# The Effects of Child Maltreatment, Genetic Factors, and HPA Axis Functioning on Internalizing Symptoms in African American Children: A Moderated Mediation Model

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## Dedication

*For my mom, Robyn.*

## Abstract

Child maltreatment is a potent relational pathogen that alters functioning across diverse developmental domains, and has been shown to increase risk for a host of mental health problems, including internalizing disorders. Similarities in the neuroendocrine profiles of individuals who develop internalizing symptoms and individuals who have been maltreated are striking, and suggest a role of neuroendocrine functioning, specifically the hypothalamic-pituitary-adrenal (HPA) axis, in the pathogenesis of internalizing disorders following child maltreatment. Risk and protective genetic factors, particularly relevant to HPA axis functioning, have been discovered, further highlighting involvement of the HPA axis and offering ideas about how some maltreated children may evade the biological impact of maltreatment. There has been movement in the field toward identifying mediators and moderators at multiple levels of analysis to best inform developmental mechanisms, which may ultimately aid in the treatment and prevention of deleterious outcomes following child maltreatment. Utilizing a large, ethnically homogenous sample, the current study employed exploratory and confirmatory factor analysis and structural equation modeling to examine associations among child maltreatment, risk across multiple HPA-related genes, daytime cortisol patterns, and internalizing symptoms in effort to clarify biological mechanisms. Results revealed that experiences of maltreatment prior to age 5 were most predictive of internalizing symptoms in African American youth, whereas maltreatment occurring at or after age 5 was most predictive of HPA axis dysregulation in the form of blunted diurnal decrease of cortisol. Genetic factors did not alter the relationship between maltreatment and cortisol,

nor were genetic risk patterns reflected in HPA functioning. There was no mediation of the relationship between maltreatment and internalizing symptoms by HPA dysfunction. Results are interpreted through a developmental psychopathology lens, emphasizing the principle of equifinality, whereby children follow multiple pathways toward internalizing symptoms. Implications for future research, particularly the need for longitudinal studies in this area, are discussed.

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# Introduction

## Overview

Child maltreatment represents an extreme failure of the evolutionary expected caregiving environment to provide basic emotional, physical, and/or psychological needs essential for optimal development (Cicchetti & Lynch, 1995). Maltreatment may involve acts of omission (failure to provide physical and/or emotional resources for the child) and/or commission (harmful acts inflicted upon the child), with subsequent implications for maturation (Cicchetti & Lynch, 1995). Extensive research with both humans and animals over the past four decades has shown that maltreatment is capable of inducing pervasive, deleterious effects across a number of developmental domains (Cicchetti & Lynch, 1995; Cicchetti & Toth, 2013; Cicchetti & Valentino, 2006). Disruptions in attachment, self-conceptualization, brain structure, brain function, neuroendocrine function, gene expression, emotion recognition, social cognition, information processing, executive functioning, self-regulation, social competence, and both peer and romantic relationships have all been linked to the experience of child maltreatment (see Cicchetti & Toth, 2015 for review). By way of developmental cascading effects, the impact of maltreatment on any one of these domains can transact with other levels of functioning and be carried forward across the lifespan to erode mental health (Masten & Cicchetti, 2010).

One mental health outcome frequently associated with child maltreatment is internalizing symptoms (Andersen & Teicher, 2008; Cicchetti & Rogosch, 2001b; Kim & Cicchetti, 2006; Manly, Kim, Rogosch, & Cicchetti, 2001; Widom, DuMont, & Czaja,

2007). Internalizing symptoms may include excessive guilt, sadness, isolation/social withdrawal, negative self-image, feelings of worthlessness/hopelessness, cognitive deficits (poor concentration and memory), irritability, anhedonia, avolition, fatigue, somatic complaints, autonomic arousal (e.g., rapid heart beat) changes in appetite, excessive worry, avoidance, rumination, or sleep disruption – all of which are captured by anxiety and depressive disorders as constructed in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5; American Psychiatric Association, 2013). Children maltreated during or prior to the preschool years seem to be most vulnerable to developing these symptoms (Cicchetti, Rogosch, Gunnar, & Toth, 2010; Hart, Gunnar, & Cicchetti,1995; Kaplow & Widom, 2007; Keiley, Howe, Dodge, Bates, & Petit, 2001; Manly et al., 2001).

Internalizing problems are of great concern given the personal and public health burden they present. Internalizing disorders are one of the leading contributors to disability worldwide (Murray & Lopez, 2002), and are associated with substantial impairment in several areas of functioning, including academic achievement, interpersonal relationships, employment, and financial stability (Rice & Miller, 1995; Wulsin & Singal, 2003). Even more, internalizing disorders such as major depressive disorder have been associated with higher prevalence of death by suicide, and have also been related to several chronic physical illnesses (e.g., arthritis, cardiovascular disease, diabetes), further contributing to increased mortality (Kessler, 2012). The development of internalizing problems earlier in life carries greater risk for experiencing chronic, recurrent psychopathology (Fombonne, Wostear, Cooper, Harrington, & Rutter, 2001),

disability (González et al., 2010; Mathers & Lancar, 2011), and poor physical health (Weissman et al., 1999) throughout the lifespan. Additionally, earlier-onset internalizing disorders are more likely to be accompanied by self-injury and behavior problems (Gomez, Vance, & Gomez, 2014; Jonsson et al., 2011; Maughan, Collishaw, & Stringaris, 2013). As such, it is important to understand the ways that maltreatment increases risk for internalizing problems in children as this information may help identify effective intervention targets and alleviate the tremendous impact on personal and public health.

Maltreatment may enhance risk for internalizing problems through its effects on the hypothalamic-pituitary-adrenal (HPA) axis. The following sections of this introduction are organized in a way that will orient the reader to basic HPA functioning and regulation, and the variety of ways that maltreatment and internalizing symptoms interact with the HPA axis. First, background information on basic HPA functioning and physiology will be provided. Second, an overview of maltreatment and HPA functioning will be presented. Third, the ways by which disrupted neuroendocrine function may contribute to internalizing symptoms, and the neuroendocrine profiles of individuals with internalizing symptoms will be discussed. Fourth, the mechanistic role of HPA function and the specificity of maltreatment will be examined. Fifth, the contribution of moderating genetic factors will be considered. Finally, rationale, aims, and hypotheses of the current study will be specified.

**Basic HPA Functioning and Physiology**

The HPA axis exists as one important component of a complex stress system network whose functioning allows organisms to meet environmental demands. Neural and hormonal signaling are responsible for initiating and inhibiting HPA activity. Generally, the HPA axis governs two relatively distinct processes: 1) mounting responses to and promoting recovery from acute stressors, and 2) maintaining a diurnal circadian cycle of hormone production to ensure appropriate arousal and restoration at different times of the day (Cone, Low, Elmquist, & Cameron, 2002). In these ways, HPA activity is critical to providing organisms with required resources to manage and respond to internal and external signals, and to maintain physiological stability.

HPA axis activity is initiated once a stressor is detected. Stressors may exist systemically (i.e., physical stress such as decreased blood pressure, low blood glucose, extreme temperatures) or psychologically (i.e., psychological threat as interpreted by the organism, recognized and processed by the corticolimbic system; Gunnar & Vasquez, 2006). Thus, maltreatment may activate the HPA axis through pathways from internal organs that carry information about physical states, and through pathways that relay information regarding expectations and interpretations of threat. With regard to response to acute psychological stressors, the first step in the stress response is threat detection by various limbic brain regions (e.g., amygdala, hippocampus) which send activating signals to the paraventricular nucleus (PVN) of the hypothalamus. With systemic stressors, signals reach the PVN traveling across brainstem pathways. Signals from psychological and systemic pathways converge on the PVN, and, when strong enough, result in

secretion of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus (PVN) of the hypothalamus. A hormonal cascade follows, whereby CRH and AVP subsequently travel to the anterior pituitary to bind receptors on corticotrophic cells, causing cleavage of the proopiomelanocortin (POMC) molecule and, consequently, the release of adrenocorticotropic hormone (ACTH) into circulation. Finally, ACTH reaches the adrenal gland, binds receptors in the adrenal cortex, and triggers the release of glucocorticoids (cortisol in humans; corticosterone in rodents).

Once released, cortisol acts on the body and brain to initiate a host of responses that allow for behavioral and physiological adaptations to stress, including: increasing the bioavailability of glucose, redistributing energy to vital targets (i.e., toward brain and muscles and away from non-essential biological processes such as digestion, physical growth, and reproduction), reducing inflammation, enhancing attention to the immediate environment, and facilitating defense-related learning and memory processes (reviewed in Gunnar, Doom, & Esposito, 2015). These effects of cortisol are essential for survival as they allow organisms to adaptively attend to the environment and have the energy to respond to and learn from threat exposure. Moreover, via the circadian diurnal cycle, controlled secretion of cortisol throughout the day allows organisms to have resources necessary to manage daily functions essential to health, such as metabolism, brain development, immune function, and cellular repair (Gunnar et al., 2015). Through the diurnal cycle, HPA activity also synchronizes other biological systems including hepatic,

circulatory, and respiratory systems, thus allowing for efficient functioning at a physiological level (Chung, Son, & Kim, 2011).

Cortisol achieves its effects primarily through binding its receptors– the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) – located in the cell's cytoplasm and distributed throughout various areas of the brain and body (reviewed in Gunnar & Vasquez, 2006). Cortisol preferentially binds MRs (Wyrwoll, Homes, & Seckl, 2011), and will bind GRs when MRs are occupied or otherwise unavailable. Given that most MRs are occupied when basal cortisol levels are exceeded, GR activity is highest when cortisol levels surpass baseline, such as early in the day when the circadian cycle is at its peak and/or when an acute stressor is introduced. In this way, cortisol's receptors work in concert to maintain basal levels and circadian production of cortisol throughout the day, and to manage responses to stress and regulation of the system back to baseline following stress exposure (see Gunnar & Vasquez, 2006).

As can be seen, cortisol and other stress hormone production is necessary and critical for health. However, cortisol and its secretagogues can be extremely detrimental when uncontrolled, both when production is too high and too low. For example, high levels of cortisol and CRH can damage nerve cells (Ábrahám, Harkany, Horvath, & Luiten, 2001) and alter neural development in important regions of the brain such as the amygdala, hippocampus, and prefrontal cortex (PFC; Heim, Owens, Plotsky, & Nemeroff, 1997). Alternatively, when cortisol levels are too low, tissue responsivity to neurotransmitters is disrupted, and glucose is not adequately delivered to the brain (see Gunnar, et al., 2015). Further, both excessive and inadequate levels of cortisol hinders

expression of genes that promote neurogenesis and neuroplasticity, impeding cellular growth and development (see Gunnar & Vasquez, 2006). Thus, regulatory processes are paramount.

Regulatory mechanisms to control HPA activity exist in many forms. One important mechanism includes negative feedback, whereby cortisol inhibits its own production by shutting down the release of its secretagogues. Negative feedback is accomplished through cortisol binding to GRs in the PVN, anterior pituitary, hippocampus, and medial PFC (reviewed by Gunnar & Vasquez, 2006). Therefore, once cortisol is produced, its presence signals inhibition of further production through receptor activity at various levels of the brain, demonstrating the importance of receptors to proper HPA functioning and regulation.

**Maltreatment and HPA Functioning**

The HPA axis is incredibly sensitive to social experiences (Dickerson & Kemeny, 2004; Flinn & England, 1995). Social relationships both shape and serve to regulate HPA activity. Relationships with caregivers have been shown to influence HPA functioning such that children do not respond as highly to stressors when in the presence of supportive caregivers (Gunnar, Brodersen, Nachmias, Buss & Rigatuso, 1996; Hostinar, Johnson, & Gunnar, 2015; Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996; Spangler & Schieche, 1998). Thus, the caregiving relationship buffers children from stress and minimizes exposure to the potentially damaging effects of cortisol. Children with poor relationships with caregivers, however, are not buffered, even from relatively minor stressors (Gunnar & Donzella, 2002).

Understanding the capacity of caregivers to regulate HPA activity is relevant to understanding how and why child maltreatment may drastically shift HPA functioning. That is, maltreated children are not only unprotected from activation of the HPA axis by mild stressors, they are also likely to experience frequent and/or prolonged activation of the HPA axis due to their caregivers' frightening and/or neglectful behavior. In this way, a maltreating caregiver's behavior may program a child's system to be stress-responsive, sending a message that the environment is not safe and will require an arsenal of HPA hormones for immediate survival. Evolutionarily, this type of programming is adaptive in the short-term, as it allows the child to meet the demands of an uncertain environment, and to build a behavioral repertoire that may allow him or her to quickly manage threat and evade harm. Over time, however, this sensitization of the system can be problematic given the negative consequences of exposure to dysregulated levels of stress hormones as discussed above.

Preclinical research has consistently shown that maltreatment induces a stress-reactive system. In rats and nonhuman primates, maltreatment promotes sustained release of CRH as well as increased ACTH and corticosterone output following stress exposure (Dent, Smith, & Levine, 2000; Gunnar & Vasquez, 2006; Pihoker, Owens, Kuhn, Schanberg, & Nemeroff, 1993). Increased circulating levels of CRH as well as increased CRH mRNA expression under resting conditions have similarly been found (Heim & Nemeroff, 1999). Moreover, reduced GR mRNA expression in the dorsal hippocampus and higher morning levels of corticosterone have been consistently demonstrated (Avishai-Eliner, Yi, Newth, & Baram, 1995; Francis, Diorio, Liu, & Meaney, 1999; Ivy,

Brunson, Sandman, & Baram, 2008; Liu et al., 1997; Plotsky & Meaney, 1993). With an abundance of CRH, ACTH, and corticosterone, as well as an attenuation of the inhibitory capacity of the GR, maltreated animal's stress systems are designed to facilitate adaptation to high environmental demand.

The idea that maltreatment sensitizes the HPA axis specifically toward a stress-reactive state is not as clear from the child literature, in part because reactivity by way of psychosocial and pharmacologic challenge is not as easily tested in maltreated children, but also because a variety of confounding variables and other methodological constraints are introduced into these investigations. For example, measuring mRNA expression in brain tissue of children is not feasible. Similarly, samples of cerebrospinal fluid (where CRH can be measured) are not readily available for examination, precluding the ability to examine more central aspects of the HPA axis. Uncertainty about the true nature and timing of maltreatment, secondary to variable assessment procedures, introduces further limitations and sources of variability. Finally, many of the studies are cross-sectional and provide only a snapshot of HPA functioning during one specific point in time of a maltreated child's life.

That being said, HPA hyper-activity has been documented in some studies of maltreated children. For example, maltreated children have exhibited hyper-activity by way of increased reactivity to a separation paradigm and higher baseline cortisol levels (Bugental, Martorell, & Barraza, 2003). In addition to hyper-activity, other forms of disruption, including hypo-activity, have been found. Different groups of older children age 10-12 and 12-16 exhibited blunted responses to stressors (Fisher, Kim, Bruce, &

Pears, 2012; MacMillan et al., 2009; Peckins, Dockray, Eckenrode, Heaton, & Susman, 2012). Other groups of older children (age 7-15) showed decreased ACTH secretion to a CRH challenge (De Bellis et al., 1994). Atypical diurnal rhythms (Cicchetti et al., 2010; Gunnar, Morison, Chisholm, & Schuder, 2001; Hart, Gunnar, & Cicchetti, 1995) characterized by lower morning cortisol (Bruce, Fisher, Pears, & Levine, 2009), higher evening cortisol (Hart, Gunnar, & Cicchetti, 1996; Kaplan, Pelcovitz, & Labruna, 1999; Kaufman et al., 1997), and minimal change in cortisol levels over the course of the day have also been documented.

The adult literature predominantly points toward hypo-responsiveness and increased negative feedback of the HPA axis following child maltreatment, though there is also evidence of hyper-activity, especially for adults with internalizing symptoms, as discussed below. A critical meta-analysis of primarily cross-sectional studies revealed lower basal evening levels, stronger suppression of cortisol following administration of an artificial version (dexamethasone) of the hormone, and blunted responses to psychosocial stressors among adults who have experienced maltreatment (Miller, Chen, & Zhou, 2007). These findings suggest a compensatory down-regulation of the stress system, presumably as a result of chronic over-drive and hyper-activity earlier in life. This “attenuation hypothesis” is supported by the only published longitudinal neuroendocrine study spanning early childhood to young adulthood, where basal cortisol levels of abused girls were initially significantly higher than non-abused girls in childhood, but began decreasing in adolescence, and were substantially lower than non-abused females during young adulthood (Trickett, Noll, Susman, Shank, & Putnam,

2010). As can be seen, maltreated individuals may initially demonstrate HPA hyper-activity but may later demonstrate hypo-activity, suggesting that developmental timing is an important variable to consider when making sense of previous findings in the child literature.

In sum, individuals who have been maltreated exhibit a variety of alterations to the organization of their stress response system and patterns of HPA axis activity. Some individuals appear to have higher levels of stress hormones when they theoretically should have lower levels, while others have lower levels than what is optimally required. These observations can differ as a function of methodological approaches, age group sampled, timing of maltreatment experiences, and a host of other factors (e.g., Banny, Cicchetti, Rogosch, & Oshri, 2013; Cicchetti et al., 2010; Davies, Sturge-Apple, Cicchetti, & Cummings, 2007; DeBellis & Zisk, 2014; Doom, Cicchetti, Rogosch, & Dackis, 2013; McCrory, De Brito, & Viding, 2010). Though a unitary neuroendocrine pattern is not uniformally observed, the important message is that deviations in hormone levels, whether too high or too low, reflect general disruption, and that both hyper- and hypo-activity can have negative consequences on health, with implications for internalizing symptoms (e.g., Tarullo & Gunnar, 2006).

**Relevance of HPA Dysfunction to Internalizing Symptoms**

HPA dysfunction in the form of altered hormone production has direct links to internalizing symptoms. For example, administration of CRH to rodents has been shown to have both immediate and delayed effects on behavior that strongly parallel internalizing symptoms, including increased physiological arousal (increased heart rate),

decreased food intake and feeding behavior, disruption of slow-wave sleep, restlessness (i.e., increased locomotion in familiar environments), suppressed exploratory behavior in novel environments, and increased distress vocalizations (reviewed by Heim & Nemeroff, 1999). Additionally, the structural brain changes that accompany exposure to elevated or suppressed stress hormones such as dendritic atrophy of the PFC, increased dendritic branching and activity of the amygdala, and reduced hippocampal volume/decreased branching of hippocampal pyramidal neurons (Makino, Gold, & Schulkin, 1994; Sullivan & Gratton, 2002; Ulrich-Lai & Herman, 2009) can shape cognition and emotion in ways consistent with internalizing disorders. Such cognitive and emotional patterns include hypervigilance, exaggerated startle, concentration difficulties, and altered processing of emotional information (e.g., Lupien, Gillin, Frakes, Soefje, & Hauger, 1995).

Furthermore, decreased tissue responsiveness to neurotransmitters following chronically low levels of cortisol can impair functioning of relevant serotonergic and dopaminergic systems, possibly resulting in changes to reward and motivation (Goff & Tottenham, 2014; Gunnar et al., 2015). Lastly, the effects of cortisol on memory formation and consolidation can promote salience and recall of fear-related, emotionally-arousing memories (e.g., Bryant, 2003; Cicchetti et al., 2010; Howe, Cicchetti, & Toth, 2006, Roozendaal, 2000) at the expense of recalling neutral and positive information (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Tops et al., 2003). Over time, such a pattern can skew perceptions of reality, and initiate and/or perpetuate negative moods.

**Internalizing Symptoms and HPA Dysfunction**

Similar to maltreated individuals, those with internalizing symptoms exhibit HPA dysfunction. In humans, hyperactivity of the axis represented by elevated morning cortisol, flattened circadian patterns, and increased urinary free cortisol over the day have all been associated with internalizing symptoms (reviewed by Nestler et al., 2002). Hyperactivity is also evident in increased reactivity to stress among animals; rodents who exhibit increased glucocorticoid (corticosterone) response to restraint stress also exhibit features indicative of animal models of depression (see Gunnar & Quevedo, 2007 for a review). Additionally, longer and larger cortisol responses to acute stress in humans, as well as reduced ability for dexamethasone to suppress plasma cortisol levels are forms of hyperactivity observed in individuals with internalizing symptoms (e.g., Lopez-Duran, Kovacs, & George, 2009; Zobel et al., 2001). Failure to suppress to dexamethasone along with longer, larger responses to stress suggests that negative feedback is compromised in individuals with internalizing problems, likely leading to the HPA hyperactivity often observed in this population (Pariante & Lightman, 2008).

In addition to elevated HPA activity, blunted HPA activity has also been associated with internalizing symptoms. For example, low basal cortisol levels were concurrently associated with emotional symptoms in 5th graders (Shirtcliff & Essex, 2008). Additionally, children exhibiting an attenuated response to a psychosocial stressor also had increased emotional/behavioral problems (Ouellet-Morin et al., 2011). In a study of younger children, those at risk for depression demonstrated hyporesponsivity to a psychological stressor (Hankin, Badanes, Abela, & Watamura, 2010), though these

children were later hyperresponsive to the same psychological stressor when assessed in adolescence (Hankin et al., 2010).

**Mechanistic Role of HPA Dysfunction**

The fact that HPA disruption has been found to precede internalizing symptoms when assessed longitudinally points toward a mechanistic role of HPA disruption in the pathogenesis of internalizing disorders. Individuals without depressive symptoms but at risk for developing depression given family history have been shown to exhibit higher and more variable morning cortisol over a 10 day period (e.g., Halligan, Herbert, Goodyer, & Murray, 2004). This pattern of cortisol predicted depression onset several years later (Halligan, Herber, Goodyer, & Murray, 2007). Additionally, a higher cortisol awakening response (CAR) in the absence of depressive symptoms predicted onset of major depressive episodes 2.5 years later in adolescents (Vrshek-Schallhorn et al., 2013). In another study of adolescents, higher morning and afternoon cortisol levels prospectively predicted an increase in depressive symptoms over time (Heim & Binder, 2012).

Reversal of both neuroendocrine dysfunction and anxious and depressed behavior by pharmacologic manipulation of stress system regulators also supports a mechanistic role of HPA dysfunction in internalizing symptoms. In rodents, both depressive behavior and HPA hyperactivity have been reversed by pharmacologic agents that increase GR function (Weaver et al., 2004). The opposite can also be achieved. That is, rats who were characteristically “relaxed” were made to exhibit anxious and depressive behavior by pharmacologically shutting down function of the GR and decreasing its expression in the

brain (Weaver et al., 2004). Administration of antidepressants in humans has been shown to improve negative feedback and increase function and expression of GR, which correlates with improvements in depressive symptoms (Pariante & Lightman, 2008). Hyporesponsivity has been shown to normalize upon clinical recovery as well (Amsterdam et al., 1988). Taken as a whole, these findings offer good reason to speculate that HPA disruption contributes to internalizing symptoms.

Despite a great deal of evidence of disrupted neuroendocrine function in individuals with internalizing symptoms, some do not exhibit HPA dysfunction at all. For example, Birmaher and colleagues (1996) found no association between depressive symptoms and HPA response to CRH challenge. Similarly, children with anxiety and/or depressive disorders did not display any evidence of dysregulation when their cortisol production was monitored over a 24h period (Feder et al., 2004). Additionally, waking cortisol, diurnal slope, and total cortisol output were unrelated to later onset of depression in adolescents (Adam et al., 2010). Such findings suggest that other factors may better account for the dysfunctional patterns of neuroendocrine function observed for some individuals with internalizing symptoms. Accumulating research has pointed toward maltreatment as being the missing link (e.g., Feder et al., 2004; Hart et al., 1996; Heim et al., 2000, 2001; Heim, Mletzko, Purselle, Musselman, & Nemeroff, 2008; Kaufman, 1991).

**Specificity of Maltreatment**

In a landmark study, when examining four different groups of women with varying degrees of overlap between childhood abuse and internalizing symptoms (i.e.,

women with both childhood abuse histories and depression; women with childhood abuse histories but without depression; women without abuse histories with depression; and women without abuse histories and without depression), Heim et al. (2000, 2001) found that only the women with abuse histories who also had depression exhibited increased production of cortisol to stress. These findings were replicated in later work by Heim and colleagues (2008) that showed depressed men with childhood abuse histories *and* current major depressive disorder diagnoses failed to suppress to the combined dexamethasone/CRH test (thus, cortisol levels were higher than they should be following this pharmacologic challenge), yet men with the same diagnosis *without* abuse histories did not evince this pattern of hyperactivity.

Similar conditional associations of maltreatment, HPA dysfunction, and internalizing symptoms have been documented for children as well. Cicchetti and colleagues (2010) found that maltreated children exhibited a blunted decrease in cortisol over the course of the day, but only in the context of concurrent internalizing symptoms. Neither nonmaltreated children with internalizing symptoms nor maltreated children without internalizing symptoms exhibited this neuroendocrine dysfunction. Similarly, other groups have shown that children who have experienced maltreatment demonstrate atypically low morning cortisol and high evening cortisol (also reflecting a diurnal flattening, as Cicchetti and colleagues (2010) found), only if they also have high levels of depressive symptoms (Hart et al., 1996; Kaplan, Pelcovitz, & Labruna, 1999; Kaufman et al., 1997).

These seminal findings, combined with evidence from preclinical research on maltreatment, have led researchers to speculate about the specificity of HPA dysfunction in the development of internalizing symptoms following maltreatment. Because dysfunction is evident primarily in individuals with internalizing symptoms and maltreatment histories, it appears that maltreatment uniquely predisposes for internalizing symptoms because of its specific impact on HPA functioning. As such, maltreatment may operate through the HPA axis to increase risk for internalizing symptoms. Additionally, there may be specific classes of internalizing disorders that exist; some of which are secondary to HPA dysfunction (i.e., those observed in maltreated individuals) with others that come about via alternative avenues (i.e., those observed in nonmaltreated individuals). Different classes may require different treatments.

**Moderating Factors**

Notably, there are children who develop neither HPA dysfunction nor internalizing symptoms. Why are some maltreated children protected against neuroendocrine dysfunction and/or internalizing symptoms while others are not? Although there have been many proposed reasons (see, for example, Banny et al., 2013; Cicchetti et al., 2010; Davies et al, 2007; DeBellis & Zisk, 2014; Doom et al., 2013; McCrory et al., 2010), genetic variation is particularly relevant to the current study. If a child possesses a version of a gene that alters the way they biologically respond to stress, the impact of maltreatment on HPA function may be favorably attenuated or unfortunately enhanced.

With this in mind, several researchers have investigated the relative contribution of stress-related genes to internalizing symptoms in the context of child maltreatment and have found significant associations (e.g., Binder et al., 2004; Bradley et al., 2008; Cicchetti & Rogosch, 2014; Cicchetti, Rogosch, & Oshri, 2011; Liu et al., 2006; Polanczyk et al., 2009). Among the genes, four are particularly common: FKBP5, CRHR1, NR3C1, and NR3C2 (Kuningas et al., 2007; Lavebratt, Åberg, Sjöholm, & Forsell, 2010; Lekman et al., 2008; Liu et al., 2006; van West et al., 2006) and will be briefly reviewed herein (see Method section for more detailed information on these genes and their involvement in HPA function).

A single nucleotide polymorphism (SNP; rs110402; see Table 2 for a glossary of genetic terms) in the *CRHR1* gene that was previously implicated in differential HPA response to stress paradigms altered risk for developing depression in maltreated adults (Bradley et al., 2008; Heim et al., 2009; Polanczyk et al., 2009). When part of a haplotype, this SNP also enhanced risk for internalizing symptoms in maltreated children and, further, interacted with other genes to amplify risk in maltreated carriers relative to maltreated non-carriers (Cicchetti & Rogosch, 2014; Cicchetti et al., 2011). Variation in *FKBP5*, a gene whose product has been shown to reduce the ability for cortisol to bind GR (Klengel et al., 2013), has also been linked to diverse associations among maltreatment and internalizing symptoms. One particular SNP (rs1360780) promoting increased FKBP5 function (thus, reduced GR activity) has consistently been found to increase risk for depressive symptoms exclusively in maltreated individuals, both children and adults, possessing this variant (Zannas & Binder, 2014).

Similarly, SNPs in genes coding for cortisol's receptors, GR and MR (*NR3C1* and *NR3C2* genes, respectively), differentially enhance risk for internalizing symptoms or endophenotypes of internalizing symptoms (i.e., amygdala reactivity) in maltreated individuals. In a group of adolescents, a functional SNP in the MR gene that was previously shown to increase reactivity of the HPA axis to stress, enhanced amygdala reactivity to threat among those reporting earlier childhood abuse (Bogdan, Williamson, & Harriri, 2011). With regard to the GR gene, few gene-environment interaction studies examining maltreatment, specifically, have been conducted, but several focusing more broadly on environmental adversity have shown associations. One study examining a SNP (*BclI*) associated with reduced cortisol production found increased emotional/behavioral problems for carriers of the minor allele who also experienced childhood adversity (Velders et al., 2012). Other indicators (i.e., DNA methylation) of functional change to the GR resulting in reduced activity have been examined with regard to internalizing symptoms in maltreated children. One study found that increased methylation of the GR gene partially mediated the effect of early adversity (including maltreatment) on internalizing problems (Parade et al., 2016). Taken together, it appears that factors operating at the genetic level can increase or decrease the likelihood that a maltreated individual will exhibit internalizing symptoms or its correlates, presumably given the alterations in HPA axis functioning that these variants confer.

## The Current Study

### *Study rationale*

Despite much speculation that HPA axis dysfunction may operate as a mechanism by which child maltreatment uniquely contributes to internalizing symptoms, and despite the belief that the relative impact of maltreatment on internalizing symptoms is dependent on genetic factors associated with differential activity of the HPA axis, no previous studies have examined these variables together in one model. Rather, a piecemeal approach has typically been executed. That is, some studies have examined maltreatment, HPA-related genes, and HPA function, but have only speculated about the implications for internalizing symptoms, framing HPA dysfunction as an endophenotype of internalizing symptoms (e.g., Heim et al., 2009; Tyrka et al., 2009). Conversely, other studies have examined maltreatment, HPA-related genes, and internalizing symptoms, but have only speculated about HPA functioning, using the candidate genes and their previously-established associations with HPA functioning as proxies for actual HPA function in the sample (Bradley et al., 2008; Grabe et al,. 2010; Zimmermann et al., 2011). Finally, other studies have examined maltreatment, HPA function, and internalizing symptoms, but have not considered genetic contributions (e.g., Cicchetti et al., 2010; Ouellet-Morin et al, 2011). One study that attempted to examine all variables together (Cicchetti et al., 2011) was ultimately unable to given cell size constraints as a function of sample characteristics. Thus, building a moderated mediation model will help clarify the nature of these relationships in ways that previous studies could not.

*Focus on Early Maltreatment*

In humans, fear-related neural systems are undergoing drastic construction especially during the first years of life (Thompson & Nelson, 2001); therefore,

maltreatment occurring during this formative period of development can greatly affect the way neural systems are structured. Indeed, maltreatment (like cortisol) is associated with changes in size and dendritic branching of the amygdala, hippocampus, and PFC (De Bellis et al., 1999, 2002; Heim et al., 2008; Petchtel, Lyons-Ruth, Anderson, & Teicher, 2014; Ulrich-Lai & Herman, 2009), and there is evidence that the amygdala is particularly sensitive to early experiences of adversity (Pechtel & Pizzagalli, 2011). Animal models have provided direct and causal evidence that timing is critical by demonstrating that the structural and functional brain changes that follow maltreatment during the first week of life persist and manifest as increased physiological and behavioral responses to stress throughout development (see Kaffman and Meaney, 2007; Lupien et al., 2009; Meaney et al., 1996). Studies in children have also highlighted the importance of timing, revealing that only children maltreated early in life exhibit HPA dysfunction and internalizing symptoms (Cicchetti et al., 2010). Thus, the theoretical relevance of timing to limbic brain development and neuroendocrine function as well as support from previous studies with children provides justification for the focus on early timing of maltreatment.

Although other aspects of maltreatment, most prominently the type of maltreatment experienced, have emerged as important predictors of the effect of maltreatment on internalizing symptoms and HPA function (e.g., Bradley et al., 2008; Cicchetti et al., 2010; Hart et al., 1996; Heim et al., 2009), it has recently been suggested that all forms of maltreatment are relatively equally damaging. That is, in a sample of over 2,200 maltreated children, different types of maltreatment were shown to have

similar effects on the development of internalizing symptoms (Vachon, Krueger, Rogosch, & Cicchetti, 2015). Thus, in effort to be most parsimonious, subtype as well as additional features of maltreatment were not of primary focus in the current investigation.

*Internalizing Symptoms vs. Specific Disorder*

Psychopathology has been broadly characterized by two main factors: 1) internalizing symptoms, and 2) externalizing symptoms (Krueger, 1999). An outcome variable combining various measures to broadly capture internalizing symptoms was thus considered more appropriate than constraining to one specific disorder cluster. Further, children in the current study were primarily between the ages of 8-10. Measures geared toward a specific disorder, such as depression, would likely be an inadequate representation of internalizing problems given that depressive disorders, singularly, are less likely to fully emerge before adolescence (Kessler et al., 2005). As such, internalizing symptoms, rather than a particular disorder, were of focus in the current study.

*Diurnal Change*

Although there are several ways to examine HPA function such as reactivity to and recovery from a psychosocial and/or pharmacologic stressor, examining reactivity was not possible given available archival data and the questionable ethics of inducing stress in maltreated children, particularly those currently experiencing maltreatment. Moreover, although hyperactivity has been frequently associated with maltreatment and internalizing symptoms, diurnal blunting has been documented more often in studies of children (e.g., Dozier et al., 2006; Fisher, Gunnar, Dozier, Bruce, & Pears, 2006).

*Multigenic Risk Score*

As noted above, multiple genes involved in HPA functioning have been found to moderate the relationship between maltreatment and internalizing symptoms. Capturing risk across a variety of genes relevant to the system will better represent the HPA axis in its entirety, rather than focusing on one "string" in the symphony orchestra. Thus, in effort to examine the HPA system as a whole, a multigenic risk index was utilized. Rationale for considering the particular genes and SNPs used in the mutligenic risk score is presented in the Method section.

*Externalizing Symptoms as a Covariate*

The focus of the current study is understanding biological mechanisms underlying the development of internalizing symptoms, specifically. However, there is often a great deal of overlap between internalizing and externalizing symptoms (Wiggins, Mitchell, Hyde, & Monk, 2015). Additionally, different patterns of diurnal activity have been found for internalizing and externalizing symptoms (Bernard, Zwerling, & Dozier, 2015). Thus, failure to control for externalizing symptoms could cloud results of the current study. Understanding the nature of HPA axis disruption that is more specific to internalizing symptoms rather than comorbid internalizing/externalizing or externalizing, exclusively, could guide treatment efforts for different populations. For these reasons, the decision to focus on internalizing symptoms while controlling for externalizing symptoms was made.

### Study Aims

1. Clarify the nature of the relationship among maltreatment, genetic factors, HPA axis functioning, and internalizing symptoms to better understand potential biological mechanisms involved in the development of internalizing symptoms in maltreated children.

2. Evaluate how genetic risk, as represented by a risk score, interacts with maltreatment to impact cortisol production across the day.

### Hypotheses

1. Children maltreated before age 5 will have higher levels of internalizing symptoms than later maltreated and nonmaltreated children.

2. Children maltreated before age 5 will have less change (i.e., blunted diurnal pattern) in cortisol from morning to evening compared to later and nonmaltreated groups.

3. The relationship between early maltreatment and internalizing symptoms will be mediated by cortisol function, such that the indirect effects of early maltreatment on cortisol and cortisol on internalizing symptoms will best explain the relationship between early maltreatment and internalizing symptoms.

4. Genetic risk will moderate the impact of maltreatment on cortisol functioning such that early maltreated children with the highest genetic risk scores will have the least amount of change in cortisol across the day.

## Method

### Participants

The current study combines participants who attended research camps between the years 2004-2012. During their time at camp, children were involved in a variety of research projects, all of which were focused on examining the impact of childhood maltreatment and other risk factors on psychopathology. Maltreated (n = 373; 47% female) and nonmaltreated (n = 366; 53% female) children with available genetic, neuroendocrine, and internalizing symptom data were culled into one racially/ethnically

homogenous sample (N = 739). African American children were of exclusive focus for this study given 1) the importance of using a racially homogeneous sample when conducting genetic association studies to avoid population stratification issues, 2) the lack of comprehensive information on the development of internalizing symptoms in maltreated African American children, and 3) the relatively larger proportion of African American children attending the summer camps.

Children ranged in age from 8 to 12 years (*M* age = 10.37, *SD* = 1.31); however, most (69%) of the children were between the ages of 8-10 years. All children were from predominantly low-income, disadvantaged families (90% received Temporary Assistance for Needy Families; TANF). Given study hypotheses and previous findings of greater impact of early maltreatment on HPA axis functioning (Cicchetti et al., 2010, 2010), maltreatment groups were subdivided into those maltreated early in life (before age 5; n = 263), those maltreated later in life (age 5 and beyond; n = 110), and nonmaltreated children (n = 366). Maltreatment groups differed by gender and age; thus, both variables were added to the structural model in order to control for potential confounding effects. Table 1 lists demographic characteristics of each maltreatment group.

## Procedure

### *Family Recruitment*

All study procedures were approved by the University of Rochester Institutional Review Board. Informed consent was obtained from parents of all participants. Child assent was also acquired prior to a child's participation. Maltreating families were recruited with the assistance of a Department of Human Services (DHS) liaison. Thus, all

maltreated children had documented experiences of child maltreatment on file with the New York DHS. The DHS liaison contacted a random sample of identified maltreating families to explain purposes and procedures involved with the research study. Interested parents signed consent for the liaison to release their contact information to the research team and to allow for comprehensive examination of their DHS record files. Nonmaltreated children were recruited from families receiving TANF. Families receiving TANF were of focus given their low-SES characteristics and the intention to create a demographically-matched comparison group, in order to remove socioeconomic status (SES) as a confound. A DHS liaison contacted nonmaltreating families and provided the contact information of interested families to the research team following consent. All families were free to withdraw from participation of the study at any time without penalty.

*Data collection*

All children attended a week-long (7 hrs/day for five days), research-based summer camp program on one occasion between the years 2004-2012. Within the camp context, children were divided into groups of eight; approximately half of the children in each group were maltreated. All groups were composed of children of the same age and gender. Each group was led by three trained camp counselors unaware of maltreatment status and hypotheses of the study (see Cicchetti & Manly, 1990, for detailed descriptions of camp procedures). Trained research assistants, also unaware of maltreatment status and study hypotheses, administered research measures/questionnaires to children during the course of the week and collected salivary cortisol and DNA samples, as detailed

below. Children also participated in a variety of recreational activities throughout the week. Clinical support by licensed providers at Mt. Hope Family Center was available for any emerging concerns regarding a child's danger to self or others.

**Measures**

The measures listed below represent only a subset of assessments administered during the camp program that are relevant to the current study's research questions. Information was obtained by multiple informants (i.e., self-, parent-, and counselor-report) and multiple methods (questionnaires, validated report measures, biological sampling).

*Demographics*

Parents (typically mothers) reported on child race and also specified their child's Latino/Hispanic ethnicity as None, Puerto Rican, Cuban, Mexican, Dominican, or Other. Race/ethnicity was coded into a single variable using the Add Health system of coding race and ethnicity (DeYoung et al., 2011). To further characterize race/ethnicity, a SNP panel of 106 ancestry-informative genetic markers (AIMS) was used to classify children into African, European, or Native American descent (see Table 2 for more information regarding AIMS and other genetic terms). This method of classification is a standard typically used to determine ancestry (Lai et al., 2009; Yaeger et al., 2007). Ultimately, the decision regarding inclusion of children into the current study's African American sample was based on the proportion of African ancestry a child possessed (i.e., children with African AIMS proportion scores at or above 0.6 were included; AIMS information was not available for 1.3% of the larger sample) rather than parent-reported

race/ethnicity. However, for a majority of children (97%), ancestral characterization overlapped with parent-reported race/ethnicity.

*Maltreatment*

Thorough searches of DHS records by the research team yielded maltreatment information, which was coded by trained research assistants, doctoral students, and clinical psychologists using the Maltreatment Classification System (MCS; Barnett, Manly, & Cicchetti, 1993; Cicchetti & Barnett, 1991b). Maltreatment classifications generated using the MCS were independent of DHS case designations. The MCS has been shown to be a highly reliable and valid method of classifying maltreatment experiences (Bolger, Patterson, & Kupersmidt, 1998; English et al., 2005), and considers various aspects of maltreatment including type of maltreatment, severity of each maltreatment incident, developmental period during which maltreatment began, number of developmental periods over which maltreatment occurred (chronicity), and frequency of occurrence of each maltreatment subtype.

Onset of maltreatment was originally coded as infancy (0-18 months), toddlerhood (19-35 months), preschool (36-59 months), early school (5-7 years), later school (8-12 years), and adolescence (13-18 years). For the current study, these onset designations were combined in effort to group children into early (before age 5: infancy, toddlerhood, or preschool) or late (age 5 or later: early school, later school, and adolescence) maltreatment given the low number of children across more specific designations and the general view that the first few years of life are most sensitive for the development of limbic areas of the brain known to influence and regulate the stress

response (e.g., Thompson & Nelson, 2001). Maltreatment variables were dummy coded into early, late, and nonmaltreatment groups such that nonmaltreated children were the reference group.

A trained research assistant interviewed identified nonmaltreating mothers using the Maternal Maltreatment Classification Interview (Cicchetti, Toth, & Manly, 2003) to verify that the family did not have undocumented experiences of maltreatment. Additionally, DHS records were examined a year following camp completion to confirm continued absence of maltreatment in the family. If families received DHS preventative services due to concerns for risk of maltreatment, or if any other questions regarding possible maltreatment arose, then those families were excluded from the current study.

*Internalizing Symptoms*

Internalizing symptoms were assessed using four measures. Two of the internalizing measures (Revised Checklist for Manifest Anxiety Scale (RCMAS) and Children's Depression Inventory (CDI), see below for description) focused on specific internalizing disorders (i.e., depression and anxiety) and their respective diagnostic structures/symptom domains, while the remaining measures (Child Behavior Checklist Teacher Report Form (CBCL TRF) and Positive and Negative Affect Schedule-Child version (PANAS-C), see below for description) captured general features (i.e., withdrawal, somatic complaints, positive and negative affect) of internalizing problems.

Because the goal was to broadly examine internalizing symptoms given the relevance of stress system functioning to various internalizing domains as well as heightened risk for general internalizing problems for maltreated individuals, a latent

variable across the various measures was created. When possible, overall scores for each measure rather than specific subscale scores (as described below) were used to create the internalizing symptoms latent variable. Only in the case of the PANAS-C were separate subscales (positive affect vs. negative affect) utilized, given that a combination of subscales (e.g., an overall affect score) was not feasible or meaningful for this measure.

*RCMAS*

Level of total anxiety symptoms were assessed using the RCMAS (Reynolds & Richmond, 1985). The RCMAS is a 37-item, self-report instrument with good reliability (internal consistency = .85; Reynolds & Richmond, 1978) designed to assess the level and nature of anxiety in children ages 6 to 19 years. Children answered a series of yes/no questions regarding different symptoms of anxiety, including social anxiety, general worry, and physiological symptoms of anxiety. A total anxiety T-score was generated. High scores indicated higher levels of anxiety, with T-scores greater than 60 indicating clinically significant anxiety concerns. Children in the current sample had mean total anxiety T-scores of 45.43 (*SD* = 11.17, range = 18-80). Total anxiety T-scores were used to create the internalizing latent variable.

*CDI*

The CDI (Kovacs, 1982, 1992) is a 27-item self-report measure regarding various depressive symptoms that school-age children report experiencing over the past 2 weeks. Internal consistency for the total scale has been reported from .71 to .89 (Kovacs, 1982). Although depressive symptoms are the primary focus of the CDI, the instrument reportedly measures a broad, multidimensional construct that overlaps with other

childhood disorders, especially anxiety disorders (Saylor, Finch, Spirito, & Bennett, 1984). Symptom areas include negative mood (sadness, tearfulness, worry, difficulty with decision-making), interpersonal problems (problems with social interaction), ineffectiveness (negative evaluation of performance and ability), anhedonia (loss of interest, low energy, trouble sleeping, changes in appetite), and negative self-esteem (self-dislike, feeling of being unlovable).

Each item is rated on a scale of 0 to 2, with 2 indicating the highest level of difficulty associated with the symptom. One item of the measure directly assesses suicidal ideation. For the current study, if a child endorsed this item, the child was evaluated by a licensed clinician at the Mount Hope Family Center. Clinicians were always on duty during camp hours. An overall depression score is provided, which can range from 0-54. Scores of 19 and above reflect clinically significant concerns (Kovacs, 1992). Scores greater than 36 reflect severe depression (Stanley et al, 2009). In the current sample, total depression scores ranged from 0 to 48 ($M$ = 7.57; $SD$ = 6.94). Total depression scores were used to create the internalizing latent variable.

*PANAS-C*

The PANAS-C (Laurent et al., 1999) was used to obtain both child- and counselor-reported information regarding general affective states of each child. The measure has demonstrated good internal consistency (.86-.90 for Negative Affect (NA) scale; .84-.87 for Positive Affect (PA) scale; Watson, Clark, & Tellegen, 1988). The PA and NA scales have also demonstrated both convergent and discriminant validity with other self-report measures of childhood anxiety and depression (Laurent et al., 1999). The

NA scale, in particular, is thought to best capture individuals experiencing both anxiety and depression, given that emotional distress is a core component of these internalizing symptoms (Clark & Watson, 1991).

Using a 5-point Likert scale (1 = *very slightly* to 5 = *extremely*), raters indicated how often 30 different feelings and emotions (15 descriptors for positive affect and 15 for negative affect) were outwardly exhibited or personally experienced by the child. PA included descriptors such as interested, alert, excited, active, proud, and daring. NA included descriptors such as sad, frightened, upset, jittery, and lonely. The highest possible score across items for each scale is 75. Mean scores across all 15 items are obtained for each scale; thus the maximum mean score for each scale is 5. For the PA scale, higher scores reflect higher positive affect. Mean (*SD*) counselor-reported PA in the current study was 3.38 (.61); mean self-reported PA was 3.99 (.76). For the NA scale, higher scores reflect higher negative affect. Mean (*SD*) counselor-reported NA in the current study was 1.47 (.39); mean self-reported NA was 1.92 (.77). Mean scores for each scale were used to create the internalizing latent variable.

*TRF*

The TRF (Achenbach, 1991) is a 118-item measure that is widely used and empirically validated for the assessment of frequency (i.e., never, sometimes, often) of behavioral and emotional disturbance (both internalizing and externalizing symptoms) from teachers' perspectives. In the current study, camp counselors were able to observe similar behaviors as teachers and were thus used in lieu of teachers given the proximity and extent of interaction between the counselors and children within the summer camp

context. The TRF has demonstrated variable reliability, especially for the internalizing scale (.56 to .84), which reflects the difficulty that observers have in rating the internal states of children (Achenbach, 1991). Three camp counselors rated each child on the TRF; counselors' scores were averaged to obtain one score for each subscale. The overall internalizing T score ($M$ = 47.67, $SD$ = 7.91; range = 36-76) captures three aspects of internalizing symptoms, withdrawal, somatic complaints, and anxiety/depression, and was used to create the internalizing latent variable.

*Exploratory Factor Analysis*

Prior to conducting exploratory factor analysis (EFA), correlations were examined to assess the relative relationships among the various measures (see Table 3 for correlations among internalizing measures and other study variables). Correlations generally suggested a weak relationship between self- and counselor-reported measures and stronger correlations within self- and counselor-reported measures. Given a lack of certainty about the underlying factor structure/dimensionality of the data and whether measures could be meaningfully combined to create an internalizing latent variable, an EFA was run on a portion of the data. A random sample (n = 369) from the full dataset (N = 739) was selected to create a training sample with which the EFA was performed. Together, all self- and counselor-report indicators of internalizing symptoms, as listed above, were used to conduct the EFA using Geomin (oblique) rotation (i.e., factors were allowed to correlate). Due to skewness of some of the variables (CDI, self-reported NA, self-reported PA, counselor-reported NA, and TRF internalizing), separate EFA with transformed and untransformed variables were run. The model using the transformed

variables did not improve model fit, so untransformed variables were used in subsequent analyses with full-information maximum-likelihood (fiml) as an estimator. Number of factors was determined using standardized factor loading values and eigen values ≥ 1.0.

To assess the goodness of fit of the EFA-generated model, the comparative fit index (CFI), Tucker-Lewis index (TLI), and root mean squared error of approximation (RMSEA) were examined. Values close to 1 for both TLI and CFI indicate good fit, while an RMSEA of .06 or less is often considered good fit (Bentler, 1990; Hu & Bentler, 1999). These guidelines were used to determine model fit for the current study.

*Confirmatory Factor Analysis*

The EFA-identified factor structure was validated on the remaining half of the sample. Thus, to confirm results of the EFA, a confirmatory factor analysis (CFA) using a 2-factor structure with oblique rotation and full information maximum likelihood estimator was run on the testing sample. After confirming the measurement model, a measurement invariance model was examined to determine whether the internalizing latent variables were operating similarly between males and females.

*Externalizing Symptoms*

Given the substantial overlap that often occurs between internalizing and externalizing symptoms (e.g., Essex et al., 2011), the significant (though weak) correlation between self- and other-reported internalizing and externalizing symptoms in the current study (r = .326 and .153, respectively), and the focus of the current study on contributions of maltreatment and stress system dysfunction solely to the development of internalizing problems, the externalizing problem scale of the TRF was used as a

covariate for both counselor-reported and self-reported internalizing symptoms. The TRF (Achenbach, 1991, see above description) was exclusively used to indicate externalizing problems as self-report measures of externalizing problems were not available. Aggression, attention problems, and rule-breaking behaviors comprised the overall externalizing T-score (*M* = 53.56, *SD* = 9.37; range = 39-82.5), which was used in analyses.

*HPA Functioning*

*Cortisol Collection*

Saliva samples were obtained within the camp context each morning upon arrival to camp at 9am, at midday (noon), and each afternoon prior to camp departure at 4pm to provide information about daily cortisol production. Three samples were consistently collected at 9am, noon, and 4pm across the camp week, resulting in a maximum of 15 total samples collected per child. All children experienced a 45-min bus ride before arriving to camp each day. Travel time to camp in addition to time spent being greeted by camp staff ensured that sample collection occurred consistently at least 1 hour after awakening, thus avoiding capturing cortisol values associated with the cortisol awakening response. Food or drink was not consumed for at least 30 min prior to saliva sample collection. Children chewed sugarless, Trident gum to facilitate saliva production and then deposited saliva through a straw into a plastic vial. Following collection, saliva samples were immediately stored at -40°C. Samples were later shipped overnight for assaying in duplicate by Salimetrics Laboratories (State College, PA) using an enzyme immunoassay (with assay sensitivity parameters ranging from 0.0007µg/dl to 1.8µg/dl

with 5% and 10% intra- and inter-assay coefficient variation, respectively). Cortisol production across the daytime period proxies the regulatory capacity of the HPA axis. Of note, daytime cortisol production patterns as used in this study are not equivalent with the diurnal cortisol rhythm. Wake time, cortisol awakening response, and bedtime values were not collected as part of the current study, precluding the ability to completely measure diurnal cortisol patterns.

*Cortisol Change Variable*

Mean raw cortisol values (in µg/dl) across camp days were as follows: *M* morning cortisol = 0.197, *SD* = 0.110; *M* noon cortisol = 0.123, *SD* = 0.054; *M* afternoon cortisol = .103, *SD* = 0.046. Morning samples were available across all five camp days for 58% of children, noon samples were available across all five camp days for 69% of children, and afternoon samples were available across all five camp days for 68% of children. A majority (97%) of children had at least 3 samples for each time point. Three children were missing all cortisol samples for morning, noon, and afternoon across all days. Given the large number of cortisol samples available per child and the intention of examining patterns of cortisol production from morning to evening, latent growth curve modeling was considered as a method to create the best-fitting slope across several data points. However, when cortisol values were collapsed across days, and morning, noon, and afternoon time points were plotted, values did not follow a linear pattern, thus eliminating this option. Instead, change in cortisol levels from morning to evening across the day was captured by a difference score.

To create the difference score, raw cortisol values were winsorized at 3 standard deviation values for the day/time in order to reduce the impact of outliers. Cortisol values were then log-transformed (log10) due to skewness. Log values were averaged across the five days for each morning and afternoon time points. For cases that did not have all data points per time period, available values were used to calculate the mean. The mean, log-transformed morning value (*M* morning cortisol = -.785, *SD* = .216) was subtracted from the mean, log-transformed afternoon value (*M* afternoon cortisol = -1.055, *SD* = .183) to create the cortisol difference score. Negative cortisol difference values represent a greater change from morning to evening, which reflects more typical cortisol production patterns whereby levels decline over the course of the day. Cortisol difference score values closer to zero thus represent a "flattening" of the typical morning-to-afternoon pattern (*M* difference score = -0.269, *SD* = 0.176; range = -0.82-0.36).

*Genetic Risk*

*DNA Collection, Extraction, and Genotyping*

Oragene collection kits and/or Epicentre Catch-All Collection Swabs were used to obtain saliva and/or buccal swab DNA samples, respectively. Buccal cells and saliva are both commonly used for genotyping DNA; both sources of DNA collection yield quality product. For buccal cells, DNA was extracted and prepared for polymerase chain reaction (PCR) amplification using the Epicentre BuccalAmp DNA Extraction Kit (Epicentre, Cat. No. BQ090155C). For saliva, DNA was purified from 0.5 ml of Oragene-DNA solution using the DNA genotek protocol for manual sample purification using prepIT-L2P. Sample concentrations were quantified using the Quant-iT PicoGreen dsDNA

Assay Kit (P7589, Invitrogen). Applied Biosystems Custom Taqman SNP Genotyping Assays were used for single nucleotide polymorphism (SNP) genotyping. TaqMan procedures were performed on several gene variants. Genotypes were identified and sequenced with the Beckman-Coulter CEQ8000 semiautomated fluorescent sequencing system, which utilizes Fragment Analysis Application and associated software. To ensure quality control, all samples were genotyped in duplicate. In addition, human DNA control samples purchased from Coriell Cell Repositories were used for each genotype. Samples that were not able to be genotyped to a 95% or greater confidence level were repeated under the same procedures up to four times.

*Risk Index*

Nine variants across four stress-system genes (*CRHR1, FKBP5, NR3C1,* and *NR3C2*) were used to create a multigenic index of risk. All SNPs included in the multigenic risk index were chosen given their involvement in regulation of HPA axis activity (see below). Additionally, selected SNPs were a function of available archival data. A multigenic index was used in order to consider variation across several genes relevant to a neurobiological system rather than one particular aspect of the system. By using information regarding linkage disequilibrium (LD), and by examining genotype distributions, relevant, available SNPs were narrowed down from thirteen to nine in effort to reduce LD and given lack of variation in genotype. LD reflects non-independence of various alleles. Predictive accuracy of polygenic risk scores decreases among linked genotypes (Vilhjalmsson et al., 2015). Thus, efforts to reduce LD were made in order to avoid collinearity among SNPs so that different SNPs within each gene could be more

effectively represented. Eliminated SNPs included rs6195, rs6189/6190 of the *NR3C1* (GR) gene and rs3800373 of the *FKBP5* gene. Within the *NR3C1* (GR) gene, children in the current sample lacked variation in genotypes such that 99% of children were homozygous for the risk allele in the case of rs6195 and 99% of children were homozygous for the non-risk allele in the case of 6189/6190. The final SNP (rs3800373 of *FKBP5*) was eliminated given documented high LD with rs1360780 (Binder et al., 2004; Paggliaccio et al., 2014).

Genotypes were coded based on characterization of risk alleles for each SNP (see below for decision-making process). In some cases (rs7209436, rs110402, rs242924 of CRHR1 gene; rs2070951 of *NR3C2* gene), individuals homozygous for the identified risk allele were coded as "1," while individuals heterozygous for risk were grouped with those homozygous for the non-risk allele and coded as "0." In other cases (rs5522 of *NR3C2* gene; rs41423247 of *NR3C1* gene; rs9296158, rs1360780, rs9470080 of *FKBP5* gene), individuals with any risk allele (i.e., homozygous or heterozygous for risk allele) were coded as "1," while those homozygous for the non-risk allele were coded as "0." That is, for the former group, a recessive model was adopted for coding whereas, for the latter, a dominant model was utilized.

Number of risk genotypes across all nine SNPs were summed (maximum score = 9) to create a multigenic risk score, where higher scores reflected higher number of risk genotypes previously associated with HPA axis dysregulation. Investigations comparing utility of summing risk scores across SNPs versus including SNPs in a haplotype have found no bias in the former approach (Paggliaccio et al., 2014). If a child had four or

fewer genotypes missing across the nine SNPs, a prorated risk score was calculated by counting the number of risk genotypes and dividing by the total number of available data points to determine percentage of risk, and applying that percentage to nine to determine overall risk score. For example, if a child was missing 3 genotypes and thus had 6 of 9 genotypes available, 3 of which were risk genotypes, then 3/6, or 50%, of genotypes were risk genotypes. Therefore, 50% of 9 (i.e., 4.5) was used as the overall multigenic risk score.

Multigenic risk scores were mean-centered to remove multi-colinearity. One child was missing 5 or more genotypes, and, as such, was excluded from analyses. For each SNP, Hardy Weinberg Equilibrium (HWE) was calculated. Deviation from HWE was observed for rs2070951. The magnitude of the p-value for this SNP suggests that this deviation is not of substantial concern (see Turner et al., 2011), obviating the need to exclude or explore this subgroup in further analyses. All other SNPs were in HWE. Neither individual SNPs nor the overall multigenic risk score were related to maltreatment status. See Table 4 for allele frequencies and coding summary.

**Gene/SNP Selection and Coding Rationale**

The primary focus of this study was to explore HPA axis functioning as a biological mechanism through which child maltreatment contributes to the development of internalizing symptoms. Although there have been associations of several of the SNPs included in this study with internalizing symptoms and/or interaction with abuse or other environmental stressors to predict internalizing psychopathology, these aspects were ultimately not considered when creating the coding system for genetic risk given an effort

to isolate *functional* risk, that is, risk in the form of direct impact on biological functioning. Given that both attenuated and enhanced cortisol/ACTH levels (either as response to stress or with respect to basal levels) are considered maladaptive (e.g., Tarullo & Gunnar, 2006), alleles were classified as risk given their demonstrated impact on both reduced and increased cortisol/ACTH levels in healthy individuals. Functioning in healthy individuals was emphasized due to confounding effects that concurrent psychopathology has on HPA axis functioning.

*CRHR1*

CRH binding to its receptor provides the signal necessary to transduce neural input into a cortisol response (Rivier & Plotsky, 1986). *CRHR1* is the primary receptor for CRH, and its activation by CRH has been found to increase ACTH and cortisol responses to stress as well as fear-related behaviors (e.g. Stenzel-Poore, Heinrichs, Rivest, Koob, & Vale 1994; Timpl et al., 1998). CRH over-expression has also been shown to increase basal levels of cortisol (Labermaier et al., 2014). Additionally, both increased *CRHR1* expression and ligand binding attributable to genetic variation in the gene have been linked to prolonged elevation of cortisol following stress, whereas a *CRHR1* antagonist reversed this same neuroendocrine pattern in mice (Timpl et al., 1998). Similarly, in humans, reduced functioning of the *CRHR1* receptor can normalize HPA axis responses to stress by reducing reactivity (Binder & Nemeroff, 2010; Hauger, Risbrough, Brauns, & Dautzenberg, 2006). Therefore, given its role in initiating the stress response and amplifying and prolonging HPA axis activation, the *CRHR1* gene was selected.

*rs7209436*

Although many studies have demonstrated links between various SNPs within the CRHR1 gene and adverse mental health outcomes both dependent and independent of environmental risk (e.g., Bradley et al., 2008; Cicchetti et al., 2010, 2011; Liu et al., 2006; Tyrka et al., 2009), very few have actually investigated the direct impact of SNPs on HPA axis functioning in healthy individuals. In studies finding interactions between maltreatment and rs7209436 on psychopathology, the T allele was implicated as the risk allele. The notion that the T allele is the risk allele with respect to HPA axis functioning was supported in the one known study investigating the impact of rs7209436 on cortisol reactivity in healthy children. That is, preschool children carrying the T allele within a haplotype had marginally higher reactivity to a psychosocial stressor (Sheikh, Kryski, Smith, Hayden, & Singh, 2013). Thus, there is emerging evidence that rs7209436 enhances risk by heightening responsivity of the HPA axis to stress. However, given lack of robust evidence to date demonstrating an effect of the T allele on HPA functioning, a recessive genetic model was utilized when coding risk in the current study. That is, only individuals homozygous for the T allele were coded as "1," which represents a more conservative approach to coding risk as it requires individuals to possess both alleles previously linked to altered functioning.

*rs110402*

The A allele of *CRHR1* rs110402 has been shown to predict increased cortisol levels in children in response to a laboratory stressor when part of a multigenic index utilizing a recessive model (Pagliaccio et al., 2014). Others have found that the A allele is

associated with attenuated response to stress (TSST) in healthy adults without psychiatric disorders (Mahon, Zandi, Potash, Nestadt, & Wand, 2013). In this study, all types (additive, dominant, recessive) of models were tested, and a recessive model emerged as the most appropriate underlying model. As a result, a recessive model was utilized to code the rs110402 SNP in the current study.

*rs242924*

In a study investigating variability in rs242924 and cortisol response in healthy adults, those homozygous for the T allele were nominally hyporesponsive to a stressor (Mahon et al., 2013). This is the only known study directly examining the impact of the rs242924 SNP on HPA axis functioning. As such, a conservative recessive model was used for coding genotypes.

*NR3C1*

Through binding its receptor, cortisol impacts various HPA axis functions, including both onset and termination of the stress response. The *NR3C1* gene codes for the GR, which serves as the primary regulatory component of the stress reaction (de Kloet, Joels, & Holsboer, 2005). Altered GR availability and/or signaling has been shown to contribute to disrupted negative feedback inhibition of the HPA axis, leading to insufficient suppression of CRH and AVP release from the hypothalamus and ACTH from the pituitary, resulting in high levels of cortisol and maladaptive physical and mental health outcomes (see Zanas & Binder, 2014, for review). The GR is needed to control HPA axis inhibition; without GR expression, exaggerated HPA responses and decreased feedback sensitivity are observed (e.g., Lupien, McEwen, Gunnar, & Heim,

2009; Weaver et al., 2004). By increasing the number and responsivity of GR receptors, endocrine profiles can be restored (Binder, 2009), thus demonstrating the profound control that GR has in influencing HPA axis functioning and highlighting its relevance to the current investigation.

*rs41423247*

Variability in rs41423247 (aka *BclI*) has been linked to altered cortisol sensitivity and variable stress responses. Individuals homozygous for the minor G allele (which produces a long fragment) have demonstrated elevated cortisol concentrations in response to stress (Rosmond et al., 2000), and a trend toward lower GR affinity for and sensitivity to dexamethasone in leukocyte cells (Panarelli et al., 1998) when compared to major allele homozygotes. In a study of children that included rs41423247 as part of a genetic profile score utilizing a dominant coding model (GG/GC vs. CC), children with higher profile scores had higher cortisol output before, during, and after a stressor (Pagliaccio et al., 2014). In another study of young men, a differential effect of number of minor alleles was observed such that minor allele *heterozygotes* demonstrated *higher* responses to stress compared to major allele homozygote controls, while minor allele *homozygotes* evinced significantly *lower* responses (Wust et al., 2004). Kumsta et al. (2007) identified sex-specific effects of genotype which could account for Wust et al.'s finding. Kumsta et al. (2007) demonstrated that although men homozygous for the minor allele had blunted stress responses, cortisol responses were heightened in women homozygous for the minor allele. Notably, all women in the Kumsta (2007) study were using oral contraceptives, which may account for findings.

Ising and colleagues (2008) found a dose-response effect (suggesting an additive model) of the minor allele such that individuals homozygous for the risk allele had lowest levels of anticipatory cortisol preceding a stressor, followed by minor allele heterozygotes, with major allele homozygotes having highest anticipatory cortisol levels. Higher anticipatory cortisol may reflect adaptive preparation of the system, while lower levels can indicate failure to mobilize necessary resources. Contrary to Kumsta's study, the attenuating effect of the minor allele on anticipatory stress did not differ between males and females in Ising's study. Lower than expected cortisol levels have also been found in a handful of studies reflecting super-suppression of the system following exposure to cortisol analogues. These studies have shown that both G-allele heterozygotes and homozygotes of both sexes show hypersensitivity of GR to cortisol based on increased suppression to dexamethasone (Stevens et al., 2004; van Rossum et al., 2003).

Despite the suggestion of enhanced, rather than reduced negative feedback – which is more commonly cited as an indicator of dysfunction and has also been found for the minor allele of rs41423247 by Rosmond et al. (2000) – for minor allele carriers, increased sensitivity of GR to cortisol is also problematic as it leads to vulnerability for increased CRH production in limbic regions (Reul & Holsboer, 2002; Schulkin, Gold, & McEwen, 1998). Additionally, it has been suggested that increased sensitivity of the GR receptor to cortisol may reflect underlying increased effect of cortisol on cells in these individuals (Weaver, Hitman, & Kopleman, 1992), meaning cortisol may be more powerful and, potentially, have greater impact on tissues in these individuals. From this

perspective, the G allele would continue to function as a "risk" allele. Although there have been various findings depending on sex, tissue type, and genotypic model, the only study relevant to children utilized a dominant model (Pagliaccio et al., 2014). For this reason, a dominant model of coding was used in the current study.

*FKBP5*

Function of the GR depends on various chaperone proteins binding to the receptor complex to either increase or decrease its functioning. The *FKBP5* gene is a negative regulator of GR function making it a relevant and necessary candidate gene in this study, particularly with regard to its impact on negative feedback. *FKBP5* codes for a protein, FKBP51, which binds to other proteins of the GR complex and changes GR signaling by reducing GR's interaction with transport proteins, impeding nuclear translocation of the receptor, and creating a receptor structure that has a lower affinity for cortisol (Chrousos et al., 1982; Denny, Valentine, Reynolds, Smith, & Scammell, 2000). With such changes to GR activity, important negative feedback mechanisms which rely on cortisol binding to GR and serve to restrain HPA activity are affected, thereby leading to elevated cortisol levels. Preclinical data has demonstrated glucocorticoid resistance and elevated levels of cortisol in primates who naturally have high levels of FKBP5 (Bamberger, Shulte, & Chrousos, 1996; Chrousos et al., 1982; Chrousos et al., 1986), and, conversely, hypersensitivity of GR to corticosterone in animals that have lost function of the *FKBP5* gene (Touma et al., 2011). Furthermore, because cortisol induces production of FKBP5 by binding to FKBP51 protein (Zannas & Binder, 2014), additional FKBP5 is produced in the presence of elevated cortisol, thus perpetuating the cycle.

*rs1360780*

The minor A allele of the rs1360780 SNP is associated with higher FKBP5 induction and reduced GR sensitivity (Binder et al., 2004; Klengel et al., 2013). Studies have shown that healthy adult carriers of the minor allele exhibit prolonged cortisol responses to stressors with insufficient/slower recovery to baseline (Buchmann et al., 2014; Ising et al., 2008), and non-suppression of the HPA axis to dexamethasone (Touma et al., 2011). Healthy Dutch infants with the minor allele have also demonstrated increased cortisol following a minor stressor – the Strange Situation Paradigm (Luijk et al., 2010) – regardless of their attachment security classification. Notably, infants with insecure attachment showed even further negative effects on cortisol following stress, capturing the true diathesis present in the minor allele (i.e., altered stress response), where effects are further enhanced in the presence of a second vulnerability factor (i.e., insecure attachment – a proxy for poor parenting). These features indicate inadequate termination of the stress reaction secondary to GR resistance, a hallmark feature of depression, and suggest risk for exacerbated cortisol production in response to stress.

In some of the studies (i.e., Ising et al., 2008; Luijk et al., 2010) the effect of the minor allele was examined individually (AA vs. AG vs. GG). Other studies (i.e., Touma et al., 2011) utilized a recessive model. Most often, only individuals homozygous for the minor allele showed effects on HPA axis functioning, with no differences between minor allele heterozygotes and major allele homozygotes, suggesting a recessive model of operation of the "risk" allele (i.e., need both risk alleles for an effect). However, in Buchmann's 2014 study, both minor allele homozygotes and heterozygotes demonstrated

similar effects on function with no difference between these genotypes but significant differences compared to major allele homozygotes, suggesting a dominant model of operation (i.e., need only one risk allele for an effect). In another study utilizing rs1306780 as part of a genetic profile score, consistent with Buchmann et al. (2014), individuals were dichotomized in accordance with a dominant model (i.e., AA/AG = 1; GG = 0) In this study (Pagliaccio et al., 2014), higher genetic profile scores were associated with higher cortisol levels in children aged 8-12 in response to a LabTAB stressor. In contrast, Luijk et al. (2010) found an additive main effect of genotype on cortisol such that cortisol reactivity increased in magnitude across genotypes as number of minor alleles increased (i.e., AA > AG > GG). As can be seen, there are differences across findings and approaches to coding. In order to be consistent with the only known study utilizing rs1360780 as part of a genetic profile score with children, the decision to follow a dominant genotypic coding model was made.

*rs9296158*

Despite extensive study of rs1306780, much less has been documented regarding other SNPs in the *FKBP5* gene. Of those that have been investigated, rs9296158 has been shown to have an impact on HPA axis activity in healthy individuals. The A allele has been found to increase FKBP5 protein and mRNA expression (Binder et al., 2004). Among psychiatric controls, A allele carriers had higher cortisol levels post-dexamethasone treatment, reflecting GR resistance (Binder et al., 2008). In this study, a dominant model was examined whereby A allele homozygotes and heterozygotes were

found to be similarly high in cortisol levels following dexamethasone administration. Thus, a dominant model was utilized to code rs9296158 genotypes in the current study.

*rs9470080*

Individuals carrying the T allele have been found to have increased FKBP5 mRNA and protein levels as well as less suppression to dexamethasone than individuals homozygous for the major C allele (Binder et al., 2004, 2008). Thus, individuals with either one or two copies of the T allele have demonstrated impaired negative feedback. As such, a dominant coding scheme was used to classify rs9470080 genotypes in the current study.

*NR3C2*

The *NR3C2* gene codes for cortisol's other receptor – the MR. The MR has a high affinity for cortisol, thus, in the brain, cortisol more readily binds MR than GR. As such, cortisol is bound to MR under basal conditions, and, in this way, MR acts to maintain baseline function while creating a stress activation threshold. MR functioning is therefore important for maintaining tonic inhibition of HPA axis activity (e.g., Ratka, Sutanto, Bloemers, & de Kloet, 1989). Studies have suggested that MR haplotypes linked to higher MR expression and lower basal cortisol also exhibit more efficient onset of HPA activity and lower perceived stress (van Leeuwen et al., 2011). Conversely, loss of MR expression coincides with less sensitivity to ACTH (van Eekelen, Rots, Sutanto, Oitzl, & De Kloet, 1991). Furthermore, in the absence of typical MR function, basal cortisol levels increase and individuals are more stress-reactive (Arvat et al., 2001; Heuser et al., 2000). Thus, effective MR action serves an important role in maintaining homeostasis

and regulating a healthy and dynamic stress response, making it a relevant gene to include as part of the multigenic risk index in the current investigation.

*rs5522*

Variability in the MR gene is linked with variable stress responses in healthy individuals. The rs5522 SNP (aka I180V) is located in an area involved in transcription and has a functional impact on amino acid production (Bogdan, Pagliaccio, Baranger, & Hariri, 2016). Individuals who carry the minor G allele of rs5522 produce the amino acid valine instead of the more typical amino acid leucine. This change affects the MR such that it is less functional in the presence of cortisol (Derijk et al., 2006). Additionally, minor allele carriers exhibit increased plasma and salivary cortisol output in response to psychosocial stressors (Derijk et al., 2006; Dickerson & Kemeny, 2004). Risk variant carriers have also been found to have higher baseline ACTH and higher cortisol during recovery periods following stress exposure (Ising et al., 2008). In these studies, a dominant genotype model was utilized such that any individual carrying a minor allele (val/val; CC or val/iso; CT) showed significant difference from individuals homozygous for the major allele (iso/iso; TT). As such, a dominant model was used to code rs5522 risk in the current study.

*rs2070951*

The G allele of the rs2070951 SNP has been associated with reduced cellular response to cortisol and less MR protein expression (van Leeuwen et al., 2011). Compared to individuals heterozygous for the G allele or non-carriers, G allele homozygotes also demonstrate greater total cortisol output as measured by AUC (Muhtz,

Zyriax, Bondy, Windler, & Otte, 2011). Additionally, individuals carrying the G allele have higher basal cortisol levels (Kuningas et al., 2007). In some studies, G allele carriers were compared against non-carriers, suggesting a dominant model, while, in other studies, only homozygotes evinced changes in HPA function, suggesting a recessive model. In effort to be most conservative, a recessive model was used in the current study.

## Results

### Data Analytic Plan

The primary analyses for the current investigation included factor analysis and structural equation modeling. For both strategies, the R statistical program version 3.3.1 was used. First, EFA and CFA were conducted to build and verify the measurement model, creating the latent variables. Model invariance to gender was examined for the measurement model to ensure the latent variables were operating similarly for males and females in the sample. Once the measurement model was optimized, latent variables were used as dependent variables in a structural equation model with maltreatment status (early vs. late vs. non) as the dummy-coded independent variable, cortisol production as a mediator of the relationship between maltreatment and internalizing symptoms, multigenic risk as a moderator of the impact of maltreatment on cortisol slope, and counselor-reported externalizing symptoms as predictors of internalizing symptoms - in order to control for the effect of comorbid externalizing symptoms in the model. Gender and age were added to the structural model to control for confounding effects given unequal distribution of gender and age across maltreatment groups. The structural model was then examined relative to a multi-group model in order to determine whether separate structural models for males and females fit the data better than the structural

model treating males and females equally. Follow-up analyses were conducted to help understand the nature of original findings. These analyses were carried out using SPSS v. 22 or the R statistical package. First, differences in mean morning and mean afternoon cortisol values among groups were tested to understand the nature of the cortisol flattening results. Next, results were examined with regard to severity of internalizing symptoms. Chronicity of maltreatment and its overlap with early and late onset of maltreatment were also examined. Finally, given differences in the literature regarding approach to coding genetic risk, three additional types of coding schemes were tested to examine the effect of different coding approaches on the original results.

**Exploratory Factor Analysis**

Of all seven internalizing symptom indicators (RCMAS, CDI, self-reported PA, self-reported NA, counselor-reported PA, counselor-reported NA, and TRF internalizing symptoms), self-reported PA significantly cross-loaded on two factors and did not load strongly on either factor (factor 1 loading = -0.285; factor 2 loading = -0.219, *p*s <.05). Model fit was poor when this variable was included in the model ($\chi^2$ (8) = 37.41, p = .000; RMSEA = .1, CFI = .958, TLI = .889); therefore, self-reported PA was excluded from subsequent models (further investigation demonstrated extremely restricted range of variability, whereby a majority of children reported high positive affect scores).

Ultimately, the best-fitting model had a 2-factor solution (eigenvalues > 1 for the first 2 factors; 2.416 and 1.908, respectively; $\chi^2$ (8) = 12.01, p = .15; RMSEA = .037, CFI = .993, TLI = .987), with RCMAS (.699), CDI (.865), and self-reported NA (.610) significantly loading on factor 1 at the .05 level (factor loadings in parentheses), and TRF

(.856), counselor-reported NA (.730), and counselor-reported PA (-.630) significantly loading on factor 2 at the .05 level. This factor structure suggests an observer effect, given that all self-report measures loaded on factor 1 and counselor-report measures loaded on factor 2. The inability to combine both self- and counselor-report measures into one latent variable is unsurprising given frequent discrepancy between self- and other- report of internalizing symptoms (Achenbach, McConaughy, & Howell, 1987). Exploratory efforts to create one latent variable by correlating residuals substantially reduced model fit.

**Confirmatory Factor Analysis**

*Measurement Model*

Using a 2-factor solution as indexed by the EFA, two different CFA models were run on the training sample; one included counselor-reported PA, the other excluded counselor-reported PA. Two models were created to better examine the relevance of PA to the counselor-reported latent variable, in light of removal of self-reported PA per EFA results (above), and interest in maintaining uniformity across self- and other- reported indicators for the PANAS-C measure.

The model without counselor-reported PA was not valid (i.e., residual variances were not significant), whereas the model with counselor-reported PA fit well ($\chi^2$ (8) = 16.86, $p$ = .032; RMSEA = .039; CFI = .991; TLI = .984). Thus, counselor-reported PA was maintained in the model. In sum, the latent variable was optimized when separated into two factors defined as follows: 1) internalizing-self with three indicators (α= .78) – RCMAS total anxiety T-score, CDI overall raw score, and self-reported NA mean score,

and 2) internalizing-counselor with three indicators (α= .75) – TRF internalizing T-score, counselor-reported NA mean score, and counselor-reported PA mean score. Standardized factor loadings resulting from the CFA are presented in Figure 1.

*Measurement Invariance*

The measurement model created via CFA was tested for measurement invariance between males and females. The first step in assessing measurement invariance is examining a configural model, which allows for the assessment of model fit of the original, fixed model compared to a model where all parameters are free to vary across the two groups. The configural model had acceptable fit ($\chi^2$ (16) = 21.41, $p$ = .163; RMSEA = .03; CFI = .996; TLI = .992), suggesting that proceeding with testing measurement model invariance was appropriate. Several additional models, including weak, strong, strict, and partial invariance models, were examined in order to identify specific aspects of the model that were or were not operating similarly among the different groups. Weak models forced factor loadings to be the same for each group, but allowed intercepts and residual variances to vary. Strong models forced factor loadings and intercepts to be the same for each group, but allowed residual variances to vary. Strict models forced all aspects (factor loadings, intercepts, and residual variances) to be equal across groups. Additionally, partial measurement invariance models were constructed in order to identify which of the intercepts and/or variances differed across groups.

The weak invariance model was nested within the configural model, allowing for comparison using a Chi Square test, where failure to reject the null hypothesis signifies

superiority of the weak invariance model over the configural model. The weak invariance model was found to be a better fit than the configural model ($\chi^2$ (20) = 25.82, $p$ = .353; RMSEA = .028; CFI = .995; TLI = .993), demonstrating that constraining factor loadings to be equal across groups did not negatively affect model structure. Next, a strong invariance model was nested within the weak invariance model. In comparison to the weak model, the strong model was not found to be a better fit ($\chi^2$ (24) = 50.13, $p$ = .000; RMSEA = .054; CFI = .979; TLI = .973). Thus, a model with constrained factor loadings fit better than a more restricted model that had both constrained factor loadings and constrained intercept values.

Lack of strong invariance indicated that the observed means (intercepts) between males and females were different. Partial invariance was next examined to understand whether *all* intercepts among the various latent variable indicators were different between males and females, or whether only some of the intercepts differed between males and females. In this way, examining partial invariance allowed for some intercept constraints to be released while others were maintained. Chi Square tests among different indicators revealed that only the intercept for the TRF internalizing indicator was significantly different between males and females ($\chi^2$ (1) = 19.12, $p$ = .000). Based on this information, the TRF internalizing indicator was released, and a modified strong invariance model (i.e., a strong, partial invariance model) was compared to the weak model. The strong partial model was nested within the weak, and when compared, was shown to fit better than the weak model as evident in failure to reject the null hypothesis

that the weak model is *not* an improvement over the strong partial model ($\chi^2$ (23) = 30.66, $p$ = .184).

The strong partial invariance model was then compared to a strict partial model and was not found to improve model fit ($\chi^2$ (29) = 47.15, $p$ = .011). Inspection of differences in residuals among indicators revealed differences for the CDI measure. Chi Square tests among different indicators revealed that the residuals for the CDI measure were significantly different between males and females ($\chi^2$ (1) = 17.96, $p$ = .000).When residual constraints for the CDI were released in the strict partial model, it was found to function better than the strong partial model ($\chi^2$ (28) = 39.52, $p$ = .11).

Thus, overall, the measurement model was found to have partial strict invariance, whereby models operated equally for males and females across factor loadings, intercept values, and residual variances, with the following exceptions: the TRF internalizing measure's intercept was not invariant and the CDI's residual variance was not invariant. This means that the CDI had different reliability for males and females and the TRF internalizing measure had different mean levels for males and females. Specifically, males had more measurement error than females on the CDI measure (male variance = 20.16; female variance = 11.70), and females had higher mean levels than males on the TRF (male intercept = 47.15; female intercept = 49.27), suggesting gender specificity of the TRF content. In sum, the measurement model for the current study was operating similarly among males and females such that the self-reported latent variable had all three factor loadings, all three intercepts, and two of three variances operating equally. The counselor-reported latent variable had all three factor loadings, two of three intercepts,

and three of three variances operating equally. An established rule is that so long as there are at least two factor loadings and two intercepts that are equal across groups, similar inferences about latent variables can be made for each group (Byrne et al., 1989). The measurement model for the current study well surpassed this metric.

**Structural Model**

The proposed structural model demonstrated good model fit ($\chi^2$ (43) = 111.26, $p$ = .000; RMSEA = .046, CFI = 0.949, TLI = 0.925); fit was reduced, but was still acceptable ($\chi^2$ (47) = 135.37, $p$ = .000; RMSEA = .050, CFI = .936, TLI = .904), when gender was added as a covariate given the gender distribution differences among maltreatment groups in the current sample. Distribution of age was also originally found to differ among maltreatment groups and was included as a covariate, but was not ultimately included in the final model given that, unlike gender, it did not significantly predict any of the outcomes, and model fit was further reduced when age and gender were both included in the structural model. Results for the structural model including the gender covariate are depicted in Figure 2, which labels significant (solid lines) and non-significant paths (dashed lines) with their respective standardized coefficients (note: for maltreatment pathways, the standardized coefficient associated with standardization of only the latent variable was used given that maltreatment is a dummy-coded variable; all other paths with continuous variables list the standardized coefficient associated with standardization of all variables in the model). Overall, the model explained 2.5% of the variability in cortisol difference scores, 4.2% of variability in self-reported internalizing

symptoms, and 11.2% of variability in counselor-reported internalizing symptoms. Specific path findings are presented below.

*Cortisol Change*

Mean differences in cortisol difference scores among the three groups are shown in Figure 3. A main effect of maltreatment on cortisol was found such that individuals maltreated later in life (age five and beyond) demonstrated a higher (i.e., more positive; thus closer to zero) cortisol difference score value ($z = 2.43$, $p = .015$, *standardized estimate* (*std est.*) = .046), meaning they evinced *less* change in cortisol values between morning to afternoon, relative to nonmaltreated children. Late maltreated children also had higher cortisol difference values when compared to early maltreated children ($z = 2.34$, $p = .019$, *std est.* = .046). Early maltreated children did not differ from nonmaltreated children in their cortisol difference value ($p = .984$). There was also a main effect of gender on cortisol difference values such that females had more negative difference score values (i.e., greater change from morning to evening) than males.

*Internalizing Symptoms*

A main effect of maltreatment on self-reported internalizing symptoms was found such that children maltreated earlier reported more internalizing symptoms than nonmaltreated children ($z = 2.42$, $p = .016$, *std est.* = .222). There were no differences between late and nonmaltreated children for self-reported internalizing symptoms ($p = .182$). When early and late maltreated children were directly compared, these groups did not significantly differ in level of internalizing symptoms ($p = .522$); see Figure 4 for mean differences in internalizing symptoms. A main effect of gender on self-reported

internalizing symptoms was found such that females had lower self-reported internalizing symptoms than males ($z$ = -2.00, $p$ = .045, *std est*. = -.166). There were no effects of cortisol slope, maltreatment, or gender on counselor-reported internalizing symptoms. Counselor-reported *externalizing* symptoms predicted both self- and counselor-reported internalizing symptoms whereby higher counselor-reported externalizing symptoms were associated with higher internalizing symptoms. There was a significant, positive relationship between self- and counselor-reported internalizing symptoms.

*Mediation and Moderation*

Cortisol change was not a significant predictor of either self- or counselor-reported internalizing symptoms. Given that simple mediation requires a relationship between the mediator and the dependent variable (Barron & Kenny, 1986), it was unlikely that any of the indirect effects of the model would be significant. Indeed, of the four indirect effects (i.e., 1: EM → Cort * Cort → internalizing-self; 2: EM → Cort * Cort → internalizing-counselor; 3: LM → Cort * Cort → internalizing-self; 4: LM → Cort * Cort → internalizing-counselor), none were significant predictors of internalizing symptoms (all *p*s >.1), reflecting lack of mediation of the relationship between maltreatment and internalizing symptoms by cortisol. Further, there was no direct effect or moderation by multigenic risk on cortisol change (main effect: $z$ = .002, $p$ = .998, *std est.* = .000; interaction with early maltreatment: $z$ = -.399, $p$ = .690, *std est.* = -.003; interaction with late maltreatment: $z$ = 1.584, $p$ = .113, *std est.* = .023). Thus, the functional, HPA axis-related SNPs included as part of a multigenic index in this study did not directly or indirectly affect daytime production of cortisol.

**Multi-group Model**

To ensure that the structural model was operating similarly for males and females, a multi-group structural model in which regression paths were restricted to be the same across males and females was fit. This model was compared to a model where the regression paths were allowed to be freely estimated across groups. The partial strict invariance for the latent variables from the measurement model was carried forward into the multi-group model. Both constrained and free models had similar and acceptable fit indices (fit measures for both models were: $\chi^2$ (64) = 127.66, $p$ = .000; RMSEA = 0.053, CFI = 0.952, TLI = 0.937). Chi Square tests comparing the less restrictive model to the fully restrictive model revealed that the less restrictive model did not improve model fit ($\chi^2$ (6) = 10.2, $p$ = 0.12). Thus, multi-group analyses demonstrated that there were no differences in regression paths by gender, meaning that the structural model operated similarly for males and females and conclusions can be applied to both groups.

**Follow-up Analyses**

*Morning and Afternoon Cortisol*

To better understand the nature of the cortisol change patterns found among maltreatment groups, separate linear regressions with mean, log-transformed morning and afternoon cortisol values as outcome variables were conducted. Maltreatment did not significantly predict morning (*M* EM = -.79; *M* LM = -.81; *M* Non = -.77) or afternoon (*M* EM = -1.06; *M* LM = -1.04; *M* Non = -1.05) cortisol values (all *p*s > .1). Although groups did not differ significantly at either time of day, as can be seen in Figure 5, there

was a slight lowering of morning and a slight increase of afternoon levels for later maltreated children relative to nonmaltreated youth.

*Severity of Internalizing Symptoms*

In light of previous findings suggesting that cortisol functioning partially depends on severity of internalizing symptoms (e.g., Hart et al., 1996; Cicchetti et al., 2010, 2011), levels of internalizing symptoms in the current sample were more closely examined to understand whether lack of prediction of internalizing symptoms by cortisol in the structural model was a consequence of a relatively low level of internalizing symptoms within the sample. To examine this, z-scores for each indicator used for the self-reported internalizing latent variable (i.e., RCMAS, CDI, self_NA) and counselor-reported internalizing latent variable (i.e., TRF, clr_PA, clr_NA) were created. The z-scores for the three measures comprising self-reported internalizing symptoms were then averaged. The same was done for counselor-reported internalizing symptoms. The overall sample was found to have a z-score range from -1.58 to 2.98 for self-reported internalizing symptoms and -1.51 to 2.91 for counselor-reported internalizing symptoms. Despite these ranges, the mean (*SD*) level of internalizing symptoms was generally quite low for each group (self-reported internalizing: EM *M* = .109 (.852), LM *M* = .061(.807), non *M* = -.100 (.828); counselor-reported internalizing: EM *M* = .085 (.043), LM *M* = -.055 (.077), non *M* = -.044 (.043)).

Given that the sample had generally low levels of internalizing symptoms overall, a more direct examination of how the severity of internalizing symptoms might impact cortisol was conducted. First, symptom level (children above median vs. children below

median) was used to predict cortisol difference scores, controlling for gender. Symptom level did not significantly predict cortisol difference scores for either self-reported ($F$ (1, 733) = .594, $p$ = .441) or counselor-reported ($F$ (1, 733) = 1.20, $p$ = .273) internalizing symptoms. Furthermore, symptom level had no bearing on morning or afternoon cortisol values for self-reported internalizing symptoms (morning: $F$ (1, 733) = .200, $p$ = .65; afternoon: $F$ (91, 734) = .036, $p$ = .849). Counselor-reported symptom level did significantly influence cortisol morning values ($F$ (1, 733) = 6.64, $p$ = .010), and, marginally, afternoon values ($F$ (1, 734) = 3.85, $p$ = .050) such that children with scores above the median had higher morning cortisol values than children with internalizing symptom scores below the median.

Secondly, effects of maltreatment on cortisol were examined only among children with clinically-significant internalizing symptom levels (i.e., z-scores $\geq$ 1). Controlling for gender, timing of maltreatment did not predict cortisol change scores for children with clinical-level self-reported internalizing symptoms ($F$ (2, 89) = 1.688, $p$ = .191) or those with clinical-level counselor-reported symptoms ($F$ (2, 81) = .394, $p$ = .676). Additionally, maltreatment did not predict differences in morning or afternoon levels for either children with clinical-level self- or counselor- reported internalizing symptoms (self-report morning: $F$ (2, 89) = 2.25, $p$ = .11, self-report afternoon: $F$ (2, 90) = .354, $p$ = .703; counselor-report morning: $F$ (2, 81) = 1.163, $p$ = .318 and counselor-report afternoon: $F$ (2, 81) = 1.02, $p$ = .366).

*Chronicity of Maltreatment*

Because it is possible that children who had an earlier onset of maltreatment may have also experienced more chronic maltreatment, simple descriptive statistics were examined. Frequencies among early and late maltreatment groups with respect to chronicity were plotted, as seen in Figure 6. There was more variability in number of development periods of maltreatment within the early maltreated group. Although modal number of developmental periods was 1 for each group (EM children experiencing maltreatment in one developmental period n = 124; LM n = 92), more children in the EM group experienced maltreatment during two (EM n = 82; LM n = 18) or three (EM n = 43; LM n = 0) developmental periods, and the difference among the distribution was significant ($\chi^2$ (4) = 794.75, $p$ = .000). Results demonstrate that early maltreatment also captures a greater range of chronicity in the current sample.

*Alternative Genetic Coding Schemes*

Given various approaches to coding genotypes prevalent in the literature, three additional coding schemes were tested to examine whether these approaches altered the structural model. As such, dominant, recessive, and additive coding schemes were created whereby all genotypes included in the multigenic risk score were coded either in accordance with a dominant (i.e., individuals carrying any risk allele were coded as "1;" non-risk homozygotes coded as "0"), recessive (i.e., individuals carrying two copies of the risk allele coded as "1;" risk heterozygotes and non-risk homozygotes grouped together as "0"), or additive (the three different genotypes were each given a separate score based on number of risk alleles – "2" for risk allele homozygotes, "1" for risk allele

heterozygotes, and "0" for non-risk allele carriers) model. There were no significant direct or moderation effects for any of the alternative coding schemes on cortisol slope when included in the structural model. Thus, alternative coding schemes did not alter the original structural model results.

## Discussion

The current study examined the association among maltreatment, genetic factors, HPA functioning, and internalizing symptoms in effort to better understand mechanisms by which disruptions to the child-caregiver relationship negatively affect mental health. Overall, results highlight the complexity of development, convey the concept of equifinality, and reiterate the need for future longitudinal research on this topic.

### Early Maltreatment

Consistent with the extant literature (e.g., Andersen & Teicher, 2008; Cicchetti & Rogosch, 2001a, b; Kim & Cicchetti, 2006; Manly et al., 2001; Widom et al., 2007), maltreatment was associated with an increased prevalence of internalizing symptoms in children above and beyond other forms of adversity (i.e., poverty). Few studies have focused exclusively on internalizing symptoms in maltreated, African American children (but see Bradley et al., 2008 and Cicchetti & Rogosch, 2014, for exceptions). Thus, importantly, results of the current study confirm that previous findings generalize to African American samples.

#### *Timing Specificity and Internalizing Symptoms*

As hypothesized, early, but not late, maltreatment predicted internalizing symptoms, suggesting time-dependent effects of maltreatment. These results echo

findings of several earlier studies demonstrating that children maltreated during or prior to the preschool years evince higher levels of internalizing symptoms than nonmaltreated youth and those maltreated later in childhood (Cicchetti et al., 2010; Hart et al.,1995; Kaplow & Widom, 2007; Keiley et al., 2001; Manly et al., 2001). Continued replication of these findings with the current sample points toward early-occurring maltreatment as being a powerful vulnerability factor for the development of internalizing symptoms, above and beyond other forms of risk. The first four years of life appears to be a sensitive period for the development of internalizing symptoms.

Maltreatment occurring within the first few years of life presents disruption during a time when relationally-based developmental processes that have been implicated in the development of internalizing symptoms are prominent and are undergoing more rapid development. This suggests that there may be specificity of stage-salient developmental processes occurring before age 5 to internalizing symptoms, and that maltreatment occurring during this time may exert its effects by substantially altering these processes.

Attachment is particularly salient to the early time period as it forms over the first year of life, and attachment patterns stabilize between 6 to 18 months of age (e.g., Ainsworth, Blehar, Waters, & Wall, 1978; Belsky, Rovine, & Taylor, 1984). Previous research has suggested attachment as a mechanism, showing that maltreated children have higher rates of insecure or disorganized attachment (Carlson, Cicchetti, Barnett, & Braunwald, 1989; Cicchetti &Barnett, 1991a), and that those with disrupted attachment patterns are at increased risk for internalizing symptoms (Groh, Roisman, van

IJzendoorn, Bakermans-Kranenburg, & Fearon, 2012). Moreover, interventions (Child Parent Psychotherapy; CPP), that support secure attachments effectively reduce internalizing symptoms in young children (Ghosh, Ippen, Harris, Van Horn & Lieberman, 2011). Attachment-based interventions have also been found to normalize activity of stress-mediating systems (Bernard, Hostinar, & Dozier, 2015; Cicchetti, Rogosch, Toth, & Sturge-Apple, 2011; Dozier, Peloso, Lewis, Laurenceau, & Levine, 2008). As such, early maltreatment may enhance risk for internalizing symptoms primarily through its impact on the attachment relationship and the many areas of development that disrupted attachment may subsequently affect, including, possibly, the HPA axis.

Another explanation for maltreatment timing effects in the current study may be its overlap with maltreatment chronicity. Results of follow-up analyses demonstrated that early maltreated children in the current study experienced maltreatment across a greater number of developmental periods than children maltreated later in life. Chronic maltreatment has been considered one of the most robust predictors of general maladaptation (Manly et al., 2001), even when considering other maltreatment characteristics such as severity of maltreatment (Bolger, Patterson, & Kupersmidt, 1998). As such, it may be that early, chronic maltreatment is the most deleterious combination given the increased chances for cumulative, cascading effects and increased likelihood that a variety of developmental tasks are negatively affected when maltreatment occurs across several developmental periods.

It is necessary to note that speculation that early maltreated children have greater internalizing symptoms given disruption to foundational developmental tasks does not imply that later maltreated children are unaffected by their experiences or may not also develop internalizing symptoms. Instead, it may be that early and late maltreatment both affect internalizing symptoms, but do so through different pathways and on different timescales. As such, conceptualizing the mechanisms for early and late maltreatment separately could help target the most effective intervention points for these different groups of at-risk children.

*Informant Differences*

Notably, maltreatment effects were evident only for self-reported internalizing symptoms. Previous studies have differed widely in methodology of assessing internalizing symptomatology (e.g., use of single self-report measures, aggregate of multiple self-report measures, composite scores incorporating both self- and other-report, etc.). Many researchers have argued for the exclusive use of self-report of internalizing symptoms (e.g., Reynolds, Anderson, & Bartell, 1985), given that internalizing symptoms are emotional experiences directed within (e.g., sadness, guilt, worry), and are not as readily expressed through behavior as other symptoms (e.g., hitting, biting, stealing, hyperactivity). Findings of the current study support this recommendation, as discrepant findings were produced depending on informant source. As such, utilization of self-report measures and consideration of these measures separately from the report of teachers, parents, or other outside observers in future studies may help clarify the nature of development of internalizing symptoms and elucidate discrepant findings that

currently exist within the literature. Of note, it may be that the children who have concordance among self- and other-report measures are the children whose symptoms are of greatest severity, given that internal distress has risen to the level of recognition by other observers.

*HPA Axis Functioning*

Contrary to hypotheses, cortisol patterns across the day did not mediate the observed relationship between early maltreatment and internalizing symptoms. Indeed, it was the late and not early maltreated youth who evidence the greater disruption in the normal daytime cortisol pattern. The developmental psychopathology principle of equifinality proposes that maltreated children can travel many different pathways to the same pathological outcome (Cicchetti & Rogosch, 1996). Thus, one explanation for the lack of mediation by cortisol in the current study is that different mechanisms are operating at this point in time to explain the impact of early experiences of maltreatment on internalizing symptoms. As stated above, some of these mechanisms may include attachment and attachment-related phenomena, which may serve as the primary pathway that children experiencing maltreatment early in life travel toward internalizing symptoms.

The hypothesis that HPA dysfunction, as represented by diurnal disruption, would mediate the relationship (suggesting its mechanistic properties) between maltreatment and internalizing symptoms was largely based on previous studies linking these three variables, in addition to the preclinical research documenting early effects of maltreatment on neuroendocrine function and the brain systems that control it. However,

it is possible that the associations found among maltreatment, diurnal patterning, and internalizing symptoms among children better reflect the impact of internalizing symptoms on cortisol, rather than vice versa. In previous studies (Cicchetti et al., 2010; Hart et al., 1996), both high levels of internalizing symptoms *and* maltreatment were required in order for the relationship to emerge. If HPA dysfunction comes after symptom onset (i.e., represents a "scar" of internalizing symptoms), lack of mediation by cortisol in the current study may be due to the fact that there had not been a long enough time from symptom onset to data collection for the effect to manifest physiologically. It may be that the distress caused by internalizing symptoms serves as a compounding stressor in the context of previous maltreatment experiences, and, together, these stressors contribute to HPA dysregulation over time.

There is some support for the idea that HPA dysregulation comes after symptom onset. In a study of adolescents, HPA dysregulation longitudinally predicted recurrence of major depressive episodes much better than it predicted first onset (Vrshek-Schallhorn et al., 2013). Findings by Doane and colleagues (2013) also support the hypothesis that internalizing symptoms, in and of themselves, are contributory to diurnal blunting and take time to manifest physiologically. That is, Doane et al. (2013) found that blunted diurnal slopes were evident for adolescents who had past episodes of depression, but were not present for those whose depression onset was within 3 months of cortisol assessment. Early-maltreated children in the current study may have had more recent onset of symptoms, precluding any significant association between cortisol and internalizing symptoms to emerge. Timing of symptom onset was not considered in the

current study, nor was the study longitudinal in nature. Furthermore, even if some of the early-maltreated children had longer-standing symptoms, there may not have been enough of these children to be able to detect the effect.

Alternatively, perhaps the relationships between diurnal functioning, maltreatment, and internalizing problems that have previously been found are more specific to depressive symptoms rather than internalizing symptoms, broadly. Some of the previous studies finding HPA dysregulation in maltreated individuals have focused on depression more specifically. Indeed, in a study where depressive symptoms and internalizing symptoms were both considered separately, maltreated children with clinically significant depression showed altered diurnal patterns, but maltreated children with internalizing symptoms, broadly, did not have any change in diurnal patterns (Hart et al., 1996).

It is also possible that previous associations between maltreatment, HPA dysfunction, and internalizing symptoms were capturing the relationship between HPA dysfunction and *externalizing* symptoms, given the frequent overlap of internalizing and externalizing problems (Essex et al., 2011). Previous research has shown that diurnal cortisol mediated the relationship between early adversity and externalizing symptoms in a sample of young children (Bernard et al., 2015). There is also evidence that lower and flatter cortisol patterns predict externalizing symptoms in internationally adopted children (Koss, Mliner, Donzella, & Gunnar, 2016). The current study controlled for the effect of externalizing symptoms, while many previous studies have not. This may suggest that

diurnal blunting is associated specifically with externalizing symptoms, and may explain lack of mediation of internalizing symptoms in the current sample.

**Late Maltreatment**

*Timing Specificity for HPA Axis Functioning*

Contrary to hypotheses, children whose maltreatment experiences began at age 5 or later exhibited more disruption to the HPA axis in that they had less change in cortisol across the day. Importantly, later maltreated children also were more likely to have experienced maltreatment recently (between 8-12 years of age), closer to the time of assessment. It may be that later maltreated children in the current study demonstrated blunted cortisol patterns because HPA effects are better captured by current adversity and/or context rather than early adversity. There has been speculation about whether diurnal effects documented in the literature represent early adversity, current adversity, or both (Tarullo & Gunnar, 2006). A previous meta analysis supports recency effects of stress on HPA activity, demonstrating that cortisol outcomes, at least with regard to hyperactivity, were more pronounced for more recently occurring stress (Miller et al., 2007). Results of the current study also suggest salience of recent life stress on diurnal patterning given the following: 1) children in the current study who were maltreated earlier in life did not evince HPA dysregulation as indexed by diurnal blunting, 2) early maltreated children did not evince blunted cortisol despite *also* having more chronic experiences, which have been linked to diurnal blunting (e.g., Boyce et al., 1995; McCormack et al., 2003), and 3) only a small percentage (20%) of the children maltreated before age 5 also had recent experiences of maltreatment, while a majority

(63%) of children whose maltreatment began at or after age 5 had recent experiences of maltreatment.

It may also be that later maltreated children demonstrated more diurnal dysregulation due to specificity of effects of later maltreatment on brain structures (e.g., amygdala, hypothalamus, hippocampus, PFC) that control HPA axis functioning. For example, although limbic system development occurs rapidly over the first few years of life (Thomson & Nelson, 2001), some researchers have suggested that peak sensitivity of amygdala development to abuse actually occurs between ages 10-11, such that even modest abuse during this age has been shown to increase volume of the right amygdala in a dose-response fashion (Petchtel, Lyons-Ruth, Anderson, & Teicher, 2014). Thus, if the greatest effects on areas of the brain relevant to HPA axis control occur at later ages, this could explain why only individuals experiencing abuse at or after age 5 would demonstrate changes to HPA axis regulation in the current sample.

Finally, the cortisol patterns of later maltreated children in the current study could be a byproduct of circadian-related areas of functioning that later, more recent experiences of maltreatment may disrupt, such as sleep. In the past, researchers have found associations between maltreatment and sleep disruption (Glod, Teicher, Hartman, & Harakal, 1997). Sleep patterns have been shown to be "protected" in younger maltreated children (Tininenko, Fisher, Bruce, & Pears, 2010), with issues not typically occurring until later developmental periods (Dahl, 1996). The nature of the disrupted diurnal cortisol patterns that have been associated with poor sleep (i.e., less change across the day, often with lower morning and higher evening levels; Palesh et al., 2008) are

similar to those found for the later maltreated children in this investigation. As sleep patterns drift, circadian patterns could follow (or vice versa). Thus, another reason that later maltreated children in this study may have demonstrated disrupted cortisol functioning could be due to the impact of maltreatment on other variables, such as sleep, and/or additional unmeasured characteristics of the late maltreatment group.

*Internalizing Symptoms*

There was no relationship between late maltreatment and internalizing symptoms. Additionally, there was no relationship between the cortisol dysfunction that later maltreated children exhibited and internalizing symptoms. Although it was not hypothesized that there would be mediation of internalizing symptoms by cortisol for later maltreated children, given the observed effects of late maltreatment on cortisol for children in the current study, the hypothesized relevance of HPA function to the development of internalizing symptoms, and the suggestion of possibly enhanced sensitivity of effects of late maltreatment on amygdala development (Petchtel et al., 2014), it is necessary to speculate about why cortisol changes evident in the late maltreatment group did *not*, then, predict internalizing symptoms in the current study.

A possible reason for the lack of relationship between cortisol and internalizing symptoms for late maltreated children could be that HPA dysregulation may not yet have had time to behaviorally manifest. Such "sleeper" effects would suggest that HPA changes may occur initially in the absence of behavioral changes, and are plausible given that HPA axis changes often predate behavioral changes in the opposite direction (e.g., delay in effect of psychotropic medication on improved depressive behavior). Heuser and

colleagues (1996) demonstrated that antidepressant treatment normalized HPA function within 1 week of administration, but symptom improvement typically requires 2-4 weeks. Thus, it may be that for those children whose primary path toward internalizing symptoms is through HPA dysfunction, the dysfunction takes time to register. If this is true, it would support the idea that HPA dysfunction is a mechanism that comes prior to symptom onset, but challenges the hypothesis that this mechanism is specific to early maltreated kids. If HPA mechanisms are more relevant for later maltreated kids, it could mean that 1) early and late maltreated kids follow different paths to internalizing symptoms, and 2) the late maltreated children in this study may not have had a long enough time since data collection for dysregulation to behaviorally manifest. In this way, it could be that past studies finding relationships among maltreatment, HPA dysfunction, and internalizing symptoms just so happened to have measured all of these things long after the onset of symptoms. Longitudinal studies of maltreatment, HPA function, and internalizing symptoms would help clarify whether HPA dysfunction better reflects risk for or impact of internalizing symptoms in maltreated children.

**Genetic Risk**

Contrary to expectation, there was neither a direct effect of multigenic risk on cortisol functioning, nor did this aspect of risk moderate the relationship between maltreatment and cortisol functioning, regardless of coding scheme utilized. It has been common in the literature to use genotype as a proxy for functional relevance, when, in fact, functional relevance is not well-established. Although the current study included only SNPs that have exhibited functional relevance, there was no observed effect on

cortisol functioning. However, it is important to note that, despite best efforts made to consider differential effects of ethnicity, gender, and tissue type when creating the coding scheme for the multigenic risk variable, this information was not readily available in many of the studies used to classify risk. Thus, it is possible that some alleles classified as risk were, in fact, not associated with biological functioning in the way it was presumed for this group of African American children.

Additionally, genes do not operate in isolation. Although the multigenic risk score captured several genes involved across a biological system, it utilized an additive score to reflect effects across genes. While there has been speculation that even complex traits exhibit additive genetic variance (Hill, Goddard, & Visscher, 2008), other studies of anxiety and depression have demonstrated the multiplicative effects of genes (e.g., Cicchetti & Rogosch, 2014). As the knowledge base about the effects of single SNPs accumulates, future studies could use this information to assign weighted scores to better capture genetic risk across a variety of SNPs.

**Limitations**

This study is the first to examine concurrent associations among maltreatment, genetic factors, HPA axis function, and internalizing symptoms in African American children using structural equation modeling. Prospective assessment of maltreatment using DHS records and use of a classification system allowing for the examination of important maltreatment parameters is an incredible advantage of this study. The sample was large, allowing for adequate power to detect effects. The use of latent variables is another strength, as it allowed for a better representation of internalizing symptoms

among various measures while minimizing measurement error. Additionally, the use of an ethnically homogeneous sample allowed for population stratification issues to be evaded, perhaps offering a more accurate picture of genetic association for this racial group.

Despite these strengths, there are limitations to consider. First, the sample, overall, was actually quite normative with regard to the level of internalizing symptoms, externalizing symptoms, and HPA axis functioning, and mean differences were generally small. Thus, the current sample may not be a highly representative sample of maltreated children, overall. More importantly, predictors explained only 4.2% of variability in internalizing symptoms and 2.5% of variability in cortisol functioning. Findings must therefore be interpreted with great caution in light of these features. Secondly, although a goal was to explore mechanisms using moderated mediation, the fact of the matter is that this is a cross-sectional study and pathways simply reflect linear associations at one point in time of development. Direction of relationships cannot, and should not, be implied.

Additionally, it was not possible to fully represent the diurnal pattern given that samples were not collected at wake or at bedtime. Although this would have been optimal, the characteristics of this very low SES sample makes home collection of salivary samples a difficult task. That is, the disorganization that often accompanies families living in poverty can introduce a range of collection error problems (e.g., time variation, failure to properly store samples following collection, etc.). Thus, having a consistent collection time and storing procedure in the context of the summer camp,

though not allowing for examination of the full diurnal picture, may actually have provided a more accurate representation than home-based assessment.

Information on wake-up time was also not obtained, precluding the possibility of controlling for this variable in analyses. Wake-up time is relevant to the daytime production pattern given that morning levels are higher after awakening due to the CAR; thus, morning levels are more so related to the time a child awoke rather than the chronological time of day. As such, without controlling for time of wake, morning values used in the current study may vary as a function of a child's wake-up time rather than due to their maltreatment status, thereby influencing associations of late maltreatment with cortisol difference scores. It is unlikely that this is the case, however, given that this possibility would have required the entire group of later maltreated children to have woken up much earlier in the day than the other groups of children.

It was also not possible to control for other factors known to influence HPA axis functioning, such as pubertal status or use of medication such as acetaminophen, oral contraceptives, and psychotropic agents. Children's cortisol levels increase during puberty (Gunnar,Wewerka, Frenn, Long, & Griggs, 2009; Stroud et al., 2009), and medication use has been shown to affect stress hormone levels. With relevance to the current study, antipsychotics have been specifically linked to flatter diurnal rhythms in children regardless of risk-status (Hibel, Granger, Cicchetti, & Rogosch, 2007). Such agents could, thus, have introduced error variance into the current investigation and impacted results especially if more children in the late maltreatment group were disproportionately utilizing antipsychotic or other medications. Furthermore, pubertal

status may have similarly confounded the observed association of maltreatment and cortisol production across the day. Although a majority of children in the study were between the ages of 8 and 10, some of these children and those that were closer to the maximum age of 12, may have already entered the pubertal stage. This is possible given that early puberty is more likely to occur in African Americans, especially girls (Herman-Giddens et al., 1997). However, despite lack of pubertal assessment, age was considered and did not change the findings when included in the structural model. Nonetheless, age is not equivalent to pubertal status, and both puberty and medication use should be considered in future projects in effort to best understand HPA function and stress-related vulnerability.

**Conclusions and Future Directions**

Development involves complex, dynamic, interactive systems that cascade and shift across the lifespan. Maltreatment introduces a potent pathogen into the developmental process and can have far-reaching effects. Two such implications for maltreatment on development includes internalizing symptoms and alterations to the typical circadian process of the HPA axis, depending on characteristics of maltreatment including timing, chronicity, and recency, as revealed in the current investigation. There are numerous pathways a child may follow toward both internalizing symptoms and HPA axis dysfunction and a host of complications that can follow the onset of each. Only through longitudinal studies, and continued preclinical research efforts, can a better understanding of the antecedents and consequences of internalizing symptoms and diurnal disruption following or preceding experiences of maltreatment be attained. Such studies will help

clarify the nature of development and, ultimately, could help guide preventive interventions.

In the context of future studies, it will be of utmost importance for researchers to embark upon prospective, longitudinal examinations of maltreatment beginning as early in life as possible. Critically, these designs should repeatedly assess psychiatric symptoms (both internalizing and externalizing) as well as other forms of current life stress. Additionally, multiple assessments of HPA function across time will be particularly fruitful. Probing multiple aspects of HPA functioning (i.e., diurnal patterns, CAR, reactivity to/recovery from pharmacologic challenge, and reactivity to/recovery from psychosocial challenge) at multiple time points could help clarify age-related patterns associated with the different aspects of the HPA axis and delineate the nature of involvement of various levels of the system (e.g., effects at the level of the pituitary, adrenal, etc.). Finally, integrative models that consider the dynamic role of other proposed mediating variables at multiple levels (e.g., early attachment, peer relationships, social support, self-esteem, etc.) will add to our knowledge base regarding the ways by which factors induce or protect against vulnerability to internalizing symptoms following experiences of maltreatment, and will help inform treatment and prevention of these problematic disorders.

## Tables

Table 1. *Demographic Characteristics*

| | EM<br>n = 263<br>*M*(*SD*) or % | LM<br>n = 110<br>*M*(*SD*) or % | Non<br>n = 366<br>*M*(*SD*) or % | Test Statistic ANOVA/$\chi^2$ |
|---|---|---|---|---|
| Age | 10.31(1.25) | 10.78(1.35) | 10.30(1.33) | $F(2, 736) = 3.38, p = .035$ |
| Gender (% female) | 44.5 | 51.8 | 54.9 | $\chi^2(2) = 6.72, p = .035$ |
| Genetic Risk | 3.50(1.89) | 3.54(1.19) | 3.63(1.82) | $F(2, 734) = .403, p = .677$ |

*Note.* EM = early maltreatment; LM = late maltreatment; Non = nonmaltreated; ANOVA = analysis of variance. Significant differences in age and gender by group: LM children were significantly older; more female children belonged to the Non group. Differences resulted in both age and gender being controlled for in analyses. However, because age was not a significant predictor of any outcome variables when included in the structural model, and because structural model fit was reduced when age was included, age was not ultimately entered into the final structural model.

Table 2. *Glossary of Genetic Terms*

| Term | Definition |
|---|---|
| Single nucleotide polymorphism (SNP) | Variation in a gene sequence at a single nucleotide base position that can sometimes result in functional changes to the gene product and may influence disease susceptibility. |
| Ancestral Informative Maker (AIMS) | A SNP whose known frequency distribution differs widely between populations from different geographical regions; information can thus be used to classify an individual's ancestral region of origin. |
| Linkage Disequilibrium (LD) | Non-random association of alleles at two or more genetic loci; alleles in LD are typically inherited together. |
| Allele | A different version of a gene at a particular genetic locus; humans have two alleles per genetic locus - one allele from each parent. |
| Genetic Locus | The location or position of an allele/SNP on a chromosome. |
| Major Allele | An allele that is more common in a population. |
| Minor Allele | An allele that is less common in a population. |
| Risk Allele | An allele associated with a deleterious outcome (e.g., depression, greater reactivity, high blood pressure). |
| Genotype | Pair of alleles at a particular genetic locus. |
| Heterozygous | Possessing two of the same alleles at a particular genetic locus. |
| Homozygous | Possessing two different alleles at a particular genetic locus. |
| Hardy Weinberg Equilibrium (HWE) | Principle in population genetics postulating that allele/genotype frequencies will remain stable within a population over time, unless evolutionary influences shift the distribution; observed distributions in a sample can be tested against expected distributions to determine whether deviations exist. |
| Additive Genetic Coding Model | Method of coding/grouping genotypes that assumes risk outcomes occur in a graded manner, such that each group (homozygous risk, heterozygous risk, homozygous non-risk) will differ from one another, with homozygous risk individuals exhibiting the most deleterious outcomes. |
| Recessive Genetic Coding Model | Method of coding/grouping genotypes that assumes risk outcome occurs only for individuals who are homozygous for the risk allele, whereas heterozygous risk individuals will not differ from homozygous non-risk individuals. |
| Dominant Genetic Coding Model | Method of coding/grouping genotypes that assumes risk outcome is evident for *any* individual (i.e., homozygous or heterozygous) carrying at least one risk allele (i.e., both heterozygous and homozygous risk genotypes will differ from those not carrying risk allele, but risk heterozygotes and homozygotes will not differ from one another). |

Table 3. *Correlations Between Latent Variable Indicators and Other Study Variables*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) EM | -- | | | | | | | | | | | |
| (2) LM | .31*** | -- | | | | | | | | | | |
| (3) Gene Risk | -.03 | -.01 | -- | | | | | | | | | |
| (4) Cort Change | -.02 | .09* | -.01 | -- | | | | | | | | |
| (5) RCMAS | .07† | .03 | .06 | .06† | -- | | | | | | | |
| (6) CDI | .11** | .00 | -.02 | .03 | .61*** | -- | | | | | | |
| (7) Self_NA | .07† | .05 | .00 | .00 | .49*** | .53*** | -- | | | | | |
| (8) TRF_int | .04 | -.04 | .04 | -.06† | .04 | .14*** | .05 | -- | | | | |
| (9) Clr_PA | -.07* | .03 | -.04 | .08* | -.02 | -.11*** | -.05 | -.46*** | -- | | | |
| (10) Clr_NA | .08* | .00 | -.01 | .04 | .11** | .20*** | .10** | .63*** | -.43*** | -- | | |
| (11) Ext | .15*** | .03 | .03 | .09* | .09* | .14*** | .11** | .14*** | -.18*** | .33*** | -- | |
| (12) Age | -.04 | .13*** | -.04 | -.04 | .07† | -.01 | -.14*** | -.02 | -.04 | .03 | -.06† | -- |
| (13) Female | .09** | -.01 | .01 | .12*** | .09* | .08* | .01 | -.07† | -.01 | .08* | -.01 | -.04 |
| Mean | -- | -- | 3.57 | -.27 | 45.43 | 7.57 | 1.92 | 47.67 | 3.38 | 1.47 | 53.56 | 10.37 |
| SD | -- | -- | 1.77 | .18 | 11.17 | 6.94 | .77 | 7.91 | .61 | .39 | 9.37 | 1.31 |

*Note.* EM = early maltreatment; LM = late maltreatment; Gene risk = multigenic risk score; Cort change = cortisol change across the day; RCMAS = Revised Checklist for Manifest Anxiety Scale (overall T-scores); CDI = Children's Depression Inventory (total raw scores); Self_NA = Self-reported negative affect (mean score) of the Positive and Negative Affect Schedule-Child version (PANAS-C); TRF = Teacher's Report Form counselor-reported internalizing symptoms (overall internalizing T-scores); Clr_PA = counselor-reported positive affect (mean score) of the PANAS-C; Clr_NA = counselor-reported negative affect (mean score) of the PANAS-C; Ext = counselor-reported externalizing symptoms (overall externalizing T-scores of the TRF). N = 716 (range = 716-739 due to missing variable data).
† $p \leq .10$, * $p \leq .05$, ** $p \leq .01$, *** $p \leq .001$.

Table 4. *Genetic Coding Summary with Genotype Frequencies by Maltreatment Group*

***CRHR1***

| **rs7209436** | | | | **rs110402** | | | | **rs242924** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| risk allele: T<br>coding type: recessive<br>HWE: $p$ = .80 | | | | risk allele: A<br>coding type: recessive<br>HWE: $p$ = .06 | | | | risk allele: T<br>coding type: recessive<br>HWE: $p$ = .33 | | | |
| Genotype Frequencies | | | | Genotype Frequencies | | | | Genotype Frequencies | | | |
| | EM | LM | Non | | EM | LM | Non | | EM | LM | Non |
| TT | 30 | 4 | 44 | AA | 35 | 6 | 47 | TT | 25 | 4 | 35 |
| CT | 106 | 54 | 159 | AG | 101 | 52 | 147 | GT | 100 | 49 | 139 |
| CC | 127 | 52 | 162 | GG | 127 | 52 | 171 | GG | 138 | 57 | 191 |

***FKBP5***

| **rs9296158** | | | | **rs1360780** | | | | **rs9470080** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| risk allele: A<br>coding type: dominant<br>HWE: $p$ = .16 | | | | risk allele: T<br>coding type: dominant<br>HWE: $p$ = .97 | | | | risk allele: T<br>coding type: dominant<br>HWE: $p$ = .11 | | | |
| Genotype Frequencies | | | | Genotype Frequencies | | | | Genotype Frequencies | | | |
| | EM | LM | Non | | EM | LM | Non | | EM | LM | Non |
| AA | 62 | 39 | 96 | TT | 48 | 30 | 68 | TT | 59 | 35 | 92 |
| AG | 128 | 46 | 174 | CT | 128 | 53 | 182 | CT | 123 | 51 | 171 |
| GG | 72 | 24 | 93 | CC | 86 | 27 | 114 | CC | 79 | 24 | 100 |

***NR3C2***

| **rs5522 (I180V)** | | | | **rs2070951 (-2G/C)** | | | |
|---|---|---|---|---|---|---|---|
| risk allele: C<br>coding type: dominant<br>HWE: $p$ = .79 | | | | risk allele: G<br>coding type: recessive<br>HWE: $p$ = .03 | | | |
| Genotype Frequencies | | | | Genotype Frequencies | | | |
| | EM | LM | Non | | EM | LM | Non |
| CC | 4 | 0 | 4 | GG | 134 | 57 | 199 |
| CT | 47 | 23 | 61 | CG | 104 | 41 | 130 |
| TT | 211 | 87 | 299 | CC | 25 | 12 | 34 |

***NR3C1***

| **rs41423247 (BclI)** | | | |
|---|---|---|---|
| risk allele: G<br>coding type: dominant<br>HWE: $p$ = .47 | | | |
| Genotype Frequencies | | | |
| | EM | LM | Non |
| GG | 7 | 2 | 23 |
| CG | 91 | 39 | 129 |
| CC | 165 | 69 | 213 |

*Note.* HWE = Hardy Weinberg Equilibrium; reported HWE p-values apply to genotype frequencies across the maltreatment groups.

**Figures**

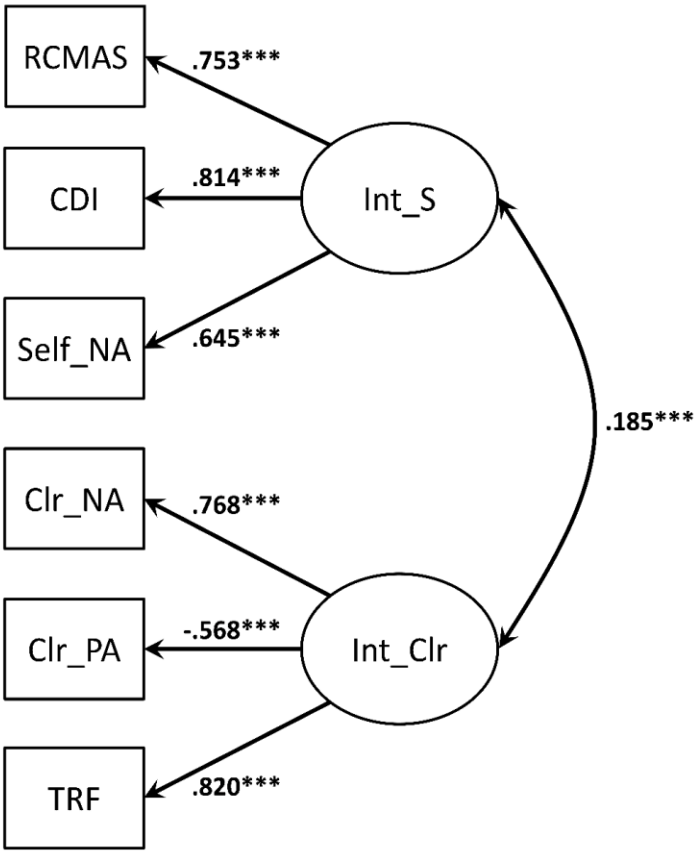


*Figure 1*. Confirmatory factor analysis final measurement model with standardized factor loadings. Note. RCMAS = Revised Checklist for Manifest Anxiety Scale (overall T-scores); CDI = Children's Depression Inventory (total raw scores); Self_NA = Self-reported negative affect (mean score) of the Positive and Negative Affect Schedule-Child version (PANAS-C); Clr_NA = counselor-reported negative affect (mean score) of the PANAS-C; Clr_PA = counselor-reported positive affect (mean score) of the PANAS-C; TRF = Teacher's Report Form counselor-reported internalizing symptoms (overall internalizing T-scores). Fit Indices: $\chi^2$ (8) = 16.86; RMSEA = .039; CFI = .991; TLI = .984. ***$p \leq .001$.

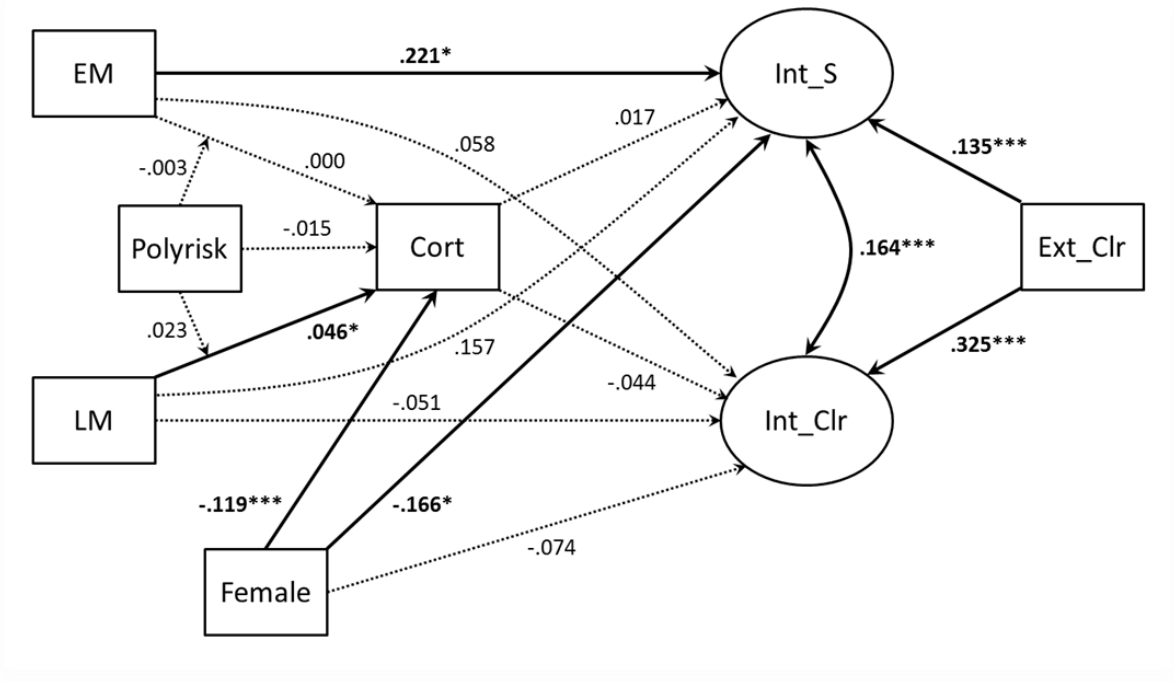


*Figure 2*. Structural equation model of maltreatment as a predictor of internalizing symptoms, controlling for gender. Note. EM = early maltreatment; LM = late maltreatment; polyrisk = multigenic risk score; cort = cortisol difference across the day; Int_S = self-reported internalizing symptoms latent variable; Int_Clr = counselor-reported internalizing symptoms latent variable; Ext_Clr = counselor-reported externalizing symptoms (TRF T-scores). Significant (solid lines) and non-significant paths (dashed lines) are labeled with their respective standardized coefficients. Fit indices: $\chi^2$ (47) = 135.37; RMSEA = .050, CFI = .936, TLI = .904. * $p \leq .05$, ** $p \leq .01$, *** $p \leq .001$.

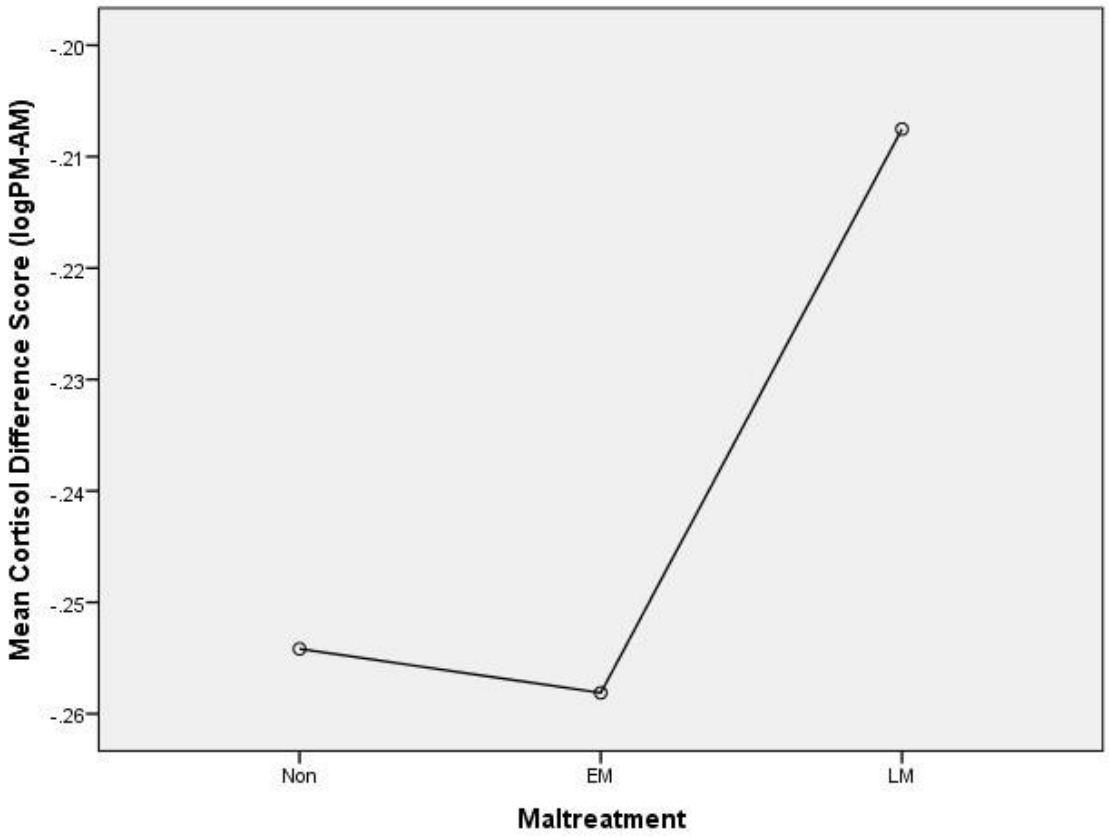


*Figure 3.* Mean cortisol difference scores among maltreatment groups. Note. EM = early maltreatment, LM = late maltreatment, Non = nonmaltreated. Values represent change in cortisol across the day as indexed by the mean (across 5 days), log-transformed morning value subtracted from the mean, log-transformed afternoon value. Higher scores (more positive, closer to zero) represent less change from morning to afternoon. Significant differences exist among groups such that LM have higher scores (less change) compared to EM ($z = 2.34$, $p = .019$, *std est* = .046) and compared to Non children ($z = 2.43$, $p = .015$, *std est* = .046). EM children did not differ from Non in their cortisol difference value ($p = .984$).

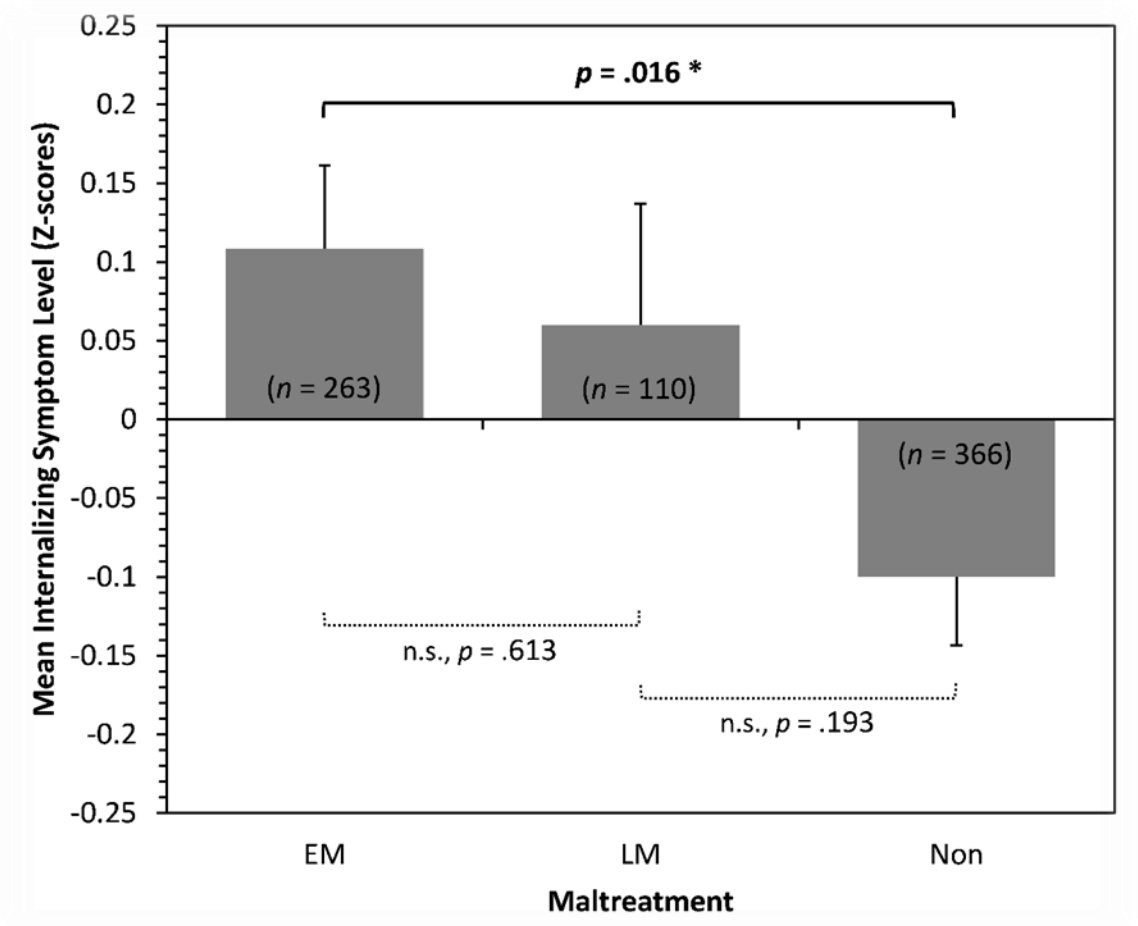


*Figure 4*. Group differences in self-reported internalizing symptoms. Note. EM = early maltreatment, LM = late maltreatment, Non = nonmaltreated. Indicator values used for the self-reported latent variable (i.e., RCMAS T-score, CDI raw score, PANAS_NA mean score) were z-scored; mean of z-scores among the three indicators are depicted in the figure.

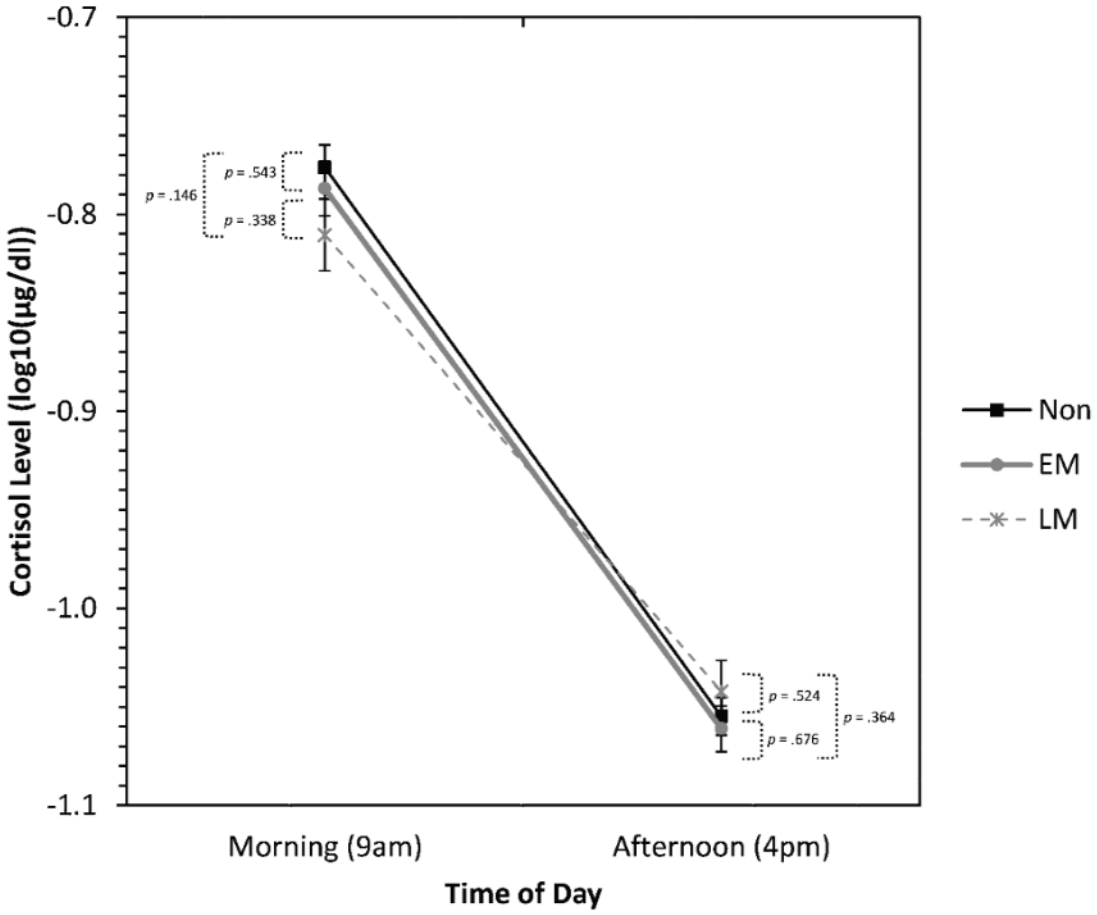


*Figure 5*. Mean morning and afternoon cortisol values and change patterns across the day by maltreatment group. Note. EM = early maltreatment, LM = late maltreatment, Non = nonmaltreated. Groups did not differ significantly at either time of day (EM vs non morning: $z$ = -.609, $p$ = .543, *std. est.* = -.011; LM vs non morning: $z$ = -1.453, $p$ = .146, *std. est*. = -.034; EM vs non afternoon: $z$ = -.418, $p$ = .676, *std. est.* = -.006; LM vs non afternoon: $z$ = .638, $p$ = .524, *std. est.* = .013). However, there was a slight lowering of morning and a slight increase of afternoon levels for LM children.

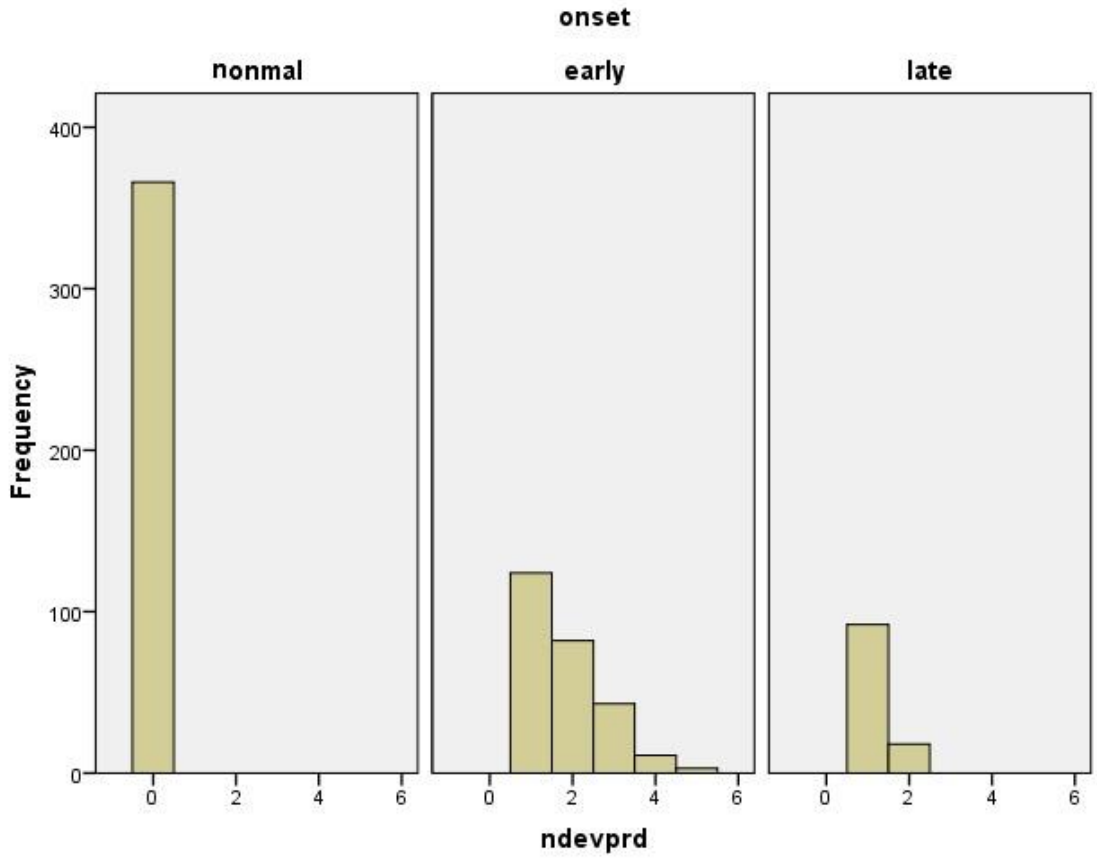


*Figure 6*. Overlap of maltreatment timing and number of developmental periods across which maltreatment occurred (chronicity). Note. Ndevprd = number of developmental periods (chronicity). More children in the early maltreatment group experienced maltreatment during two (EM n = 82; LM n = 18) or three (EM n = 43; LM n = 0) developmental periods. The difference between distributions was significant ($\chi^2$ (4) = 794.75, $p$ = .000).

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