of previous studies that have called for a more gender-sensitive treatment approach for behavior problems in youth (Winiarski et al., 2016). As mentioned above, the moderating effects of callousness on the relationship between ELS and school-age aggression also highlight the importance of screening for these traits in children who may be identified as at-risk for aggression.

Moreover, the finding that lower cortisol levels in response to a frustration task predict to school-age aggression above and beyond caregiver ratings of aggression speaks to the clinical relevance of improving screening methods for earlier detection of clinically significant behavior problems. Though the importance of early detection has been suggested in the past, most interventions for aggression are introduced in middle childhood (Webster-Stratton, 2005). Mere observations of aggression can oftentimes be problematic given the difficulty some caregivers may have in distinguishing normative aggression from clinically-significant levels of aggression. Therefore, having an additional predictor (in this case, lower cortisol) could inform earlier

detection and intervention efforts. Moreover, given that previous studies have found that blunted cortisol in response to experimentally-induced stress among youth with disruptive behaviors is related to higher CU traits (Stadler et al., 2011), it may be that the males in this sample for whom cortisol is a better predictor of aggression than observation alone represent the unique profile of children who will exhibit serious behavior problems over the lifespan (Frick & White, 2008). Of course, these youth need to be followed up later in development to better understand long-term effects, and additional studies are needed to more clearly elucidate which measures of stress reactivity may be stable markers of future aggressive behavior.

Potential Limitations and Future Directions

Though these findings illustrate the importance of a gender-sensitive approach to treating emerging behavioral problems in school-age and demonstrate a link between ELS and aggression across childhood, they should nevertheless be interpreted in light of several limitations. Most notably, the sample of mothers and children in this study were of higher SES, and most mothers were either receiving mental health interventions at the time of the study or had a longstanding history of mental health intervention. Although previous research has found higher rates of aggression among children in lower SES homes, less is known about the risk factors for the development of aggression among higher SES samples. It is possible that the effects of ELS on stress reactivity were buffered by mothers' mental health treatment and ability to model adaptive behavior in response to stress as a result of their treatment. Future studies may want to explore the link between ELS, stress reactivity, and aggression using a sample of patients with diverse socioeconomic and mental health treatment profiles.

Given the sex moderated effects observed in the ELS-stress reactivity and stress reactivity-aggression relationships, it is important for future studies to more carefully evaluate how sex

plays a role in shaping aggression. Specifically, future studies should more carefully distinguish between the different *types* of aggressive behavior. Due to the structure of the measure used in the current study (Child Behavior Checklist), we looked at overall aggression, and did not distinguish between proactive and reactive subtypes (Dodge & Coie, 1987; Raine et al., 2006), nor did we distinguish between physical and relational aggression. Because previous research suggests that relational and overt aggression can be reliably distinguished from one another as early as preschool (Crick, Casas, & Mosher, 1997) and that physiological reactivity differentially relates to relational aggression among males and females (Murray-Close et al., 2014), future studies with this sample need to more carefully tease apart the possibility that different measures of stress reactivity relate to distinct types of aggression.

Because stress reactivity did not serve as a mediator in the ELS-aggression relationship, it is important to consider other potential mechanisms for this association. Previous studies have illustrated the importance of parenting in the development of aggression across childhood (Kopala-Sibley et al., 2015), suggesting that parenting measures may be worthy of study in this regard. As children become older and social relationships become more prominent, it would also be important to examine peer affiliation as a moderator of the relationship between dysregulation and aggressive behavior. It is possible that children who are more dysregulated may be more likely to affiliate with deviant peers, and also more susceptible to the influence of these peers. This may further exacerbate their dysregulation and contribute to problems with aggressive behavior.

Our findings may also have been limited by the sole use of maternal report concerning ELS. Future studies of this type should include both objective and subjective measures of stress from

not only the mothers, but from their children as well. This would allow for an examination of how children's perceptions of stress interact with their reactivity to predict to outcomes.

Conclusions

This study explored the mediating effects of physiological and behavioral stress reactivity on the relationship between ELS and aggression, both across early childhood and in school-age. Sex and callous-unemotional traits were explored as moderators of the aforementioned relationships. The results of several PROCESS models demonstrated a link between ELS and both school-age and early childhood persistent aggression. Contrary to prediction, reactivity did not act as a mediator in these associations. However, as predicted, sex was found to moderate the relationship between ELS within the last six months and anger, such that the association was stronger for females than for males. Sex also moderated the relationship between stress reactivity and aggression, such that higher anger, lower sadness, and lower cortisol during the preschool period were associated with higher levels of school-age aggression for males. Likewise, ELS was only related to school-age aggression among children with high levels of callousness. These findings should be considered as new iterations of treatments are developed for children with behavioral problems, and suggest that researchers should specifically evaluate levels of callousness among youth as well as target stress reactivity among youth in a gender specific fashion.

Examining amygdala activity in response to emotional stimuli as a mediator of the relationship between early life stress and school-age aggression

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Abstract

Previous studies have demonstrated that certain limbic structures, most notably the amygdala, are particularly sensitive to the effects of early life stress (ELS). Similarly, deficits in amygdala functioning have been differentially associated with proactive and reactive aggression. Little research has been done to bridge these parallel literatures, and explore neural circuitry as a mechanism by which ELS relates to childhood aggression. The aim of the present study was to test this association among a sample of 20 school-aged children recruited from the community. In the first phase of the study, children participated in an fMRI task and cognitive tasks, whereas mothers completed measures of child ELS. In the second phase of the study, mothers completed additional rating forms assessing their children's levels of aggression. Overall results did not support our mediator model hypothesis. However, findings did suggest a trend in the relationship between six-month ratings of ELS and greater amygdala activity in response to emotional stimuli for females, but not for males. There was also a trend for a negative relationship between right amygdala activity in response to emotional stimuli and higher levels of proactive aggression for both males and females. Clinical implications and directions for future research are discussed.

Examining amygdala activity in response to emotional stimuli as a mediator of the relationship between early life stress and school-age aggression

The mechanisms that link early life stress (ELS) and aggression can be investigated at the behavioral, physiological, and neural levels, but the neurobiological mechanisms by which early life stress contributes to childhood aggression have not been fully elucidated. The principal aim of the second study in this dissertation project was to examine how early life stress relates to amygdala responses to emotional stimuli, and how these differences subsequently relate to varied patterns of aggression among school-age children. What is known is that certain structures of the brain, including the amygdala, are particularly sensitive to chronic stress exposure. The amygdala has also been independently linked to aggression through its role in the processing of emotional stimuli. This study proposes to connect these bodies of literature by testing a model in which differences in amygdala activity mediate the relationship between ELS and school-age aggression.

The Effects of Early Life Stress on Brain Development

There is evidence to suggest that ELS adversely influences the hypothalamic-pituitary adrenal (HPA) axis, resulting in altered glucocorticoid secretion (Lupien, McEwen, Gunnar, & Heim, 2009). Over time, these alterations may influence sensitive brain regions that are especially dense in glucocorticoid receptors, such as the limbic area (Allwood et al., 2011; Popma et al., 2006; Sondeijker et al., 2007; Tyrka et al., 2012). Data from animal studies illustrate that excessive or prolonged activation of glucocorticoid receptors can result in structural changes in limbic structures of the brain (McEwen, 2007), one of which is the amygdala. Given its role in affective processing (Phelps, 2006) and behavioral regulation (Kalat, 2006), the amygdala is often implicated in the stress response, and is also theorized to be

particularly vulnerable to the effects of early childhood stress (Lupien et al., 2009). Among adult samples, researchers have theorized that "abnormal amygdala functioning" increases risk for criminal behavior given this brain region's involvement in fear conditioning (Gao, Raine, Dawson, et al., 2010).

Abnormal neural functioning has been conceptualized differently across studies. Recent findings have demonstrated associations between early stressful environments and activation patterns in the amygdala and ventrolateral prefrontal cortex in young adults, such that the relationship between amygdala activation and prefrontal activation was significant and positive among children exposed to ELS, but negative among control children. The authors concluded that ELS contributed to the development of atypical neural processing of emotional stimuli (Taylor et al., 2006). Other studies have shown that early caregiver deprivation is associated with increased amygdala activity in response to fearful faces (Maheu et al., 2010). Recent evidence in the field of molecular science also suggests that prolonged exposure to stressors results in dendritic arborization in the amygdala, which is then subsequently linked to increases in aggression (McEwen & Chattarji, 2004).

At present, there is evidence to suggest that chronic and early stress exposure have neurodevelopmental consequences. Similarly, previous studies have linked changes in amygdala structure and function to aggressive behavior. The aim of the present study is to both bridge these parallel literatures and to overcome the limitations across these literatures, namely poor distinctions between different subtypes of aggression and underrepresentation of young children. An understanding of how the stress aggression relationship emerges earlier in development is essential for the development of appropriate preventative and treatment tools. This study will

attempt to link ELS to childhood aggression through differences in amygdala functioning in a community sample of school-aged children.

Linking the Amygdala to Aggression

Given what is known about the link between emotion processing deficits and the development of aggression (Davidson, Putnam, & Larson, 2000; Kim & Cicchetti, 2010; Lamm, Granic, Zelazo, & Lewis, 2011; Roberton, Daffern, & Bucks, 2012), and given the amygdala's role in the processing of emotional stimuli, it seems plausible to explore the amygdala as a neural mechanism through which ELS can be linked to aggression.

A recent longitudinal study found that self- and teacher-reported childhood aggression was related to lower amygdala volume and higher rates of aggression among adult men (Pardini et al., 2014). Lesion and imaging studies from human and animal literatures implicate damage to the amygdala with observable behavioral deficits, such as difficulty recognizing distress and fear cues (Gross & Canteras, 2012; LeDoux, 2003; Phillips et al., 2004). Although there is evidence linking lower amygdala volume to aggression among clinical (Fairchild et al., 2011; Sterzer, Stadler, Poustka, & Kleinschmidt, 2007) and nonclinical samples (Matthies et al., 2012), there are also several studies that do not replicate this finding (Barkataki, Kumari, Das, Taylor, & Sharma, 2006; Gregory et al., 2012). Raine and colleagues (2005) proposed that these inconsistencies may be explained by the particular importance of amygdala deficits to persistent violent behaviors. Relatedly, very few of these types of studies distinguish between proactive and reactive aggression, subtypes that (as summarized below) are presumed to have different neural correlates.

Distinguishing Behavioral and Neural Features of Proactive and Reactive Aggression

In addition to linking ELS to neural changes in school-age children, the second study will also seek to better understand how these neural variations underlie unique patterns of reactive and proactive aggression. Reactive aggression is characterized by high responsiveness to social provocation. In contrast, proactive aggression is a more premeditated and instrumental form of aggression that is characterized by a lack of emotion in response to others' distress (Crick et al., 1996; Dodge et al., 1990; Dodge & Coie, 1987; Raine et al., 2006), and is often conceptualized as "cold-bloodedness" characteristic of "psychopaths" (Raine et al., 2006).

Previous studies have found differential amygdala reactivity in individuals with proactive aggression versus those with reactive aggression. In general, these studies find that proactive aggression is typically associated with blunted amygdala responding, whereas reactive aggression is associated with an exaggerated amygdala response (e.g., Pardini et al., 2014). For example, in a study among adults with intermittent explosive disorder (IED), which is characterized by impulsive and affectively-driven aggression, an exaggerated amygdala response to anger faces was found among adults diagnosed with IED compared to matched non-clinical controls (Coccaro, McCloskey, Fitzgerald, & Phan, 2007). Lozier and colleagues (2014) found that, among a sample of clinically-referred juveniles with conduct problems and community controls, reductions in amygdala activity in response to an implicit facial emotion processing task mediated the relationship between callous-unemotional traits and proactive aggression. Together, Coccaro's and Lozier's findings illustrate that the intensity of amygdala responsivity (blunted vs. exaggerated) is dependent on subtype of aggressive behavior.

The authors of the present study attempted to address some of the methodological limitations represented in the studies summarized above. Previous studies were conducted using samples of

clinically referred adolescents or adults. To the authors' knowledge, this is one of the first studies to examine the link between different subtypes of aggression and amygdala responses to emotional stimuli in a non-clinically referred sample of school-age children. A better understanding of how neural differences in processing of emotional stimuli manifest in patterns of aggression earlier in development might help inform existing treatments for different types of externalizing behavior problems.

Examining Child Sex as a Moderating Variable

Sex is an important, and often overlooked, moderator in studies of aggression. Males are generally overrepresented in studies examining clinically-significant levels of aggression, and small sample sizes preclude moderation analyses. Nonetheless, this gap in the literature makes it difficult to understand the developmental and neural precursors of aggression in females. Recent studies have noted sex differences in the functional connectivity of the amygdala in males and females, as well as in the regulatory effects of the stress hormone cortisol on amygdala circuitry (Kogler et al., 2016). Specifically, cortisol was negatively associated with resting state functional connectivity of the amygdala with other limbic brain structures among healthy adult female participants. The opposite finding was observed among males in the same sample.

Researchers have previously suggested that aggression in females may manifest differently than among males (Crick, 1995; Crick, & Grotpeter, 1995), and have demonstrated that psychosocial and mental health factors may differentially predispose men and women to aggression (Krakowski & Czobor, 2004). For example, a recent study found that the *OXTR* rs1042778 TT genotype was associated with increased right amygdala activity to angry facial expressions, and was also uniquely related to higher levels of antisocial behavior among men, but not among women (Waller et al., 2016). This is an area of the literature that has nevertheless

been given insufficient attention. Given the small sample size (N=20), this study is underpowered to statistically test moderation by sex; nevertheless we present results separated by sex as an exploratory step in this regard.

The Current Study

At present, there are distinct bodies of literature linking (a) ELS to amygdala activity, (b) amygdala activity to aggressive behavior, as well as (c) ELS with aggression later in life. Nevertheless, there is no common thread linking these parallel literatures. Therefore, this study will be the first to explore how ELS relates to reactivity differences at the neural level, which can then be further linked to levels of proactive and reactive childhood aggression. We hypothesize that children with greater exposure to ELS will demonstrate higher amygdala activity in response to emotional stimuli. Furthermore, we predict that children with higher amygdala activity will display increased levels of reactive versus proactive aggression. Given the small sample size, child sex will be explored as a moderator of the aforementioned relationships in an exploratory manner.

Method

A total of 33 women and their school-aged children (ages 7-10) were recruited from the Emory University Child Study Center database for participation during the first phase of this study. Inclusion criteria were: a) right-hand dominant, b) medically healthy, and c) metal free (for fMRI participation). Exclusion criteria were: a) adoption, b) seizures, c) severe developmental disabilities, d) current use of stimulant medications, and e) current use of psychiatric medications (e.g., SSRIs, SNRIs). Mothers were contacted by phone to determine study eligibility. All recruitment and study protocols for both phases of the study were approved by the Emory University Institutional Review Board.

Participants came into the lab for a total of two visits during the first phase of the study. During the first visit, mothers completed child behavior and stress rating forms, and children were given a series of cognitive tests to assess IQ, working memory, and processing speed. The child was also acquainted with the scanner and the tasks he or she would perform while in the scanner. If a child could not demonstrate an understanding of the tasks or exhibited considerable movement during the mock scan, he or she was not invited to participate in visit 2. The MRI scan (described in detail below) was completed during the second visit. Mothers provided consent for their participation, and children provided assent. Children were compensated with a gift card for successful completion of each visit. Mothers were paid for their participation in each lab visit. In addition, mothers and their children were given a CD with selected images from the brain scan completed during the second visit.

During the second phase of the study, which took place six months to one year following children's participation in phase one, mothers were re-contacted via email to complete additional ratings of child behavior via REDCap, a secure online database. The email received by participants included a direct hyperlink to the online measures. Participants were instructed to click on the link, read consent information thoroughly, and complete the online questionnaires if they agreed to all stated study details. Participants were not required to complete all questionnaires in one sitting, and were re-contacted if measures were left incomplete for longer than 30 days. Mothers were compensated with a gift card following completion of the online questionnaires.

Demographics of the Sample

Of the original sample recruited for the first phase of the study, participants were excluded from final analyses due to a lack of fMRI button-press data (N=1), a lack of follow up aggression

data (N=1), failure to scan due to performance issues (N=2), and significant motion (N=9). Therefore, a total of 20 (60.6%) children were included in the final analyses. As presented in Table 5, the children included in the analyses differed from those who were not included in terms of mothers' subjective lifetime ratings of ELS (t=-3.271, p=0.003) and maternal ratings of depression during the follow-up (t=2.605, p=0.020). Overall, maternal ratings of both 6-month and lifetime subjective stressors were more negative, and maternal depression was higher for children excluded due to incomplete data. The children included in the final sample did not significantly differ from those not included on other demographic measures (e.g., annual reported income), ratings of aggression, IQ, processing speed, or working memory ($p \geq 0.111$). Table 5 illustrates demographic characteristics of included and excluded participants.

Measures

Early Life Stress. To assess early life stress, mothers were asked to complete a 57-item self-report measure called the Life Experiences Survey (Sarason, Johnson, & Siegel, 1978) during their lab visit, endorsing the specific number of events they had experienced in the past 6 months or since their pregnancy with the enrolled child (i.e., child's "lifetime"), as well as subjectively evaluating how positively or negatively those events impacted them. The kinds of stressful life events included characteristics of the family environment, such as parental conflict or divorce, financial difficulties, low maternal social support, and major change in occupation or living conditions (Bronfenbrenner, 1979). The 6-month and lifetime subjective ratings of ELS were used in all analyses.

Maternal Reports of Aggressive Behavior. Mothers completed the 23-item reactive-proactive aggression questionnaire (RPQ; Raine et al., 2006), which is designed to measure both proactive and reactive aggression among children. Mothers were asked to rate their child's

frequency of engaging in certain aggressive acts on a scale of 0 (never) to 2 (often). Reactive and proactive aggression items were summed to form proactive and reactive scale scores. Consistent with previous studies (Dodge & Coie, 1987; Raine et al., 2006), proactive aggression scores were significantly and highly correlated with reactive aggression scores in this sample (see Table 6 for intercorrelations among study variables). Both proactive and reactive scale scores were used in statistical analyses.

Neural (fMRI) Measures of Emotion Regulation/Reactivity. Patterns of neural activation in response to different types of emotional faces were measured using a standard go/no-go fMRI task paradigm. During the initial lab visit, children were acclimated to a mock scanner to familiarize them with the noise of the scanner, the confined space, and the necessity to remain still during the session. Children also practiced the go/no-go task in and outside the mock scanner to be sure they understood the instructions and were able to complete the task.

During the second visit, children underwent fMRI scanning during the go/no task, in which visual stimuli were presented using a laptop interfaced with an LCD projector. Images were projected into the magnet bore and were visible to the subject via a small mirror on the head coil. Responses were acquired with a fiber optic button response box interfaced to the computer. Between runs, participants communicated with the control room through a microphone. Participants were instructed not to talk or move their heads or bodies during the scans, and if need be, to use a hand-held squeeze-bulb alarm in case they needed the technician to discontinue the scanning procedures. A form-fitting pillow and foam padding were used to reduce head motion. Experimenters monitored participant button presses to ensure that they attended to and performed the task.

Children's emotional reactivity and regulation were measured via amygdala activity during

one run of a computerized affective go/no-go task. During the task, participants viewed happy or neutral face stimuli that were surrounded by different colored picture frames (i.e., blue, yellow, orange). See Figure 2 for an example of a neutral face stimulus. Participants were required to respond as quickly as possible to neutral and happy faces surrounded by one of two target picture frame colors ("go trials"), while refraining from responding to faces surrounded by the non-target picture frame color ("no-go" trials). Target and non-target frame colors were counterbalanced. Happy and neutral facial expressions were matched, with 120 randomized trials (stimulus duration 1000ms; mean ISI 2000ms). Accuracy in responding was high (≥70%). Studies using similar paradigms have demonstrated that this task can be successfully used in young children and adolescents to discriminate age-based differences in emotional reactivity and control(Hare et al., 2008; Tottenham et al., 2011).

MRI Acquisition Parameters. Scanning sessions were conducted using a 3.0 T Siemens fMRI scanner. Whole-brain structural T1 images were acquired with a gradient-echo T1-weighted pulse sequence (TR =2.30s, TE=30ms, 1x1x1mm voxel size), which allowed the subsequent superposition of functional imaging results with anatomy. EPI functional scans were acquired using an interleaved sequence (TR =2.00s, TE=30ms, 3x3x3 mm voxel size), with 38 3-mm axial slices, to allow full-brain coverage. Order and timing of scanning tasks was as follows: situating participant in scanner (10 minutes), localizer (3 minutes), resting (8 minutes), fMRI (12 minutes) and structural (6 minutes).

fMRI Data Preparation. Functional data were assessed using Statistical Parametric Mapping (SPM8) software. Data were preprocessed using algorithms for slice timing correction, spatial realignment and unwarping, normalization to a standard EPI template using SPM8's segmentation and normalization method, and spatial smoothing. Subsequently, checks on MRI

image quality were conducted using ArtRepair (v4) program (Mazaika, Whitfield-Gabrieli, Reiss, & Glover, 2007), including inspection for signal spikes and motion artifacts. ArtRepair was then implemented to impute values for slices where movement disrupted the signals; in cases where data imputation was not adequate to repair the brain image, or where validity checks failed, the participant's fMRI data were dropped from further analyses.

fMRI Analysis of First Level Models. The general linear model was used to model effects of interest and confounding nuisance effects (e.g., head movement parameters from the realignment stage). Low-frequency noise was removed using the default SPM8 high-pass filter. Statistical parametric maps for each comparison of interest were calculated on a voxel-by-voxel basis.

Contrast maps of the t-statistic were assessed at every voxel in the brain by applying two linear contrasts to the parameter estimates for the conditions of interest (happy vs. neutral; and the interaction of NoGo vs. Go with happy vs. neutral). The first contrast was designed to measure whether children had greater amygdala reactivity in response to emotional stimuli (happy versus neutral faces). The second contrast measured the children's response to emotional stimuli in the presence of an incongruous behavioral task demand (e.g., a happy face, NoGo condition).

We then used these contrasts to examine neural activity in our region of interest (ROI), the amygdala (right, left, and bilateral). The values for each participant and for each ROI were calculated by taking the average contrast value for all voxels within the specified ROI. ROI data were extracted using the REX toolbox. All region-of-interest (ROI) variables were anatomically defined from the Wake Forest University (WFU) Pick Atlas software, SPM Toolbox (http://fmri.wfubmc.edu).

Statistical Approach

Determining Confounds

Prior to analyses, all data were assessed for data entry errors, outliers, and missing responses using SPSS 23.0. During the first lab visit, children were given several processing speed and working memory subtests of the Differential Ability Scales (DAS-II), as well as two subtests from the Wechsler Intelligence Scale for children (i.e., Matrices and Vocabulary; WASI-II). During the follow-up REDCap assessment, mothers completed a basic demographic questionnaire (e.g., income, ethnicity), as well as the Beck Depression Inventory, 2nd Edition (BDI-II). Demographic and maternal mental health variables were tested as potential covariates for aggression variables. Processing speed, working memory, child IQ, and demographic variables were tested as potential covariates for amygdala activity. Only household income was significantly and negatively related to proactive (p=0.021) and reactive aggression (p=0.007) variables. Therefore, income was controlled in all analyses examining aggression. There were no significant covariates for amygdala activity.

Analyses relevant to hypothesis testing were conducted by running a series of correlations between ROI contrasts and subjective lifetime and 6-month measures of ELS, as well as between ROI contrasts and proactive and reactive aggression ratings. If the correlations suggested potential indirect effects, SPSS PROCESS models were to be utilized to test for mediation. Given the small sample size, the researchers were underpowered to test for interactions, but in order to explore the potentially moderating role of sex, all analyses were run on the entire sample (N=20), as well as separately for both males (N=12) and females (N=8).

Results

Intercorrelations between study variables are presented in Table 6. Contrary to our hypothesis, ELS was not correlated with either proactive or reactive aggression measures in our sample.

Tables 7 and 8 present the data from correlational analyses assessing the relationships between measures of ELS and amygdala activity, and amygdala activity and measures of reactive and proactive aggression, respectively. Overall, there were no significant associations between the independent variable and the purported mediator and the mediator and the outcome, so we did not follow up with PROCESS model analyses.

Of note, two statistical trends were observed in our data. First, there was a trend for an inverse relationship between negative subjective ratings of ELS within the past six months and greater bilateral amygdala activity for females, but not males (Table 7). There was also a statistical trend for a negative relationship between right amygdala activity and ratings of proactive aggression for both males and females (Table 8). Both trends were observed for the second contrast of interest in which emotional responses were measured in the context of a conflicting behavioral task demand.

Discussion

This study tested for mediating effects of functional amygdala activity on the relationship between early life stress and aggression in a school-age sample of children recruited from the community. Our mediation model was not supported, and ELS was unrelated to aggression in our sample. The overall lack of association between ELS and aggression was unexpected, and may have been due to a loss of data from participants in the sample who had experienced higher levels of ELS.

We noted a trend for a negative relationship between 6-month ratings of ELS and amygdala activity among females, suggesting that maternal reports of more subjectively negative stressors in the past six months may be related to greater amygdala activity in response to the Go/NoGo task for females, but not for males. This finding is consistent with previous studies demonstrating a greater sensitivity to ELS in females in comparison to males (Rudolph & Hammen, 1999). There was also a statistical trend noted for a negative relationship between right amygdala activity and proactive aggression among both males and females. This suggests that higher rates of proactive aggression are related to blunted amygdala reactivity in the interaction contrast (contrast 2). This finding is consistent with Lozier and colleagues (2014), who found that CU traits moderated the relationship between right amygdala hypoactivation and proactive aggression. Contrary to our hypotheses, there was no relationship between amygdala responses to emotional stimuli and child reactive aggression.

The two statistical trends in the present study were both observed in the second contrast, which represents the child's emotional responding in the presence of an incongruous behavioral task demand. Specifically, emotional responses are measured in the presence of a happy face when a child is presumably primed to respond, but is simultaneously told (via the color of the frame) not to respond. The second contrast may be a better measure of emotional responses in a context of competing contingencies. The negative trend observed for females suggests that girls who experienced more negative stressors within the past six months had greater difficulty with the task, which may be indicative of problems with emotional control. However, these differences in emotional responding at the neural level did not increase their risk for aggressive outcomes. The finding that higher levels of proactive aggression related to reduced reactivity in the amygdala in this second contrast is also not unexpected given what is known about the

construct of proactive aggression. Specifically, proactive aggression is associated with more "calculated" behaviors (Raine et al., 2006), thereby making youth less reactive to emotional stimuli. In future studies, it would be important to assess the role of callous-unemotional (CU) traits in these relationships, as previous studies have demonstrated the moderating effects of CU traits on associations between amygdala functioning and aggression, particularly proactive aggression (e.g., Lozier et al., 2014).

There are several strengths to this study, including its longitudinal design and exploration of the link between ELS and different subtypes of aggression. As discussed in greater detail above, there are parallel literatures linking neural changes in response to ELS (Mueller et al., 2010; Taylor et al., 2006) as well as differences in neural structure and functioning with proactive (Lozier et al., 2014) and reactive (Coccaro, Mccloskey, Fitzgerald, & Phan, 2007) aggression, but this is among the first studies to link these bodies of literature. Similarly, previous researchers have highlighted the importance of distinguishing between different subtypes of aggression (Dodge & Coie, 1987; Raine et al., 2006), given the presumed differences in risk factors and neural underpinnings of these subtypes. Though underpowered, this study also attempted to tease apart sex differences in the associations between ELS and amygdala activity, as well as amygdala activity and aggression.

Limitations and Potential Future Directions

The most serious limitation in the present study was the small sample size. Nearly 40% of the originally-recruited sample was lost due to motion/lack of button press data. However, given that the sample was young, high drop-out rates for movement are not unexpected. The restricted sample size resulted in reduced power to detect effects. In addition, both 6-month and lifetime subjective ELS ratings indicated higher levels of stress in those participants who were excluded,

suggesting the observed associations are likely conservative estimates of true associations. Future studies of this type should seek to initially recruit a larger sample of children, and to recruit participants with a wider range of exposure to ELS. Similarly, our findings may also have been limited by the sole use of maternal report concerning ELS. Future studies of this type should include both objective and subjective measures of stress from not only the mothers, but from their children as well. This would allow for an examination of how children's direct experiences of stress interact with their emotional reactivity to predict to outcomes of aggression.

Along the same vein, it is important to consider these trends in the context of several unique demographic characteristics of the sample. The children recruited for this study were largely of higher socioeconomic background, ethnically homogenous, and recruited from a pool of community volunteers at the Emory University Child Study Center. Though this is not a sample typically associated with high rates of clinically significant levels of physical aggression, it is nevertheless important to better understand how aggression develops in youth without existing clinical diagnoses of behavioral pathology. The associations between income and aggression observed in this particular sample underscore the importance of diversifying socioeconomic and ethnic diversity in future studies of this type.

It was surprising that there was no relationship between reactive aggression and amygdala reactivity, especially because there appeared to be adequate variability in parent-reported levels of reactive aggression in this sample. However, it is possible that other regions of the brain are more involved in the manifestation of reactive aggression. For example, previous studies have reported mediating effects of the orbital frontal cortex on reactive aggression (Blair, 2004). In addition, recent research suggests that differences in aggression relate not only to functional differences in specific regions of the brain, but to processing differences across more complex

neural pathways. One of the most often-cited pathways in the literature is between the amygdala and prefrontal cortex. In a recent study of 14 adolescents with psychopathic traits and 14 controls, youths with psychopathic traits demonstrated lower amygdala activity as well as less functional connectivity between the amygdala and orbitofrontal cortex during performance on a moral judgment task (Marsh et al., 2011). Given the roles of the amygdala and prefrontal cortex in emotional processing and inhibition, respectively, exploring the functional connectivity between these two brain areas, particularly as they relate to reactive aggression (which is based more on impulsivity and poor control), is an important next step in future research. Moreover, as was stated above, the only trending findings observed in this study were related to the contrast representing emotional responding in a task requiring behavioral control, further suggesting possible involvement of frontal lobe functioning.

A further limitation of the present study concerns the specific stimuli used in the fMRI task. As can be seen in Table 5, the negative mean value for the happy versus neutral contrast indicates that, contrary to what we expected, the children in our sample had more amygdala activity in response to the neutral faces than the happy faces. A recent meta-analysis demonstrated that while the amygdala is involved in the processing of happy, fearful, and sad faces, its sensitivity is greatest for fearful faces (Fusar-Poli et al., 2009). Therefore, future researchers exploring the mediating effects of amygdala reactivity in the relationship between ELS and aggression may consider utilizing a wider range of emotion faces, including positive, neutral, and negative faces. There is an established link in the literature, for example, between amygdala activation and processing of fear stimuli (Adolphs, 2002; Whalen et al., 2001) and, somewhat less consistently, anger stimuli (Adams, Gordon, Baird, Ambady, & Kleck, 2003). However, these studies collectively lack a clear measure of the "why" as it relates to deficits in

emotional processing. Therefore, a clearer link to the potential causal factors relating to these deficits, such as impairments related to ELS, would be an important direction for future study.

Finally, although we expected proactive and reactive aggression to be related to one another (Dodge & Coie, 1987; Raine et al., 2006), the high correlation (r=0.600) between these two variables raises questions about the distinctiveness of the proactive and reactive constructs in this study. Future studies with larger sample sizes should continue to explore the unique risk factors for both proactive and reactive aggression, using statistical methods that can better parse risk factor associations with these two overlapping behavioral outcomes.

Conclusions

This study explored associations between ELS, amygdala activity, and reactive and proactive subtypes of aggression. Though we were underpowered to test moderating effects of child sex on the aforementioned relationships, each hypothesis was explored separately for males and females. The results suggested that ELS was not related to amygdala activity in the sample as a whole, but there was a trend for ELS to relate to stronger amygdala activity in response to emotion among females, but not males. There was also a statistical trend noted for a negative relationship between right amygdala activity in response to emotion and proactive aggression for both males and females. The results of this study suggest that sex differences may need to be more closely evaluated when examining the effects of early stress on neural processes. Moreover, these results call for further studies with larger, more demographically diverse samples that examine the unique neurobiological factors predicting to different subtypes of aggression.

General Discussion

The two studies in the current dissertation examined stress and emotional reactivity as potential mechanisms by which early life stress (ELS) may be linked to the development of school-age aggression. In the first study, behavioral and physiological stress reactivity were examined as potential mechanisms in a sample of children exposed to clinically significant maternal mental illness. In the second study, the potential mediating role of amygdala activity in the link between ELS and proactive versus reactive aggression was tested in a community sample of school age children. In addition to testing the aforementioned mediators, the first study also assessed the moderating effects of child sex and callousness, whereas the second study examined sex differences in an exploratory manner.

The development of this dissertation project grew out of the identification of several limitations and missing links in the literature. One of the major gaps in the extant literature is the lack of studies that prospectively assess *how* early childhood stress is linked to later measures of aggressive behavior, and more specifically, studies that test emotional reactivity as a mediator. Another neglected area of research is the study of the potential role of personality factors, particularly callous-unemotional traits, in predicting which children may be more vulnerable to the impacts of early life stress and the development and persistence of aggression. An additional gap in the literature concerns the lack of distinguishing differential risk factors for different subtypes of aggression. Relatedly, females tend to be excluded from many analyses of aggression because males tend to demonstrate higher rates of clinically significant physical aggression (Crick, 1997; Krakowski & Czobor, 2004) than females. Given that clear sex differences in emerge when exploring different subtypes of aggression (Bjorkqvist, Lagerspetz, & Kaukiainen, 1992; Murray-Close et al., 2014), this is an important area of further exploration.

Collectively, both of the studies in this dissertation contribute to the existing literature by: (a) examining early childhood aggression through a longitudinal design that not only includes observational, maternal report, physiological, and neural data, but also explores the moderating effects of both callousness and child sex, thus combining parallel literatures to better elucidate the mechanisms by which stress shapes the development of aggression; (b) extending the existing literature by exploring how amygdala activity differentially mediates the relationship between ELS and reactive versus proactive aggression; and (c) illustrating the importance of redefining both theoretical and intervention frameworks to more carefully account for sex differences in both stress sensitivity and the development of aggression in children at risk.

There was no support for the mediational model across both studies, suggesting that, although ELS relates to measures of reactivity across both studies and reactivity further relates to aggression across both studies, reactivity does not appear to be the mechanism by which ELS leads to aggressive behavior. As mentioned above, future studies with more diverse samples are needed to further explore how emotion reactivity might relate to ELS and aggression in a more stressful socioeconomic context. Furthermore, additional research in this sample and in others should be done to evaluate other potential mediators of the ELS-aggression relationship, such as parenting (Kopala-Sibley et al., 2015) or peer affiliation/social isolation (Farmer et al., 2003). Moreover, given that emotion regulation is a dyadic process earlier in development (Sroufe, 1995), future studies should explore whether parenting and emotion regulation interact to mediate the pathway from ELS to aggression.

Several intriguing sex differences emerged in Study 1. Specifically, females in the sample appeared to be more vulnerable to the effects of ELS, such that those with higher levels of stress within the past six months displayed greater levels of anger during the frustration task in the

preschool phase of the study. This finding was mirrored in Study 2 by the trend association between ELS and amygdala responses to emotional stimuli noted in females, but not males, in the sample. Taken together, our findings across Study 1 and Study 2 are in line with previous research that has suggested stronger associations between ELS and stress reactivity among females relative to males (Rudolph & Hammen, 1999).

In Study 1, sex moderated the relationship between preschool stress reactivity and aggression. Higher anger, lower sadness, and lower cortisol reactivity during the preschool period predicted to higher levels of aggression for males, but not females. We also noted the absence of an association between emotional reactivity and aggression among the girls in Study 2. Again, our findings are consistent with previous research noting stronger relationships between stress and aggression (Vaillancourt et al., 2007) and anger and aggression (Broidy & Agnew, 1997) for males relative to females. Future research should aim to more carefully explore the variables that predict to differential developmental patterns of aggression, such as sex role socialization, peer rejection, individual-level factors (i.e., temperament and personality), and co-occurring trajectories of maternal mental health problems.

The finding that ELS was significantly related to aggression among children high on callousness also builds on existing findings in the literature. Previous research has suggested that CU traits have more utility in predicting ongoing behavioral problems within-subjects rather than between-subjects (Frick & White, 2008). In other words, among children with clinically significant behavioral problems, those who also have higher levels of callous-unemotional traits are more likely to evidence continued problems with behavior. Future research should continue to evaluate the moderating effects of callousness to determine whether this variable can distinguish a unique trajectory of aggressive children. If this trend (ELS relating to aggression

among children high on callousness) were to replicate, it would support the utility of screening for this trait at younger ages for children with high levels of ELS.

Lastly, an additional aim of the first study was to illustrate that measures of stress reactivity in preschool age could predict above and beyond caregiver reports of aggression during that same developmental period. The finding that lower cortisol levels in response to a frustration task predicted to school-age aggression above and beyond caregiver ratings of aggression speaks to the clinical relevance of improving screening methods for earlier detection of clinically significant behavior problems. It could be that parents have difficulty in distinguishing normative preschool disruptive behavior from clinically significant behavior problems, and that adding an additional predictor may help identify and treat at-risk children earlier.

Study Strengths

There are several notable strengths across the two studies in this dissertation. The first is the longitudinal examination of childhood aggression. Study 1 examined the development of aggression from preschool age to school age. Study 2 focused specifically on different potential impacts of ELS (amygdala activity, behavioral ratings of aggression) across two time points in middle childhood. The examination of the school age period is important because many important developmental changes occur that can set the foundation for future problematic behavior in adolescence and adulthood. Therefore, a greater understanding of not just the type of aggression trajectories that exist, but of what their unique predictors are, is important.

The incorporation of multiple assessment methods (physiological, fMRI, participant observation, maternal and alternate caregiver report) is an additional strength of the present study. Although the authors had initially conceptualized "stress reactivity" as a unitary factor comprised of intercorrelations between behavioral and physiological measures, our behavioral

measures were not correlated with our physiological measures in Study 1. This is in line with previous research suggesting that between-individual analyses (i.e., if someone responded strongly on one emotional measures and responded equally strongly to a different measure of that emotion as well) might not be the best test of response coherence (Mauss & Robinson, 2009). The fact that each of the different measures was uniquely related to ELS and aggression in some way is illustrative of two important points. First, there should be a greater push in the field to come to a clearer consensus about the definition of "emotion reactivity," and the different ways this concept can be evaluated. Secondly and related to the first point, until future research more clearly defines this construct, researchers should be careful to measure multiple indicators of reactivity, particularly in studies examining the moderating effects of sex differences.

An additional limitation of previous research is retrospective self-reporting of early life stress. This is complicated by issues of development (child's age and memory formation) as well as accuracy (potential bias in participant self-report). To address this issue, we asked mothers to rate the child's exposure to stress. To further reduce the potential bias of reporting in the first study, we asked both mothers and alternate caregivers to complete rating forms of each child's behavior, and the correlations across both maternal and alternate caregiver reports were high. Subjective ratings of stress were intentionally selected as they most accurately reflect maternal *response* to the stressors experienced by the child and his or her family. Even if the total number of stressors a family experiences is low, if these stressors are all perceived as being extremely negative, this may point to the poor coping that is being modeled to the child (and may also possibly relate to a genetic predisposition toward negative emotionality and poor coping).

Study 2 overcomes some of the limitations of Study 1 (as well as many other studies of aggression in the literature) by exploring the effects of ELS on different subtypes of aggression.

Previous studies have found associations between differential amygdala activation patterns among adults and adolescents with clinical levels of aggressive behavior (Coccaro et al., 2007; Lozier et al., 2014), but this is among the first studies to explore this relationship in a school-age sample of children. Furthermore, to the authors' knowledge, this is the first study to attempt to link ELS and neural responses to emotional stimuli to the development of aggression in childhood.

Study Limitations

The findings from both studies need to be evaluated in light of some important limitations. The first limitation is sample size. In Study 2, we lost many participants from the original sample due to movement/lack of button press data. Given the size of the final sample, we were underpowered in our sex moderation analyses. Moreover, a small sample size and little variability in rates of callousness in our community sample precluded us from examining the moderating effects of callousness on the mediational model in Study 2. Because previous studies have indicated that child callousness may moderate the different subtypes of aggression (Marsh et al., 2008), this would be an important variable to examine in future studies.

Secondly, the participants in both studies were demographically homogenous, and these results may therefore not be generalizable to individuals who are racially and socioeconomically diverse. Nevertheless, most research to date has focused on lower socioeconomic groups in studies of aggression given the high base rate in this population (e.g., Haapasalo & Tremblay, 1994; Shaw, Gilliom, Ingoldsby, & Nagin, 2003) and less is known about whether similar trends in aggression emerge across all socioeconomic groups. Another important feature of the sample in Study 1 is that most of the children were exposed to psychotropic medications prenatally and had mothers suffering from past and/or current mental illness. Maternal mental illness is in and

of itself a risk factor for later psychopathology (Goodman & Gotlib, 1999), though the exact mechanisms by which maternal mental health variables influence aggression (e.g., parenting, inherited disposition, etc.) have not been fully elucidated. Future studies with more diverse samples can begin to carefully tease apart the cumulative and interactive effects of different social, familial, and individual-level variables on the development and persistence of aggression in childhood and into adolescence.

Additional limitations across both studies concern the maternal ratings of ELS. The first limitation is the lack of variability in measures of ELS in the Study 2. Secondly, both studies rely on maternal reports of subjective stress, which can introduce bias in reporting. Future studies should attempt to obtain measures of ELS from multiple reporters, including the child. In addition, future studies should attempt to incorporate more objective ratings of ELS, such as emerging biosensor technologies or ecological momentary analysis (Shiffman, Stone, & Hufford, 2008), which can improve ecological validity and reduce recall bias in reporting of stressful life events.

Although Study 1 began to examine childhood aggression in more detail, replications with larger sample sizes are needed, as well as future studies that explore additional subtypes of aggression, such as relational aggression. One recent study, for example, found that among girls, relational aggression was strongly related to blunted physiological reactivity, whereas the reverse was true for males (Murray-Close et al., 2014). Together with the existing literature, the results of both dissertation studies suggest that aggression should not be studied as a general construct, and that it may be useful to break aggression down into different subtypes that may have sex-specific risk factors.

Implications and Future Directions

The current study highlights the need for additional longitudinal studies examining the differential trajectories of childhood aggression specifically, and psychopathology more broadly. Though our studies did not directly test existing criminological theories, such as General Strain Theory (GST; Agnew, 2007), they can nevertheless inform the existing literature and guide future iterations of the theory. Although GST does emphasize the importance of the social environment in "pressuring" individuals to partake in crime, it does not explain what individual-level biological factors make some individuals more susceptible to their social environments (and therefore more likely to engage in crime; Rebellon, Barnes, & Agnew, 2015). Given that there are countless heritable, environmental, and temperamental factors that likely work together to shape the emergence of behavior problems, more work is needed to not only explore the interactions of these variables, but also the risk pathways whereby they exhibit their effects.

Moreover, the intriguing sex differences that emerged across both studies speak to the importance of examining how strain differentially impacts males and females. It is possible that environmental stress might more adversely affect females' emotion reactivity, but that different socialization factors emerge around school-age that alter the behavioral outcomes of males and females who are both exposed to stress. GST argues that strain is linked to crime through the negative emotions that an individual experiences in response to the strain. This highlights the importance of understanding the underpinnings of emotional processing and regulation, as well as the variables that place children at risk of developing poor emotion regulation, thereby making them more susceptible to the effects of strain. One possible reason why females are seen as less likely to exhibit criminal behavior than males is that females are thought to internalize strain, thereby experiencing higher levels of depression than aggression/criminality (Broidy & Agnew,

1997). The results of the present study, however, suggest that females exposed to ELS have higher rates of anger in preschool age than do males. Since the outcomes of this difference are not yet apparent in school age, additional follow-ups are necessary to understand how this stress reactivity manifests across development, and what potential variables mediate and moderate the outcome.

Finally, ELS should be examined across a more socio-demographically diverse population. The items on the Life Events Survey represent a broad range of stressors, including death of a family member or friend, recent marriage or birth of a child, as well as incarceration. Though the measure appropriately represents a wide range of stressors and attempts to account for how stressful the event was perceived to be (through subjective ratings), more research is needed to understand how different combinations of stressors impact the development of emotion regulation and aggression. It is possible that the link between ELS and emotion, for example, might be higher among children from more disadvantaged backgrounds who are disproportionately exposed to higher levels of stress, and have fewer environmental supports in place.

Conclusion

In conclusion, the two studies from the current dissertation project examined stress reactivity as a potential mechanism linking early life stress and school age aggression. These studies collectively highlight the importance of utilizing a framework that accounts for sex differences in the development of aggression across early development. Additionally, these studies highlight the need for future research to account for different subtypes of aggression, particularly when examining the effects of child sex on the development and maintenance of externalizing behavior problems. Lastly, these studies underscore the need for future research to explore the

developmental trajectories of children exposed to varying levels of early life stress, and to examine potential mechanisms for this risk process beyond the realm of stress or emotional reactivity.

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Table 1.

Intercorrelations for Study Variables

Variable	1	2	3	4	5	6	7	8	9	10
1. Cortisol	-	0.085	-0.027	-0.019	-0.129	-0.095	0.168	0.074	-0.056	-0.093
2. CB Anger		-	0.220*	0.133	0.073	0.186*	-0.092	-0.099	0.162	0.148
3. CB Sadness			-	-0.028	-0.110	-0.041	-0.036	0.031	-0.011	0.044
4. Preschool-Age Aggression				-	0.630**	0.698**	-0.149	-0.134	0.391**	0.042
5. School-Age Aggression					-	0.791**	-0.213*	-0.141	0.494**	0.003
6. Persistent Aggression						-	-0.301**	-0.228*	0.410**	0.021
7. LES 6-Month Subjective Rating							-	0.681**	-0.090	-0.067
8. LES Lifetime Subjective Rating								-	-0.132	-0.031
9. Callousness									-	0.010
10. Child Sex										-

** p<0.05* ***p<0.01*

Table 2.

Summary of Direct and Indirect Effects of Overall Mediation Analyses

School-Age Aggression				
	t (p)	*Coefficient (SE)*	*LLCI*	*ULCI*
6-Month ELS	-2.07 (**0.04**)	-0.0361 (0.0174)	-0.0706	-0.0016
Cortisol		-0.0035 (0.0039)	-0.0143	0.0016
CB Anger		-0.0017 (0.0035)	-0.0138	0.0019
CB Sadness		0.0010 (0.0026)	-0.0024	0.0090
Lifetime ELS	-1.27 (0.21)	-0.0129 (0.0102)	-0.0331	0.0073
Cortisol		-0.0011 (0.0016)	-0.0068	0.0006
CB Anger		-0.0011 (0.0019)	-0.0080	0.0008
CB Sadness		-0.0005 (0.0016)	-0.0059	0.0013
Persistent Aggression				
	Z (p)	*Coefficient (SE)*	*LLCI*	*ULCI*
6-Month ELS	-2.82 (**0.005**)	-0.1572 (0.0557)	-0.2663	-0.0480
Cortisol		-0.0058 (0.0100)	-0.0324	0.0089
CB Anger		-0.0087 (0.0121)	-0.0411	0.0098
CB Sadness		0.0016 (0.0064)	-0.0046	0.0245
Lifetime ELS	-2.24 (**0.025**)	-0.0671 (0.0300)	-0.1258	-0.0083
Cortisol		-0.0025 (0.0040)	-0.0182	0.0028
CB Anger		-0.0053 (0.0061)	-0.0264	0.0020
CB Sadness		-0.0007 (0.0038)	-0.0140	0.0032

Note: bolded values indicate significant findings

Table 3.

Summary of Child Sex Moderation Analyses

6-Month ELS*Sex → Stress Reactivity				
	t(p)	*Coefficient (SE)*	*LLCI*	*ULCI*
Cortisol	1.2794 (0.2033)	0.0467 (0.0365)	-0.0256	0.1191
CB Anger	2.9174 (**0.0043**)	0.0832 (0.0285)	0.0267	0.1398
CB Sadness	-0.1727 (0.8632)	-0.0054 (0.0311)	-0.0670	0.0563
Lifetime ELS*Sex → Stress Reactivity				
Cortisol	0.5782 (0.5643)	0.0132 (0.0228)	-0.0320	0.0584
CB Anger	1.5907 (0.1144)	0.0286 (0.0180)	-0.0070	0.0642
CB Sadness	-0.7306 (0.4665)	-0.0139 (0.0191)	-0.0518	0.0239
Reactivity*Sex → School-Age Aggression				
Sex*Cortisol	2.1476 (**0.0339**)	0.3829 (0.1783)	0.0296	0.7362
Sex*CB Anger	-2.9183 (**0.0043**)	-0.7123 (0.2441)	-1.1960	-0.2286
Sex*CB Sadness	2.3809 (**0.0190**)	0.5256 (0.2208)	0.0881	0.9631
ELS→*Sex School-Age Aggression				
Sex*6-Month ELS	-1.3340 (0.1849)	-0.0517 (0.0387)	-0.1284	0.0251
Sex* Lifetime ELS	0.5537 (0.5809)	0.0131 (0.0236)	-0.0337	0.0599
Reactivity*Sex → Persistent Aggression				
	Z (p)	*Coefficient (SE)*	*LLCI*	*ULCI*
Sex*Cortisol	1.0564 (0.2908)	0.5608 (0.5308)	-0.4797	1.6012
Sex*CB Anger	-1.8812 (0.0599)	-1.3309 (0.7075)	-2.7176	0.0557
Sex*CB Sadness	2.0736 (**0.0381**)	1.7152 (0.8272)	0.0940	3.3365
ELS*Sex →Persistent Aggression				
Sex*6-Month ELS	0.6229 (0.5334)	0.0729 (0.1171)	-0.1566	0.3025
Sex*Lifetime ELS	1.0670 (0.2860)	0.0700 (0.0656)	-0.0586	0.1986

Note: bolded values indicate significant findings

Table 4.

Summary of Child Callousness Moderation Analyses

6-Month ELS*Callousness → Stress Reactivity				
	t(p)	*Coefficient (SE)*	*LLCI*	*ULCI*
Cortisol	0.3198 (0.7497)	0.0017 (0.0054)	-0.0089	0.0123
CB Anger	1.9306 (0.0560)	0.0082 (0.0042)	-0.0002	0.0165
CB Sadness	-0.0467 (0.9629)	-0.0002 (0.0045)	-0.0092	0.0087
Lifetime ELS*Callousness → Stress Reactivity				
Cortisol	0.3226 (0.7476)	0.0013 (0.0039)	-0.0064	0.0090
CB Anger	1.3523 (0.1789)	0.0041 (0.0031)	-0.0019	0.0102
CB Sadness	0.8445 (0.4001)	0.0027 (0.0032)	-0.0037	0.0091
Reactivity*Callousness → School-Age Aggression				
Callousness*Cortisol	0.8810 (0.3802)	0.0246 (0.0279)	-0.0307	0.0798
Callousness*CB Anger	-0.1565 (0.8759)	-0.0043 (0.0274)	-0.0586	0.0501
Callousness *CB Sadness	1.1556 (0.2504)	0.0421 (0.0364)	-0.0301	0.1142
ELS*Callousness →School-Age Aggression				
Callousness*6-Month ELS	-2.2074 (**0.0293**)	-0.0107 (0.0048)	-0.0202	-0.011
Callousness* Lifetime ELS	-0.9453 (0.3466)	-0.0033 (0.0035)	-0.0104	0.0037
Reactivity*Callousness → Persistent Aggression				
	Z (p)	*Coefficient (SE)*	*LLCI*	*ULCI*
Callousness *Cortisol	0.9902 (0.3221)	0.1068 (0.1078)	-0.1046	0.3181
Callousness *CB Anger	0.1920 (0.8477)	0.0286 (0.1492)	-0.2638	0.3211
Callousness *CB Sadness	0.6745 (0.5000)	0.1125 (0.1668)	-0.2144	0.4395
ELS*Callousness →Persistent Aggression				
Callousness*6-Month ELS	-0.8705 (0.3840)	-0.0219 (0.0252)	-0.0712	0.0274
Callousness*Lifetime ELS	0.2485 (0.8037)	0.0037 (0.0150)	-0.0258	0.0332

Note: bolded values indicate significant findings

Table 5.

Sample Characteristics

	Final Sample (N=20)	Excluded Participants (N=13)
Income: median(SD)	110,000 (53,760.78)	145,000 (68,920.85)
Child Sex		
Male (N)	12	9
Female (N)	8	4
Child age in years: mean (SD)	10.05 (1.19)	9.92 (1.31)
Child Ethnicity		
Caucasian %	55%	61.5%
African American %	20%	15.4%
Asian %	5%	-
Other %	15%	23.1%
Child IQ: mean (SD)	119 (15)	116 (14)
Aggression Ratings		
Proactive: mean (SD)	1.4 (1.8)	1.2 (1.6)
Reactive: mean (SD)	8.1 (3.2)	7.7 (2.5)
Subjective Early Life Stress		
6-month	0.85 (3.91)	-0.31 (0.95)
Lifetime	1.60 (5.10)	-5.24 (6.91)
Mother's BDI score: mean (SD)	4.90 (5.37)	12.83(9.69)
ROI Values: mean (SD)		
Con 1: Bilateral Amygdala	-0.383 (1.58)	-
Con 1: Left Amygdala	-0.022 (1.87)	-
Con 1: Right Amygdala	-0.702 (1.59)	-
Con 2: Bilateral Amygdala	0.241 (1.19)	-
Con 2: Left Amygdala	0.357 (1.51)	-
Con 2: Right Amygdala	0.139 (1.24)	-

Note: Means for income, age, maternal depression and aggression for excluded participants are based on cases of available follow-up data (N=12).

Table 6

Intercorrelations for Study Variables

	1	2	3	4	5
1. Proactive Aggression		0.600**	-0.057	0.074	0.272
2. Reactive Aggression		-	-0.070	0.074	-0.092
3. 6-Month Subjective ELS			-	0.116	0.353
4. Lifetime Subjective ELS				-	-0.016
5. Child Sex					-

***p<0.01*

Table 7.

Correlations between ELS and Neural Activity

	Contrast 1 (bilateral amygdala)	Contrast 1 (left amygdala)	Contrast 1 (right amygdala)	Contrast 2 (bilateral amygdala)	Contrast 2 (left amygdala)	Contrast 2 (right amygdala)
6-Month ELS	-0.19	-0.23	-0.12	-0.33	-0.27	-0.29
	(0.41)	(0.33)	(0.61)	(0.16)	(0.25)	(0.20)
Lifetime ELS	0.04	0.07	-0.01	0.12	0.21	-0.001
	(0.88)	(0.75)	(0.96)	(0.60)	(0.37)	(0.99)
			Females			
6-Month ELS	-0.24	-0.20	-0.22	-0.65	-0.62	-0.59
	(0.57)	(0.64)	(0.59)	(0.07)	(0.10)	(0.12)
Lifetime ELS	0.04	0.07	0.005	-0.17	-0.51	0.09
	(0.91)	(0.86)	(0.99)	(0.68)	(0.19)	(0.82)
			Males			
6-Month ELS	-0.35	-0.36	-0.30	-0.06	-0.08	-0.01
	(0.26)	(0.24)	(0.34)	(0.84)	(0.79)	(0.96)
Lifetime ELS	0.03	0.08	-0.01	0.21	0.36	-0.03
	(0.91)	(0.80)	(0.96)	(0.50)	(0.24)	(0.91)

Note: Values listed include correlation coefficient, r, and p-value in parentheses. The first contrast measures whether children had greater amygdala reactivity in response to happy versus neutral faces. The second contrast measures the child's response to emotional stimuli in the presence of an incongruous cognitive task demand (e.g., No-go, Happy).

Table 8.

Correlations between Neural Activity and Aggression

	Contrast 1 (bilateral amygdala)	Contrast 1 (left amygdala)	Contrast 1 (right amygdala)	Contrast 2 (bilateral amygdala)	Contrast 2 (left amygdala)	Contrast 2 (right amygdala)
Proactive Aggression	-0.07 (0.76)	-0.25 (0.29)	0.13 (0.59)	-0.24 (0.31)	-0.01 (0.94)	-0.42 (0.06)
Reactive Aggression	-0.10 (0.67)	-0.14 (0.56)	-0.04 (0.85)	-0.23 (0.32)	-0.09 (0.70)	-0.33 (0.16)
			Females			
Proactive Aggression	-0.11 (0.80)	-0.39 (0.38)	0.25 (0.58)	-0.50 (0.25)	-0.20 (0.66)	-0.65 (0.11)
Reactive Aggression	-0.15 (0.73)	-0.39 (0.37)	0.17 (0.70)	-0.59 (0.16)	-0.48 (0.27)	-0.58 (0.16)
			Males			
Proactive Aggression	-0.08 (0.80)	-0.02 (0.94)	-0.13 (0.69)	-0.09 (0.78)	0.14 (0.67)	-0.35 (0.28)
Reactive Aggression	-0.17 (0.61)	-0.19 (0.57)	-0.13 (0.69)	-0.14 (0.67)	-0.01 (0.96)	-0.26 (0.43)

Note: Values listed include correlation coefficient, r, and p-value in parentheses. Data presented are partial correlations controlling for household income. The first contrast measures whether children had greater amygdala reactivity in response to happy versus neutral faces. The second contrast measures the child's response to emotional stimuli in the presence of an incongruous cognitive task demand (e.g., No-go, Happy).

Figure 1.

General Conceptual Moderated Mediation Model

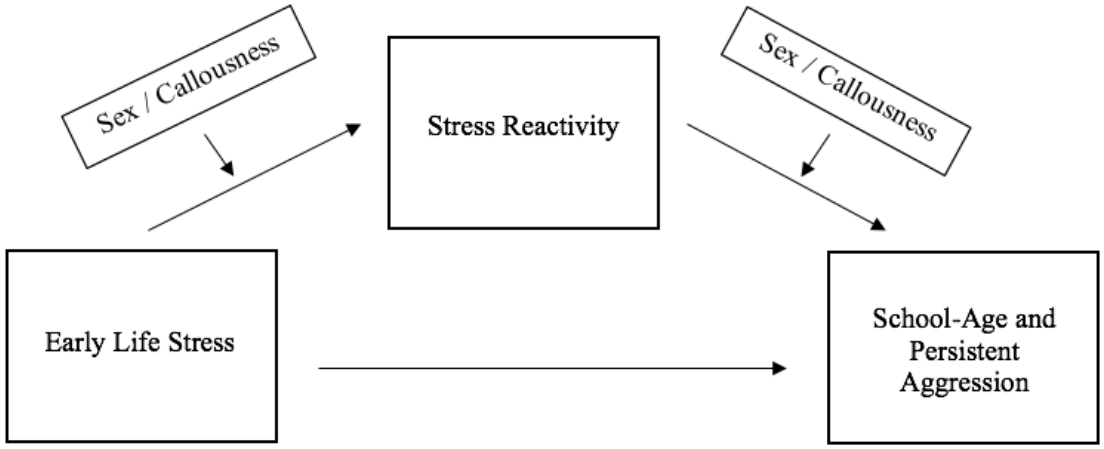

Figure 2.

Sample neutral face stimuli with instructions from Go/NoGo task.