INTERACTION OF POLYMORPHISMS IN THE FKBP5 GENE & CHILDHOOD ADVERSITY ON THE CORTISOL RESPONSE TO A PSYCHOSOCIAL STRESS TASK IN ADOLESCENTS AND YOUNG ADULTS

by

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Abstract

Childhood adversity is often associated with devastating physical, cognitive, and psychosocial outcomes, and is a major public health problem in terms of its prevalence and economic cost. Childhood adversity is associated with increased risk for psychopathology, as well as with dysregulation of the neurobiological stress response. An additional factor known to alter neuroendocrine functioning and increase psychopathology risk is polymorphisms within the FKBP5 gene. The goal of the current study was to examine the gene-environment interaction of childhood adversity and variation in the FKBP5 gene on the cortisol response to a psychosocial stress task (i.e., the Trier Social Stress Test). The final sample consisted of 90 depressed and non-depressed adolescents and young adults (11 - 21 years). Childhood adversity was assessed using the Childhood Experience and Abuse Scale (CECA; Bifulco et al., 1994), and was defined as the presence versus absence prior to 18 years of age of severe physical, sexual, or emotional abuse or neglect, witnessing to domestic discord/violence, or peer-perpetrated bullying. Participants were genotyped at the rs1360780 site of the FKBP5 gene and grouped according to whether they had at least one risk T allele (i.e., TT or TC genotype versus the CC genotype). Controlling for depression and anxiety psychopathology, I found a significant interaction of FKBP5 and childhood adversity status such that individuals with the FKBP5 risk allele (i.e., TT or TC genotype) and a history of childhood adversity showed a distinct cortisol response pattern characterized by decreasing cortisol from baseline and less cortisol output compared to individuals without childhood adversity. This relationship was specific to the experience of severe adversity and appeared to be strongest when adversity was defined as witnessing domestic discord/violence. These results are consistent with a diathesis-stress model in which the FKBP5 risk allele leaves individuals vulnerable to neurobiological dysregulation in the face of severe adverse experience in childhood. The implications of this research for understanding stress-
related psychopathology and the limitations of this gene-environment interaction design are discussed.
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Chapter 1

Introduction

Early adverse experience can have devastating and long-lasting consequences. A history of adverse experiences in childhood, including physical, sexual, or emotional abuse, peer victimization, or witnessing domestic violence, have been associated with all forms of psychopathology including major depression, anxiety disorders, substance use disorders, psychotic disorders, and personality disorders (Cohen, Brown, & Smailes, 2001; Green et al., 2010; Kessler et al., 2010; Read, Bentall, & Fosse, 2009). Further, early adversity has been associated with a host of persistent social, emotional, and physical challenges, including negative peer interactions, low self-esteem, social anxiety, poor emotion regulation, and obesity (El-Sheikh & Harger, 2001; Felitti et al., 1998; Gottman & Katz, 1989; Hawker & Boulton, 2000; Kessler, Davis, & Kendler, 1997; Kim & Cicchetti, 2003; Taylor, Repetti, & Seeman, 1997).

The association of childhood adversity and the onset of stress-related disorders such as depression and anxiety in adolescence and adulthood is especially well documented (Kendler et al., 2000; Kessler, Davis, & Kendler, 1997; MacMillan et al., 2001). For example, childhood abuse and neglect significantly predict the onset of mood and anxiety disorders in adulthood (Clark, Caldwell, Power, & Stansfeld, 2010). Further, adolescents with a history of abuse or neglect are at a 2 to 5 fold increased risk for depression in comparison to adolescents without this history (Harkness & Lumley, 2008). Other forms of adversity including bullying and witnessing domestic violence have similarly been associated with depression and anxiety in childhood and early adolescence (Arseneault, et al., 2008; Crawford, Cohen, Midlarsky, & Brook, 2001). In addition to an increased risk for the onset of psychopathology, a history of adversity affects the
clinical course of depression and anxiety disorders. Brown and Moran (1994) found an association of physical or sexual abuse and parental indifference with greater chronicity of depressive episodes. Moreover, in a five-year prospective study on depression and anxiety, patients with a history of sexual abuse in childhood were less likely to remit from their disorder than those without an abuse history (Zlotnick, Mattia, Zimmerman, 2001). Individuals with a history of childhood abuse and/or neglect are also significantly more likely to suffer a recurrence of their depression even following successful treatment than those without this history (Harkness, Bagby, & Kennedy, 2012).

Although it is difficult to estimate the prevalence of childhood adversity broadly defined, the pervasiveness of early adverse experience is apparent in the estimates of more specific experiences. In Canada, the incidence of reported and substantiated child abuse and neglect is 22 per 1000 children; however, this is an underestimate, as only a minority of abuse cases are reported to authorities (Trocmé et al., 2005). The retrospective self-reported prevalence of physical and sexual abuse suggest that these experiences are much more common. In a random sample of nearly 10,000 Ontario residents, 31.2% of men and 21.1% of women self-reported a history of childhood physical abuse, and 4.3% of men and 12.8% of women reported a history of childhood sexual abuse (MacMillan et al., 1997; Tonmyr & Bartholomew, 1998). Canadian estimates of children witnessing violence in the home are also alarming. In a sample of over 35,000 Canadians, 8% of parents reported that their children witnessed physical violence in the home; however, this estimate was inclusive of violence between any household members including siblings (Dauvergne & Johnson, 2001). Finally, bullying has also been identified as a pervasive problem with approximately one-third of adults reporting a history of peer-perpetrated bullying during their school years (Kim & Leventhal, 2008).
Altogether, the above statistics suggest that adversity is prevalent among children and adolescents and presents a significant risk for the development and persistence of ongoing psychopathology. Not only is childhood adversity a major public health problem, it is also an immense economic burden to society. The annual costs of child abuse alone are estimated at nearly $16 billion in Canada (Bowlus, McKenna, Day, and Wright, 2003). These costs include judicial and social services expenditures as well as long-term mental health costs. Given the pervasiveness of childhood adversity and its implications for mental health, it is important to understand the etiological processes that might underlie its relation with psychopathology in order to identify priority prevention and treatment targets.

In addition to the social and emotional consequences of the abovementioned childhood experiences, research on the biological correlates of early adverse experience have determined that childhood adversity has critical consequences for the developing brain. By corroborating experimental evidence from animal models on the adverse effects of early life stress, research in humans has determined that childhood adversity leads to extensive structural and functional changes in the brain (see Teicher, Tomoda, & Andersen, 2006). In particular, studies using structural magnetic resonance imaging (MRI) have identified differences between individuals with and without a history of adversity in childhood in the medial prefrontal cortex, hippocampus, and corpus callosum, such that those with a history of adversity have lower gray matter volume in these critical cortico-limbic circuits than those without (e.g., Andersen et al, 2008; Teicher, Anderson, & Polcari, 2012).

Early life adversity is believed to produce neurotoxic effects in the above brain areas by interfering with the processes of neurogenesis and synaptic overproduction and pruning that occur in critical periods across development (Teicher et al. 2006). In particular, excessive release of
glucocorticoids in the face of stress has been proposed as a key neurotoxic mechanism (Andersen & Teicher, 2004; Meaney, Stewart, & Beatty, 1981; Sapolsky, 1996; Schapiro, 1971).

Glucocorticoids, including the hormone cortisol in humans, are the end products of activation of the body’s neurobiological stress response, which is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, and further moderated by cortico-limbic circuits.

The seminal work by Meaney and colleagues was the first to show how early environmental experience can affect HPA axis functioning long after the offset of early life stress using maternal separation models in rodents (see Francis & Meaney, 1999). Since then, long-term changes in functioning of the HPA axis as a result of early adversity have also been well established in humans (see Hart & Rubia, 2012 and Tarullo & Gunnar, 2006). Although the majority of studies on early adversity in humans have focused on childhood maltreatment (i.e., childhood abuse and neglect), other forms of early adversity including bullying, and witnessing domestic violence are associated with similar outcomes. Following from the animal literature, researchers have suggested that one mechanism through which childhood adversity heightens vulnerability to stress-related disorders is that the HPA axis becomes sensitized from overstimulation during early critical periods of development. This results in a reduced threshold of proximal stress necessary for the emergence of psychopathology (Hammen, Henry, & Daley, 2000; Heim, Plotsky, & Nemeroff, 2004; Lupien, McEwan, Gunnar, & Heim, 2009; Monroe & Harkness, 2005).

The above model provides a compelling account for the onset of psychopathology in the face of adverse childhood experience. However, not all individuals with a history of childhood adversity go on to develop psychopathology; thus, additional vulnerability factors have been proposed that predispose individuals to adverse outcomes in the face of early stressful
experiences. In particular, variants of specific genes (i.e., polymorphisms) that modify the function of hormonal receptors involved in the neurobiological stress response represent a logical target. Specifically, the gene-environment moderation model (i.e., a diathesis-stress model) of psychopathology states that individuals with specific genetic polymorphisms will be vulnerable to the effects of adverse environments such that they are more prone to developing psychopathology, whereas those without the genetic vulnerability will be resilient to adversity. Studies investigating gene-environment interactions in disorders such as depression have proliferated widely over the last decade. Most of these studies have focused on genes in the serotonin system (e.g., the serotonin transporter gene [5-HTTLPR]) and have found significant support for gene-environment interaction (see Karg et al., 2011 for a meta-analytic review). However, as outlined above, the neurobiological stress response system is a primary neurobiological mechanism for psychopathology risk. Therefore, genetic variants that code for aspects of this system deserve greater empirical attention. For example, the FKBP5 gene codes for one of several proteins that regulate the negative feedback mechanism of the HPA axis.

Thus, the goal of the current study is to examine the interaction of FKBP5 gene variants and childhood adversity on the neurobiological stress response. By examining this interaction, this study seeks to investigate whether dysregulation of the neurobiological stress response follows a diathesis-stress model of pathophysiology, such that only individuals with both genetic vulnerability and early environmental risk show abnormal stress responsivity. Specifically, I hypothesize that individuals with the FKBP5 risk variant are more vulnerable to dysregulation of the HPA axis in response to stress than those without genetic risk at FKBP5, but only in the context of a history of childhood adversity. I will evaluate neurobiological stress response functioning by assessing cortisol release following a laboratory-induced psychosocial stress task.
that is known to stimulate the stress response – the Trier Social Stress Task (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). This study differs from more conventional tests of the diathesis-stress model, in which the ‘disease’ outcome is conceptualized as the presence versus absence of a particular psychiatric diagnosis (e.g., major depression). Instead, here, the outcome is the pathophysiology of the neurobiological stress response. As such, the design of this study is consistent with a transdiagnostic approach to psychopathology by focusing on domains of neurobiological functioning that cut across traditional psychiatric disorders. As such, results of this study may have implications for understanding the etiology of the spectrum of stress-related disorders, including major depressive disorder and anxiety disorders.

**Physiology and Measurement of the Neurobiological Stress Response**

When the neurobiological stress response system is initiated in reaction to acute environmental stress, the HPA axis becomes activated (see Figure 1). This triggers the release of corticotropin releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus to the anterior pituitary via the hypothalamo-hypophyseal portal system. At the anterior pituitary, CRH stimulates the release of adrenocorticotropic hormone (ACTH), which in turn stimulates the production and release of glucocorticoids from the adrenal glands. The most prominent of these glucocorticoids is cortisol. Upon cessation of the stressor, cortisol acts in a negative feedback mechanism to inhibit further CRH secretion. When cortisol binds to glucocorticoid receptors (GRs) in the hypothalamus, a cellular cascade is initiated, which prevents further CRH secretion and contributes to termination of the stress response (Tsigos & Chrousos, 2002). The typical pattern of cortisol output in response to an acute stressor is a peak in cortisol 20 to 30 minutes following the onset of the stressor (cortisol reactivity) and a decrease in cortisol concentration toward baseline up to 60 minutes following the offset of the stressor (cortisol recovery; Dickerson
Figure 1. A basic representation of the Hypothalamic-Pituitary-Adrenal (HPA) axis. Upon activation of the neurobiological stress response, corticotropin-releasing hormone (CRH) is released from the hypothalamus. CRH stimulates the anterior pituitary to release adrenocorticotropin hormone (ACTH), which in turn stimulates the production and release of glucocorticoids, including cortisol, from the adrenal glands. Cortisol acts in a negative feedback mechanism to prevent further HPA axis activation and cortisol release by interacting with glucocorticoid receptors in the hypothalamus (adapted from Tsigos & Chrousos, 2002).
The acute neurobiological stress response is an adaptive reaction to stress that is designed to recruit the necessary resources to respond to a stressor; however, prolonged activation of this response system can be damaging due to hormonal overexposure (McEwen, 2000). For example, high levels of glucocorticoids have been shown to induce hippocampal atrophy in animal models (see Sapolsky, 1996; Sapolsky, Uno, Rebert, & Finch, 1990).

HPA axis function can be measured through (a) basal cortisol levels (e.g., diurnal change), (b) cortisol release following pharmacological challenge (i.e., dexamethasone suppression test [DST], ACTH or CRH challenge), or (c) cortisol release following a laboratory-induced stressor such as a public speaking task (e.g., the TSST). Basal measures of cortisol and pharmacological challenge studies have been criticized for failing to assess the system’s endogenous response to environmental stress. Basal cortisol measures are an index of HPA axis function at rest and typically assess change in resting cortisol across the day, and pharmacological challenge tests artificially induce the stress response through the administration of exogenous glucocorticoids. Dexamethasone is a synthetic glucocorticoid that provides negative feedback to the pituitary, thus suppressing a cortisol response under normal conditions; however, it poorly crosses the blood-brain barrier (De Kloet, 1997). Consequently, although the DST provides valuable information about glucocorticoid receptor functioning at the pituitary, it does not provide information about differences in processing at higher brain levels. Specifically, the DST captures HPA axis “dysregulation” with respect to negative feedback inhibition, but it does not provide insight into the exertion of suprapituitary effects on the HPA axis. In contrast, psychosocial stress tasks provoke activation of the complete neurobiological stress response, including higher order brain systems.
The TSST and similar laboratory stress challenge paradigms address the above limitations. In the TSST, participants perform a public speaking task after which they are surprised with a mental arithmetic challenge. Participants are instructed that a panel of two confederate research assistants will be evaluating their performance on the tasks. For this reason, the TSST is a more ecologically valid provocation of the neurobiological stress response than pharmacologic challenge tests such that it allows for between-group comparisons of cortisol output as would be expected in response to life’s stressors. Variability also exists within the domain of psychosocial challenge tests. Dickerson and Kemeny (2004) found that tasks that include uncontrollability and social evaluative threat, such as the TSST, elicit the greatest cortisol response. In the current study, the TSST paradigm was used to assess the neurobiological stress response. Therefore, the following literature review examining the relation of childhood adversity and FKBP5 polymorphisms on HPA axis function will focus primarily on studies that employ laboratory-induced psychosocial stress challenge procedures such as the TSST.

**Childhood Adversity and the Neurobiological Stress Response**

The majority of studies on childhood adversity and the cortisol response to a laboratory – induced psychosocial stress challenge have found blunted cortisol in those with a history of adversity. That is, individuals with a history of adversity had cortisol concentrations that did not peak as greatly following the laboratory stressor than individuals without a history of adversity. For example, when adversity was defined as childhood maltreatment (i.e., a history of physical or sexual abuse or neglect), adversity was associated with blunted cortisol in healthy adults (Carpenter et al., 2007) and in female adolescents (MacMillan et al., 2009), in both studies controlling for depression and anxiety symptoms. Maltreated or frequently bullied 12 year olds
also showed a blunted pattern of cortisol release (Ouellet-Morin et al., 2011), as did adolescents whose parents had high marital conflict (Lucas-Thompson, 2012). None of these studies found differences in baseline cortisol between those with and without childhood adversity.

Not all studies have associated childhood adversity with blunted cortisol, however. Heim et al. (2000) found no difference in cortisol output in healthy women with and without a history of maltreatment in response to a psychosocial stress task. However, among women with major depression included in this study, a history of maltreatment was associated with greater cortisol output following the stress challenge. A major factor that may account for the discrepancy between these results and those reported above is that Heim and colleagues focused on women with depression whereas the studies above were conducted primarily on healthy individuals. Thus, the presence of psychopathology may moderate the effect of childhood adversity on the cortisol response. In support of this contention, and consistent with the results of Heim and colleagues a recent study including adolescents with depression reported that those with a history of maltreatment in childhood had greater output following the TSST than those without this history (Harkness, Stewart, & Wynne-Edwards, 2011). However, this was only the case for adolescents whose depression symptoms were in the mild/moderate range. Adolescents with severe depression showed a blunted cortisol response regardless of whether they had a history of childhood maltreatment. The results of the studies reviewed above make clear that the relation of early experience to brain functioning is complex and is significantly moderated by other contextual factors such as the presence of current psychopathology.

The reason why psychopathology, such as depression, moderates the relation of childhood adversity to neurobiological stress response functioning is unclear at present. However, there is emerging evidence to suggest that at least part of the answer may lie in the examination of
vulnerability factors that are common to heightened risk for both psychopathology and exposure to adversity – namely, genetic variations that govern the stress response. The current study will examine variants of a specific gene associated with glucocorticoid receptor sensitivity (the FKBP5 gene) as a moderator of the relation of childhood adversity to the neurobiological stress response.

**FKBP5 Gene and the Neurobiological Stress Response**

Several studies have implicated polymorphisms of glucocorticoid receptor (GR) genes as determinants of variability in the cortisol response to a psychological stressor, which is not surprising given the critical role of GRs in terminating the cortisol response (DeRijk & de Kloet, 2008). However, additional determinants of GR function include proteins that modulate the affinity of the GR for glucocorticoids. One of these proteins, the FK506-binding protein 51 (FKBP51), inhibits the ability of cortisol to bind to GRs, thus interfering with the ultra-short negative feedback loop within the cell, and ultimately negative feedback of the HPA axis more broadly. Polymorphisms in the FKBP5 gene, which codes for FKBP51, have also been implicated in the cortisol response to pharmacologic and psychosocial stress challenge. Expression of the FKBP5 gene, and production of FKBP51, is induced by cortisol through activation of glucocorticoid response elements (Vermeer et al., 2003). FKBP51 is a co-chaperone protein that interacts with heat-shock protein 90 to decrease the affinity of GRs for binding glucocorticoids. Given that GRs are a major regulator of the HPA axis, GR insensitivity (i.e., GR resistance) due to FKBP51 results in an increase in cortisol levels, as demonstrated in studies with New World Monkeys (Denny, Valentine, Reynolds, Smith, & Scammell, 2000; Wochnik et al., 2005). Rodent
models have also suggested a direct role for FKBP5 gene function and stress responsiveness, such that FKBP5 knockout mice show an impaired neuroendocrine response (Touma et al., 2011).

Several different genetic variants (i.e., single nucleotide polymorphisms [SNPs]) within the sequence of DNA that comprises the FKBP5 gene have been examined in relation to stress-related psychopathology and neuroendocrine function. These sites are identified by their reference number (rs; e.g., rs1360780). For every SNP, an individual has two alleles – one they inherit from their mother and one from their father – and these alleles can take one of two forms. Thus, for every SNP, there are three possible genotypes (e.g., at rs1360780: TT, CT, and CC). Alleles may be referred to as “risk” alleles when associated with negative outcomes. Alternatively, because of the uneven distribution of alleles in the population, alleles are sometimes referred to as minor or major alleles based on their relative population frequency. The former terminology will be used in the current study.

Consistent with the hypothesis of an interaction of FKBP5 polymorphisms and childhood adversity on neurobiological stress response functioning, a number of recent studies have found evidence for an interaction of FKBP5 SNPs and early adverse experience on risk for stress-related psychopathology, namely depression and posttraumatic stress disorder (PTSD). Appel et al. (2011) identified an interaction between retrospective reports of physical abuse during childhood and variation at rs1360780 of the FKBP5 gene in predicting lifetime depression diagnosis among adults, such that homozygous carriers of the risk T allele who were exposed to physical abuse in childhood were at increased risk for depression compared to those with only one or no risk alleles. No main effects for abuse or FKBP5 genotype were detected. In two independent prospective studies, Zimmermann et al. (2011) examined the interaction of childhood maltreatment, assessed at different points across childhood, and polymorphisms of the FKBP5
gene in predicting depression onset. Participants homozygous for the risk allele at rs1360780 and who had a history of childhood maltreatment were most likely to report a major depressive episode in adulthood. Further, although the main effect of childhood maltreatment was significant, there was no main effect of the FKBP5 gene. This interaction was not replicated in the second sample. The authors hypothesized that the reason for the discrepant results across samples may have been because of differences in how childhood maltreatment was defined. Binder et al. (2008) also found an interaction between four FKBP5 SNPs (rs1360780, rs9296158, rs3800373, and rs9470080) and retrospective reports of child abuse in predicting current adult PTSD symptoms. The interaction for each SNP was consistent with an additive model such that PTSD symptoms increased as a function of the number of FKBP5 risk alleles, but only in the presence of severe abuse. Similar to the Zimmerman et al. study in depressed samples, child abuse directly predicted PTSD symptoms but there was no direct effect for any of the FKBP5 SNPs. Finally, Roy et al. (2010) found an interaction of three FKBP5 SNPS, including the T risk allele at rs1360780, and retrospective reports of childhood trauma on suicide attempts among patients seeking treatment for substance dependence and in healthy controls. The genetic main effect for variation at rs1360780 was not significant; although childhood trauma was associated with suicide attempts. Altogether, the balance of this evidence suggests that risk alleles of the FKBP5 gene are insufficient to impart risk for stress-related psychopathology directly, but that childhood adversity does have a direct relation with psychopathology risk. More importantly, however, these studies show that the main effect of childhood adversity is further qualified by an interaction with the FKBP5 gene, such that risk for depression, PTSD, and suicide is greatest for those with FKBP5 genetic vulnerability and early adversity exposure. In the current study, I hypothesize an analogous relationship with respect to dysregulation of the neurobiological stress
response. That is, I hypothesize that differences in the cortisol response to the TSST emerge for individuals with the FKBP5 risk allele but only given a history of childhood adversity.

The direct effect of FKBP5 polymorphisms on the neurobiological stress response has received very little study to date, and results are mixed. Ising et al. (2008) found that three variants in the FKBP5 gene (rs1360780, rs3800373, and rs4713916) were associated with elevated cortisol during the recovery phase of the cortisol response to a psychological stressor. In contrast, Mahon et al. (2012) failed to find an association between the rs1360780 or rs3800373 SNPs of the FKBP5 gene and cortisol recovery. However, this group did find heightened baseline differences in those with at least one A allele at rs7757037, and heightened peak and lower total cortisol output in those with at least one C allele at rs4713902.

The literature reviewed above examining FKBP5 polymorphisms and childhood adversity on the neurobiological stress response is full of inconsistencies in terms of the detection (i.e., significant effect or not) and direction (i.e., higher or lower cortisol output) of main effects of FKBP5 SNPs and childhood adversity on the cortisol response. These inconsistencies are difficult to resolve given the current state of research, which is complicated by the heterogeneity of early adverse experience, the number of identified risk FKBP5 polymorphisms, and the numerous ways the neurobiological stress response can be assessed using different indices and methodologies. Further, the paucity of research examining cortisol following psychosocial stress challenges, specifically, makes it impossible to identify methodological trends that might account for these discrepancies. Nonetheless, as it stands, studies generally show that childhood adversity is associated with a blunted cortisol response; though, again, this effect is moderated by factors such as the presence of psychopathology. In direct contrast, polymorphisms of the FKBP5 gene
are generally associated with greater cortisol release; though, again, this effect varies depending on the FKBP5 polymorphisms and indices of the cortisol response being studied.

What these latter gene-association studies tell us is that there is an inconsistent relation between variation of the sequence of the FKBP5 gene (i.e., FKBP5 genotype) and the broader cortisol response ‘phenotype’. This inconsistency is not surprising because of evidence that the environment can further moderate translation of genotype to phenotype – for example, by exposure to early adversity – in addition to the cortisol response being polygenetically determined.

Epigenetic mechanisms might provide a molecular basis for how individuals with the same genotype can go on to develop different phenotypes in the face of different environmental exposure. Epigenetic mechanisms are, by definition, the processes by which the structure of DNA changes without changing the sequence of the DNA itself. These modifications to DNA structure have functional relevance for cellular processes via changes in gene expression. These changes in cellular phenotype presumably lead to broader phenotypic differences, for example, depression and anxiety psychopathology. Studies that provide evidence of epigenetic mechanisms are important because they suggest that gene-environment interactions are not merely statistical artifact, but rather that there is a molecular mechanism to account for how environments affect genes. However, epigenetic modifications are only one of several different mechanisms that might account for gene-environment interactions; thus, investigation into other possible mechanisms such as gene-gene interactions, genetic lesions, and changes in transcription factors are necessary to complement studies examining epigenetic mechanisms. Nonetheless, evidence of epigenetic modification of the FKBP5 gene has interesting implications for understanding neurobiological functioning.
In a series of studies, Klengel and colleagues (2012) were the first to demonstrate a plausible epigenetic mechanism for psychopathology risk for the FKBP5 gene. Their research is directly relevant to the current study because they provided evidence that the structure of the FKBP5 gene varied according to exposure to childhood adversity. More importantly, these variations in genetic structure translated to functional differences in cellular processes that are associated with changes in the cortisol response to stress. Prior to the Klengel et al. study, it was hypothesized that changes in the coating of methyl groups attached to the DNA sequence (i.e., DNA methylation) were responsible for the modulation of the FKBP5 gene by adverse environmental exposure. However, the Klengel et al. study was the first to provide evidence for how genotypic variation at the rs1360780 site of the FKBP5 gene could be translated into environment-dependent differences in DNA methylation, gene structure, and gene expression. The molecular differences associated with the FKBP5 risk T allele at rs1360780 along with childhood maltreatment (i.e., enhanced gene transcription and decreased DNA methylation), ultimately led to changes in the ultra-short feedback loop with the glucocorticoid receptor (i.e., increased glucocorticoid receptor resistance). Therefore, these results were consistent with changes that would be associated with greater cortisol output. Further, Klengel et al. proposed that the changes to this cellular negative feedback loop would lead to long-term changes in the overall neurobiological stress response system. By providing a molecular mechanism to account for the interaction of the FKBP5 gene and childhood adversity, this study offers indirect but compelling evidence that neurobiological stress response functioning, as assessed via the cortisol response to a psychosocial stress task, will also vary as a function of these two factors. Furthermore, it provides a basis from which to derive hypotheses about the direction of change in
cortisol release expected (i.e., greater or lesser cortisol output) in relation to the interaction of FKBP5 rs1360780 genotype and childhood adversity status.

In summary, the research reviewed above provides converging evidence for why an interaction between polymorphisms of the FKBP5 gene and childhood adversity on the cortisol response would be expected. First, at the behavioural level, the FKBP5 gene interacts with childhood adversity to increase risk for psychopathology. Second, the study by Klengel et al. suggests that the FKBP5 gene may interact with childhood adversity at the molecular level to produce functional changes that are consistent with increased cortisol output. However, missing from the literature is whether these two factors interact at the neurobiological level. Thus, the question remains whether molecular changes to gene structure and function, which are believed to lead to overall changes in the stress response system, will be present in the face of stressors that also engage higher brain processes. The current study aims to answer this question by examining the interaction of FKBP5 polymorphisms and childhood adversity on the neurobiological stress response, as assessed by cortisol output following a psychosocial stress challenge. Further, this study aims to reconcile some of the inconsistency observed in the existing literature on the main effects of these variables on the cortisol response by understanding how these factors may interact to affect the cortisol response.

**Objectives & Hypotheses**

The purpose of the current study, then, is to examine whether polymorphisms of the FKBP5 gene interact with childhood adversity to affect neurobiological stress response function. By examining this gene-environment interaction, this study will examine whether dysregulation of the neurobiological stress response system is consistent with a diathesis-stress model of
pathophysiology. Although previous studies have provided compelling evidence that specific genetic variants of the FKBP5 gene leave individuals differentially vulnerable to the effects of adverse early life experience at the molecular level and in terms of predicting later psychopathology, no studies have examined the relation of FKBP5 polymorphisms and childhood adversity on stress-induced activation of the neurobiological stress response. Activation of this system may be indexed by assessing the cortisol response to a stressor that activates higher order brain processes in addition to the HPA axis itself. Thus, the current study will examine the interaction of polymorphisms in the FKBP5 gene and childhood adversity on the cortisol response to a psychosocial stress challenge using the TSST. This research question will be examined in a community sample of depressed and non-depressed adolescents. Given that depression and anxiety are associated with childhood adversity, FKBP5 risk allele status, and cortisol responsivity, the ideal design for this study would be to test whether psychopathology further moderates the relation of FKBP5 polymorphisms and childhood adversity on the cortisol response. However, the current study lacks sufficient power to examine this three-way interaction. Instead, I will statistically control for the effects of psychopathology by including depression and anxiety symptomatology as covariates in the statistical models. This will ensure that any shared variance in the cortisol response between the FKBP5 gene, childhood adversity, and depression or anxiety is accounted for. Childhood adversity in the current study is operationalized as the presence versus absence prior to age 18 of severe maltreatment (i.e., emotional, physical, or sexual abuse or neglect), witnessing of severe domestic violence and/or verbal discord, and/or severe peer-perpetrated bullying. The rs1360780 SNP of the FKBP5 gene was selected because it has been associated with the cortisol response to psychosocial stress (Ising et al., 2008), stress-related psychopathology (e.g., Appel et al., 2011), and there is evidence
that it is amenable to epigenetic changes that correspond to changes in glucocorticoid sensitivity (Klengel et al., 2012).

In addition to the primary goal of identifying overall group differences in the cortisol response to stress, stratified by FKBP5 risk allele and childhood adversity status, I outline below two additional goals to examine this relation more closely. These goals are to (a) use more targeted indices of the cortisol response to better characterize differences in the cortisol trajectories, and (b) account for characteristics of childhood adversity on the observed effects of the FKBP5 polymorphisms and cortisol parameters.

The proposed study has a number of strengths in comparison to previous studies that have independently investigated the relation of childhood adversity and FKBP5 polymorphisms on neuroendocrine functioning. First, this study will employ rigorous methods to assess childhood adversity by using a contextual interview and standardized rating system to determine objective data regarding the presence and severity of childhood experiences of physical, sexual, and emotional abuse, neglect, witnessing domestic discord/violence, and peer-perpetrated bullying (i.e., the Childhood Experience of Care and Abuse (CECA); Bifulco, Brown, & Harris, 1994). Previous research examining childhood adversity and the cortisol response have used less reliable measures, such as self-report checklists, which are subject to recollection bias and are unable to capture the severity of adversity (Elzinga et al., 2009; Ouellette-Morin et al., 2011; MacMillan et al., 2009). Second, this study will use a laboratory-induced stress paradigm (i.e., Trier Social Stress Test) to assess HPA axis function. As stated previously, this method provides the most ecologically valid measurement of the neurobiological stress response, such that it allows for the effects of higher order processes. Finally, this study will be the first to explore gene-environment
interactions between variation in the FKBP5 gene and childhood adversity in relation to the cortisol response.

**Goal 1: The Relation of FKBP5 and Childhood Adversity to the Overall Cortisol Response to the TSST**

I predict that significant between-group differences in the cortisol response across time will emerge as a function of the interaction of the rs1360780 polymorphisms of the FKBP5 gene and childhood adversity. I hypothesize that participants with a history of childhood adversity and at least one risk allele at FKBP5 (TT or CT genotype) will show a dysregulated cortisol response. Furthermore, I hypothesize that variation at this FKBP5 genetic site and childhood adversity will exert these effects above and beyond any changes in neurobiological activity that may be attributed to stress-related psychopathology. This hypothesis is consistent with a diathesis-stress model in which risk associated with exposure to adverse environments is dependent on genetic vulnerability. Given the inconsistent and opposing direction of cortisol output in relation to FKBP5 polymorphisms and childhood adversity when these factors are assessed independently, it is difficult to formulate a hypothesis about the direction of the dysregulation (i.e., heightened or blunted). Instead, the rationale for the direction of my hypothesis extends from the results reported by Klengel et al. (2012) that provide evidence of molecular changes associated with the interaction of the FKBP5 risk allele and childhood adversity. I elected to derive my hypothesis from this study because it is the only study to provide evidence for a possible mechanism for understanding the interaction of the FKBP5 gene and childhood adversity. In this study, Klengel et al. identified changes in DNA structure that were specific to individuals with the FKBP5 risk allele and a history of childhood adversity. These changes corresponded to decreased sensitivity of the intra-cellular negative feedback mechanism, thereby setting the system up for *heightened*
cortisol release. Following this line of evidence, I also expect to find greater cortisol release following the TSST in individuals with the same risk genotype and history of childhood adversity. The effects observed by Klengel et al. (2012) were at the cellular level and were independent of activation of the HPA axis by suprapituitary influences, such as those that might be expected to affect the neurobiological stress response in individuals with psychopathology (e.g., negative cognitive biases). Thus, I hypothesize that the effect of FKBP5 genetic variants and childhood adversity on the cortisol response will remain even after controlling for the effects of psychopathology status. However, as discussed above, one possibility that I will not be able to address in the current study is whether psychopathology moderates the effect of childhood adversity and the FKBP5 gene via the effects of other FKBP5 SNPs, other genes governing HPA axis function, or suprapituitary influences.

**Goal 2: FKBP5 and Childhood Adversity in Relation to Specific Indices of the Cortisol Response to the TSST**

The cortisol response to a psychosocial stress task can be assessed using several different indices that focus on particular aspects of the neurobiological response. These include baseline cortisol, cortisol reactivity, cortisol recovery, and cortisol output. Baseline cortisol is the cortisol concentration prior to exposure to the stressor and is an index of cortisol levels at rest. Cortisol reactivity and recovery are indices of the change in cortisol before and after the expected peak. Cortisol output is an estimate of overall cortisol secretion, which is calculated as the area under the curve (AUC). Although there is general agreement in the literature about what aspects of the cortisol trajectory these different terms refer to, the mathematical calculations or statistical methods to derive and assess different indices of the cortisol response vary across studies. For
example, many studies fail to control for individual differences in baseline or peak cortisol; thus, indices of cortisol reactivity, cortisol recovery, or cortisol output are confounded by individual differences in baseline or peak cortisol. The current study will control for these individual differences by including baseline or peak cortisol in the statistical models used to assess cortisol reactivity and recovery (i.e., autoregressive models) and by calculating cortisol output using the ‘AUC with respect to increase’ formula outlined by Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). Thus, in the current study, cortisol reactivity is an index of the initial neurobiological reaction to the stressor, whereas cortisol recovery is an index of how quickly the neurobiological system returns to basal functioning after the offset of the stressor, regardless of start point. Finally, cortisol output (AUCb) is an estimate of the amount of cortisol secreted over the course of the stress response above and beyond baseline cortisol concentrations. By controlling for baseline differences, AUCb is also an index of change in cortisol. The cortisol parameters reported in the results were selected by referencing the overall cortisol response trajectories, stratified by FKBP5 risk allele and childhood adversity status, to determine the appropriate cortisol indices for further analysis. Consistent with the role of the FKBP5 gene in the negative feedback mechanism of the cortisol response, I hypothesize that differences in cortisol output will be specific to the recovery phase of the cortisol response, with a corresponding increase in the relative cortisol output (AUCb). The negative feedback mechanism is the regulatory process by which the neurobiological stress response “shuts down” the acute stress response. Improper functioning of this system could lead to prolonged cortisol release, and therefore prolonged cortisol recovery. Because variation in the FKBP5 gene specifically affects the sensitivity of this negative feedback mechanism, it stands to reason that differences in the
cortisol response will be observed in the recovery period, and not at baseline or the reactivity phase.

**Goal 3: FKBP5 and Specific Childhood Adversity Characteristics on the Cortisol Response to the TSST**

Within the experience of childhood adversity exists considerable heterogeneity in terms of the type and severity of adverse experience. Thus, it is reasonable to assume that the relation of FKBP5 polymorphisms and childhood adversity on the cortisol response may vary as a function of specific childhood adversity characteristics. Consequently, to explore this hypothesis I will examine two characteristics of childhood adversity, including (a) the form the adversity takes, and (b) the severity of adversity.

In the current study, childhood adversity is a composite variable that includes experiences of severe childhood maltreatment (i.e., physical, sexual, and emotional abuse or neglect), witnessing domestic discord/violence, and/or peer-perpetrated bullying. There is some limited evidence that these different forms of adversity are differentially related to psychopathology and pathophysiological mechanisms. Studies bearing on this question may have implications for interventions and treatments aimed at normalizing neurobiological functioning by providing insight into whether it is appropriate to develop ‘one-size fits all’ interventions for the different forms of adversity. For example, Gibb, Butler and Beck (2003) found that emotional abuse better predicted depression symptoms and diagnostic status than physical abuse, whereas physical abuse better predicted anxiety symptoms and diagnostic status. Further, sexual abuse in childhood is more strongly associated with comorbid anxiety in depression than is either emotional or physical abuse (Harkness & Wildes, 2002; Levitan, Rector, Sheldon, & Goering, 2003). In two separate studies, Carpenter et al. (2009, 2011) found that emotional abuse and physical abuse were
associated with decreased cortisol output independent of any other form of childhood maltreatment. Most studies, however, do not report their results broken down according to the form of adversity, and no studies have directly compared experiences of parent-perpetrated maltreatment (i.e., abuse) to peer-perpetrated bullying or the witnessing of violence on psychopathological or pathophysiological outcomes. Therefore, this will be the first study to examine the different contribution of these varied forms of adversity to the neurobiological stress response, and to determine their moderation by variation in the FKBP5 gene. This research question is largely exploratory given the absence of prior literature.

The second characteristic of childhood adversity I will evaluate is the severity of adverse experience. Early adverse experience ranges on a continuum from relatively commonplace forms of adversity (e.g., infrequent spankings in the context of an otherwise loving parent-child relationship) to severe abuse that would warrant intervention by social services (e.g., frequent physical beatings by the parent with an implement that produce noticeable lacerations). It is important to understand the type and threshold of adversity required to exert effects on neuroendocrine functioning when considering the implications of this research. For example, intervention strategies require ways of screening or identifying at-risk populations. However, if less severe forms of adversity, which are the experiences that largely go unreported to authorities, are still sufficient for neurobiological dysregulation, it could mean that a large portion of children may not have the opportunity to benefit from such interventions.

Research that has examined the relation of childhood adversity and the cortisol response either did not query for the level of detail needed to discern severity (Elzinga et al., 2009; Ouellet-Morin et al., 2011; MacMillan et al., 2009), or did not report whether results held when childhood adversity was defined more or less conservatively (Carpenter et al., 2007; Heim et al.,
Presumably, however, severe experiences of adversity, such as those that would prompt reports to the authorities, have the greatest potency for adverse outcomes. Indeed, severe forms of adversity, such as sexual trauma (e.g., rape) and severe neglect (e.g., institutionalized orphans), have been studied most frequently with respect to risk for psychopathology and brain development. The question of interest, then, is not whether severity moderates the interaction of childhood adversity and FKBP5 polymorphisms, but rather, at what threshold do adverse experiences have the potency to affect neurobiological function? Do less severe, more commonplace experience have similar effects on neurobiological function, or are the pathological effects of adversity reserved only for severe cases?

The CECA interview provides ratings of the various forms of adversity on a 4-point scale from 1-marked, 2-moderate, 3-some, and 4-little/none, and these ratings are determined with respect to anchoring examples in the CECA manual. Consistent with the conventions of the CECA, experiences rated 1-marked or 2-moderate are considered ‘severe.’ Table 1 lists some examples of ‘severe’ adversity for each type of adversity, as drawn from the CECA manual. Therefore, the analyses conducted to assess the primary goal of the study will operationalize adversity as the presence or absence of ‘severe’ (level 1 or 2) adversity. However, to assess the issue of threshold, in this secondary analysis I will loosen the definition of adversity to include also those cases rated 3-some (see Table 1). I hypothesize that the interaction of polymorphisms of the FKBP5 gene and childhood adversity on the cortisol response will be present only when childhood adversity is defined as the experience of severe adversity; I do not expect to find an interaction when childhood adversity is defined as including those experiences rated 3-some.
Table 1

*Examples of Severe (Level 1 & 2) and Non-Severe (Level 3) Childhood Adversity Taken from the CECA Manual*

<table>
<thead>
<tr>
<th>Form of Childhood Adversity</th>
<th>Severity of Childhood Adversity</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Abuse</td>
<td>Severe</td>
<td>Child is hit with a belt on the bare bottom on a weekly basis between the ages of 5-10 by father as punishment.</td>
</tr>
<tr>
<td></td>
<td>Non-Severe</td>
<td>Child is slapped across the face twice by mother for being rude.</td>
</tr>
<tr>
<td>Sexual Abuse</td>
<td>Severe</td>
<td>Child’s genitals are fondled on a monthly basis between the ages of 8-12 by father.</td>
</tr>
<tr>
<td></td>
<td>Non-Severe</td>
<td>Child is held in a forceful manner and kissed on the lips by an adult friend of father twice.</td>
</tr>
<tr>
<td>Emotional Abuse</td>
<td>Severe</td>
<td>Example 1: Mother says to child on a daily basis “You are an idiot, I wish I had never had you”; Example 2: Father is very critical of child and frequently compares child to sibling: “you’ll never be as smart as your sister.”</td>
</tr>
<tr>
<td></td>
<td>Non-Severe</td>
<td>Father is frequently critical of child “You need to do a better job on your homework” and argues frequently with child, but criticism is not hostile and child does not feel unwanted.</td>
</tr>
<tr>
<td>Witness to Domestic Discord/Violence</td>
<td>Severe</td>
<td>Mother and father argue with raised voice weekly in front of children, and mother throws objects at father.</td>
</tr>
<tr>
<td></td>
<td>Non-Severe</td>
<td>Mother and father argue with raised voices every few weeks. Some of these arguments are in front of child. No violence.</td>
</tr>
<tr>
<td>Peer-Perpetrated Bullying</td>
<td>Severe</td>
<td>Example 1: Child is attacked by a group of peers armed with scissors and bats that resulted in injury; Example 2: Child’s peer group spreads rumours that child is gay and child is subsequently taunted on a daily basis by all of the children in her class.</td>
</tr>
<tr>
<td></td>
<td>Non-Severe</td>
<td>Child is teased and called names (e.g., “fattie”) weekly by peers at school.</td>
</tr>
</tbody>
</table>

*Note. Even within the scale anchor points (Levels: 1-marked, 2-moderate, 3-some, and 4-little/none) of the CECA, there is a broad range of adversity experiences that these examples do not capture; CECA = Childhood Experience of Care and Abuse scale (Bifulco et al., 1994).*

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Chapter 2

Methods

Participants

A total of 206 participants (55 male and 151 female) were recruited as part of a larger study on stress and depression in adolescents. Participants ranged in age from 11 to 21 years and approximately 41% of the original sample met criteria for a major depressive disorder. Non-depressed participants were recruited from the Queen’s University Psychology Developmental Database, local high schools and poster advertisements in Kingston, Ontario, Canada. In addition to these recruitment strategies, depressed participants were recruited from mental health agencies and family physicians. Non-depressed participants recruited through the schools were first screened using an online version of the Beck Depression Inventory (BDI; Beck, 1996) that was submitted through the Limestone District School Board. The students were asked to complete the online BDI and told they may be contacted later about participating in a study about “stress and mood”. Non-depressed participants were contacted if they had a BDI score of less than 6.

Exclusion criteria for this study were the presence of a current psychotic disorder, bipolar disorder, developmental or conduct disorder, substance dependence, or a medical condition that could cause depression.

The final sample was comprised of 90 participants from the overall sample (see Figure 2 for inclusion/exclusion flow chart). Eleven participants were excluded based on the diagnostic exclusionary criteria. The majority of participants were excluded from the initial sample because of missing genetic data. Although genetic samples were collected for the full sample, the genetic
Figure 2. Flow chart of inclusion and exclusion of participants.
samples for the first 104 participants were collected using cotton swabs, as recommended by the original genetics co-investigator on this project. However, these samples ended up being unable to be assayed, and this geneticist left the project and the University. For the remainder of the project we collaborated with Dr. James Kennedy, head of the Psychiatric Neurogenetics Lab at the Centre for Addiction and Mental Health. We attempted to re-contact the original 104 participants to obtain a new genetic sample. Twenty-seven samples were re-assayed, but genetic data were unavailable for the remaining participants. The remaining genetic samples were collected using Oragene®•DNA sample saliva collection kits. In total, genetic data were available for 116 participants meeting the study inclusion criteria. An additional 20 participants were excluded because of missing childhood adversity and cortisol data. Finally, six participants were excluded because at least one of their cortisol samples was identified as an outlier (i.e., +/- 3 SD from the mean). This left a final sample with usable data of 90.

Demographic and clinical characteristics for the final sample stratified by childhood adversity and FKBP5 genotype are presented in Table 1. The final sample did not differ from the excluded participants in terms of sex, $\chi^2(1) = 1.64, p = .201$, or ethnicity, $\chi^2(2) = 2.64, p = .268$. However, the final sample was older ($M = 16.52, SD = 2.17$ and $M = 15.87, SD = 2.34$; $t(204) = 2.05, p = .041$) and of higher SES ($M = 2.99, SD = 1.67$ and $M = 3.67, SD = 2.02$; $t(199) = 2.56, p = .011$).

Nearly half of the final sample met current criteria for at least one DSM-IV Axis I diagnosis (48%; $n = 43$), including: (1) MDD ($n = 23$); (2) Dysthymia ($n = 7$); (3) Depressive Disorder Not Otherwise Specified ($n = 1$); (4) Adjustment Disorder with Depressed Mood ($n = 3$); (4) Post Traumatic Stress Disorder ($n = 1$); (5) Generalized Anxiety Disorder ($n = 9$); (6) Social Phobia ($n = 7$); (7) Specific Phobia ($n = 3$); (8) Panic Disorder ($n = 3$); (9) Obsessive-
Table 2

Descriptive Statistics of Demographic and Clinical Characteristics Stratified by FKBP5 Genotype and Childhood Adversity

<table>
<thead>
<tr>
<th></th>
<th>TT/TC</th>
<th></th>
<th>CC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA</td>
<td>No CA</td>
<td>CA</td>
<td>No CA</td>
</tr>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 39)</td>
<td>(n = 13)</td>
<td>(n = 29)</td>
</tr>
<tr>
<td>Sex (Female), n(%)</td>
<td>6(66.7)</td>
<td>32(82.1)</td>
<td>9(69.2)</td>
<td>23(79.3)</td>
</tr>
<tr>
<td>Ethnicity (White), n(%)</td>
<td>7(77.8)</td>
<td>34(87.2)</td>
<td>9(69.2)</td>
<td>26(89.7)</td>
</tr>
<tr>
<td>Age, M(SD)</td>
<td>16.44(2.07)</td>
<td>16.74(2.33)</td>
<td>16.23(1.88)</td>
<td>16.38(2.18)</td>
</tr>
<tr>
<td>Tanner Score, M(SD)</td>
<td>4.71(.38)</td>
<td>4.45(.63)</td>
<td>4.65(.41)</td>
<td>4.52(.45)</td>
</tr>
<tr>
<td>Hollingshead Index, M(SD)</td>
<td>3.78(1.99)</td>
<td>2.46(1.48)</td>
<td>4.04(1.94)</td>
<td>3.00(1.39)</td>
</tr>
<tr>
<td>BDI Score, M(SD)</td>
<td>25.19(17.07)</td>
<td>11.84(10.83)</td>
<td>12.72(10.45)</td>
<td>22.07(14.23)</td>
</tr>
<tr>
<td>AA score, M(SD)</td>
<td>39.11(14.57)</td>
<td>25.98(7.07)</td>
<td>28.15(11.20)</td>
<td>30.22(13.05)</td>
</tr>
<tr>
<td>Current DSM-IV Dxs, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressive Disorder</td>
<td>7(77.8)</td>
<td>11(28.9)</td>
<td>7(53.8)</td>
<td>8(29.6)</td>
</tr>
<tr>
<td>Anxiety Disorder</td>
<td>4(44.4)</td>
<td>6(15.4)</td>
<td>3(23.1)</td>
<td>6(20.7)</td>
</tr>
<tr>
<td>Other</td>
<td>1(11.1)</td>
<td>1(2.7)</td>
<td>3(23.1)</td>
<td>2(6.9)</td>
</tr>
<tr>
<td>Current Psychotropic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication, n(%)</td>
<td>4(44.4)</td>
<td>2(5.1)</td>
<td>4(30.8)</td>
<td>6(20.7)</td>
</tr>
</tbody>
</table>

Note. AA = Anxious Arousal; BDI = Beck Depression Inventory; CA = Childhood Adversity; Dxs = Diagnosis.

\( ^a \) Significant main effect of CA, \( p < .05 \).

\( ^b \) Significant interaction such that there was a simple main effect of CA for TT/TC but not CC.
Compulsive Disorder \((n = 4)\); (10) Attention-Deficit Hyperactivity Disorder \((n = 1)\); (11) Oppositional Defiant Disorder \((n = 1)\); (12) Substance/Alcohol Abuse \((n = 5)\); (13) Bulimia Nervosa \((n = 1)\). Sixteen participants met criteria for more than one DSM-IV diagnosis. The remaining 47 participants did not meet current or past criteria for any DSM-IV diagnosis.

**Measures**

**Demographic Questionnaire and Socioeconomic Status.** The demographic questionnaire included questions about participants’ age, sex, ethnicity, and parental occupation. To evaluate socioeconomic status (SES), two independent judges rated parental occupations on a 1- to 8-point scale according to the Hollingshead Index of Social Position (Hollingshead, 1975). An average of the parent ratings was used as an index of the participant’s SES. Higher scores on this scale represent a lower social position.

**Diagnostic measure.** The presence of current and/or past DSM-IV Axis I diagnoses were evaluated with the full child and adolescent version of the Schedule of Affective Disorders and Schizophrenia (K-SADS; Kaufman, Birmaher, Brent, Rao, & Ryan, 1996). The K-SADS is a semi-structured diagnostic interview. Optional probes are provided to elicit pertinent information and objective criteria are used to rate symptoms of psychopathology according to DSM-IV criteria. For the purposes of this study, the K-SADS was administered directly to the participant; parent reports were not solicited. Studies have found that agreement for internalizing disorders assessed via parent and adolescent report is poor, and because reliance on parent report would have resulted in missed diagnoses (Cantwell, Lewinsohn, Rohde, & Seeley, 1997), diagnoses were made based only on the participant’s report of their symptoms. Questions about past and current psychiatric treatment (i.e., medication and/or therapy) were also included. Interviews
were administered by clinical psychology graduate students trained to “gold-standard” reliability by Dr. Kate Harkness (see Grove, Andreasen, McDonald-Scott, Keller, & Shapiro, 1981). The process of achieving this status required interviewers to observe and be observed by trained interviewers, and to match diagnoses on at least three consecutive interviews for each. Students also conferenced every case with Dr. Harkness prior to determining the final diagnosis.

**Depression Symptoms.** The Beck Depression Inventory-II (BDI; Beck, 1996) was administered to all participants to assess for the presence and severity of depressive symptoms in the preceding two weeks. The BDI consists of 21 self-report items rated on a 4-point scale from 0 to 3. Studies of adolescent and young adult depression have shown excellent reliability and validity using the BDI (e.g., Krefetz, Steer, Gulab, & Beck, 2002). The internal consistency estimate for the BDI in the current sample was high (*Cronbach’s alpha* = .95). The following interpretive ranges are reported by Beck et al. (1996): 0-13 – minimal depression, 14-19 – mild depression, 20-28 – moderate depression, and 29-63 – severe depression.

**Anxiety Symptoms.** The anxious arousal (AA) subscale from the Mood and Anxiety Symptom Questionnaire (MASQ; Watson & Clark, 1991; Watson et al., 1995) was completed by all participants to assess the presence and severity of anxiety symptoms in the preceding week. The MASQ consists of 90 self-report items, of which 17-items comprise the AA scale. The AA items assess somatic and arousal symptoms specific to anxiety and not depressive disorders. Items are rated on a five-point scale according to the extent the participant experienced each symptom from 1 (*not all*) to 5 (*extremely*). Research with adolescents and young adults has shown good reliability and validity with the MASQ (e.g., Hankin, 2008; Hankin, Wetter, Cheely, & Oppenheimer, 2008). The internal consistency estimate for the AA subscale in the current sample was high (*Cronbach’s alpha* = .90).
**Pubertal Status.** Pubertal status was assessed using the Tanner stages of sexual maturation (Tanner, 1962). Participants self-reported on breast (females) and pubic hair (males and females) development by selecting among five illustrations. The illustrations have been validated against physician assessment for this age group (Taylor et al., 2001). Scores can range from 1 to 5, with higher scores representing later stages of development.

**Childhood Adversity.** Childhood adversity (CA) was assessed with the Childhood Experience of Care and Abuse scale (CECA; Bifulco et al., 1994). The CECA is a retrospective semi-structured contextual interview and rating system that asks questions about experiences of emotional, physical, and sexual abuse, witnessing of domestic discord/violence, and peer-perpetrated bullying before 18 years of age. The CECA consists of seven scales: (a) antipathy – hostility, criticism, and/or coldness directed toward the child by parents; (b) indifference – neglect of the child’s physical and/or emotional needs; (c) physical abuse – violence directed toward the child by parents; (d) sexual abuse – non-consensual sexual contact directed toward the child by any perpetrator; (e) psychological abuse – verbal or nonverbal acts of cruelty directed solely at the child by someone in a position of power or having responsibility for the child (e.g., parent, teacher, coach) (f) witness to domestic discord/violence – child witnessing tension/arguments, and/or interpersonal violence (e.g., hitting, punching, slapping) and/or non-personal violence (e.g., smashing or throwing things) between parents; and, (g) bullying – same-age peer-perpetrated verbal and/or physical aggression and/or social exclusion (see Appendix A for sample questions).

Clinical psychology graduate students interviewed participants and the interviews were audio-recorded for later rating by a team of baccalaureate research assistants blind to the participants’ diagnostic status. Interviewers were trained not to query for participants’ subjective
responses to their experiences or the effect of their experience on their mental health status. Each of the seven scales was rated on a scale of 1-marked, 2-moderate, 3-some, or 4-little/none. The CECA manual contains hundreds of case examples that are used to anchor the ratings. Further, interviewers and raters received extensive training and ongoing supervision in the Bedford College procedure by Dr. Kate Harkness. Specifically, Dr. Harkness conferenced each case with the raters prior to determining the final rating.

Consistent with the conventions of the CECA, each scale was dichotomized to form severe (ratings of 1-marked or 2-moderate) versus ‘at least some’ adversity (ratings of 3-some or 4-little/none) categories. A composite ‘childhood adversity’ (CA) variable was defined as the presence or absence of a severe level of any one of the seven forms of adversity. Childhood adversity characteristics for the sample are shown in Table 2. As would be expected, the types of CA do not add up to 100% because many participants experienced more than one form of adversity. Participants were categorized as having comorbid CA if they had at least two different forms of severe CA.

**Genotyping.** Saliva samples were collected by passive drool using the Oragene®•DNA sample collection kit. These kits include a buffer solution that allows for the saliva samples to be stored at room temperature. Samples were mailed to Dr. James L. Kennedy’s Neurogenetics laboratory at the Centre for Addiction and Mental Health in Toronto, Ontario for assay. For the current study, two single nucleotide polymorphisms (SNPs) in the FKBP5 gene region were selected for genotyping (rs1360780 and rs3800373). The three allelic variations at each site are TT, TC, and CC (rs1360780), and GG, GT, and TT (rs3800373). Given the genetic linkage between these two sites, nearly all participants with the risk allele at the rs1360780 site also possessed the risk allele at rs3800373. Because of this redundancy and given the location and
### Table 3

Descriptive Statistics for Childhood Adversity Stratified by FKBP5 Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TT/CT (n = 48)</th>
<th>CC (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe CA(^a) (Yes), n(%)</td>
<td>9(18.8)</td>
<td>13(30.9)</td>
</tr>
<tr>
<td>At least some CA, (Yes), n(%)</td>
<td>28(58.3)</td>
<td>23(54.8)</td>
</tr>
<tr>
<td>Form of CA, n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional Abuse(^b)</td>
<td>5(10.4)</td>
<td>6(14.3)</td>
</tr>
<tr>
<td>Physical Abuse</td>
<td>2(4.2)</td>
<td>1(2.4)</td>
</tr>
<tr>
<td>Sexual Abuse</td>
<td>0(0)</td>
<td>4(9.5)</td>
</tr>
<tr>
<td>Witness to Domestic</td>
<td>6(12.5)</td>
<td>5(11.9)</td>
</tr>
<tr>
<td>Discord/Violence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bullying</td>
<td>5(10.4)</td>
<td>5(11.9)</td>
</tr>
<tr>
<td>Comorbid CA</td>
<td>4(8.3)</td>
<td>5(11.9)</td>
</tr>
</tbody>
</table>

Note. CA = Childhood Adversity.

\(^a\) Severe CA is defined as the presence versus absence of childhood adversity rated 1-marked or 2-moderate; At least some CA is defined as the presence versus absence of adversity rated at least 3-some.

\(^b\) Emotional abuse includes neglect, indifference, antipathy and psychological abuse.
known biological function of rs1360780, only the results for the rs1360780 site are presented in
the current study (Binder et al., 2004; Klengel et al., 2012).

In the sample, there were 42 participants with the CC genotype, 40 with TC genotype,
and 8 homozygous for the risk allele – TT. The observed genotype frequencies were compared to
the expected population genotype frequencies based on the Hardy-Weinberg equation. The
Hardy-Weinberg equation is used to test the principle that allele and genotype frequencies in a
population will remain constant across generations, in the absence of outside influences. Using an
online calculator (Rodriguez, Gaunt, & Day, 2009), allele-frequencies were found to be in
accordance with Hardy-Weinberg-equilibrium, $\chi^2(1)= .12, p = .941$. This suggests that the
assumptions of the Hardy-Weinberg model have not been violated (e.g., nonrandom mating) and
that allele frequencies are generally stable in the current sample.

The three possible genotypes were recoded as a dichotomous variable defined as whether
participants had at least one risk allele or were homozygous for the non-risk allele for the
rs1360780 site of the FKBP5 gene (i.e., TT/TC versus CC for rs1360780). This method of
dichotomization is consistent with previous research (Klengel et al., 2012; Menke et al. 2013).

**Cortisol Collection and Stress Task**

**Trier Social Stress Task.** The Trier Social Stress Task (TSST; Kirschbaum, Pirke, &
Hellhammer, 1993) began with a 10-minute rest period, after which the first saliva sample
(Sample A) was collected. The participants were then led into another room and introduced to
two research assistants. The participants were told to prepare a 5-minute speech as part of a mock
“job interview” on why they would make an excellent candidate for a position in a retail clothing
store. The research assistants were introduced as members of a selection committee from the
human resources department and the participants were told that their speech would be videotaped.
The participants were taken back to the previous room and given 10 minutes to prepare their speech, after which a second saliva sample (Sample B) was taken. The participants were brought back into the room with the research assistants and delivered their speech to the research assistants who were instructed to maintain neutral facial expressions. Participants who completed their speech in under 5 minutes were instructed that they had time remaining, and if they were unable to continue, were asked a series of standardized questions. Upon completion of this portion of the task, the participants were surprised with an arithmetic test. The participants were asked to subtract by 13 starting from 2083 as quickly as possible and without making mistakes. If a mistake was made, the participants were told, “I’m sorry, that is incorrect. Please start again”. Upon completion of the TSST (which lasts approximately 20 minutes), the third saliva sample (Sample C) was collected. Forty minutes later a fourth sample was collected (Sample D) followed by the fifth and final sample (Sample E) 40-60 minutes later.

**Hormone Determinations.** Saliva samples were collected in 5-mL polypropylene vials (Rose Scientific Ltd, Edmonton, Alberta) by passive drool (Shircliff, Granger, Schwartz, & Curran, 2001) and were immediately transferred to short-term freezer storage. Samples were mailed on ice in batches to Dr. Katherine Wynne-Edwards’ lab at the University of Calgary where they were stored in cold storage (-20°C) before assay. Samples were thawed in a 20°C water bath under gentle shaking. Mucopolysaccharides and detritus were removed after centrifugation at 1500g for 15 min. The resulting supernatant was analyzed using an enzyme-linked immunoassay designed specifically for saliva (1-0102/1-0112; Salimetrics LLC, State College, PA). To control for quantification error because of inter-assay variability, all samples from one individual were placed on the same plate. Each sample was quantified in duplicate at 25µl, and triplicate high and low controls (4-COO1) were distributed across each plate to track
precision and accuracy. Samples that had a coefficient of variation ≥15% were repeated on another plate. Repetition was used to reject one of the original duplicates but was not used in analyses. This procedure is used regularly as it yields high precision and low measurement error (3-7%), with all samples expected to fall within the linear portion of the binding curve (Berg & Wynne-Edwards, 2001). Salivary cortisol measured by this method is highly correlated with serum cortisol \( r = 0.96; \) Salimetrics validation). Samples were assayed in eight batches. The high control, measured at 1.071 µg/dL, had an intra-assay coefficient of variation of 3.6 % and an inter-assay coefficient of variation of 5.0 %. The low control, measured at 0.103 µg/dL had an intra-assay coefficient of variation of 6.2 % and an inter-assay coefficient of variation of 9.8 %.

**Procedure**

The study procedures were approved by the Health Sciences Research Ethics Board of Queen’s University in Kingston, Ontario. Written informed consent was obtained from all participants, as well as from a parent or guardian if the participant was under 18 years of age. Participants were compensated $40 for their participation. Participants who entered the study by self-referral and who were suffering from an untreated psychiatric condition were referred for treatment.

Interviews and questionnaires were administered by clinical psychology graduate students in the Mood Research Lab at Queen’s University. Each participant completed two 2-hour assessments separated by approximately one week. During the first session, participants completed the demographic and K-SADS interviews and self-report questionnaires. In the second session, participants completed the TSST and CECA interview. The second session was scheduled between 3 and 5 pm to control for diurnal changes in cortisol (Gröschl, Rauh, & Dorr, 2017).
Participants were instructed not to eat or drink one hour prior to their arrival at the lab and they learned of the speech task at the second session. The diagnostic interview was administered prior to the childhood adversity interview to prevent interviewer bias in determining diagnostic status.

**Data Analysis**

Statistical analyses were performed using the Statistical Program for the Social Sciences (SPSS) software, Version 21. Preliminary univariate analyses were conducted to examine the relation of sex, age, Tanner score, SES, BDI score, and AA score to the FKBP5 genotype, childhood adversity (CA), and the cortisol indices. Significant demographic variables were included as covariates in the primary statistical models.

The independent variables in all analyses were FKBP5 rs1360780 genotype (presence [TT/CT] or absence [CC] of at least one risk allele) and childhood adversity (presence or absence of severe childhood adversity). The dependent variables were the cortisol sample values and parameters. In addition to analysis of the five cortisol samples across time, additional cortisol parameters were used in more targeted analysis of the cortisol response (i.e., baseline cortisol, cortisol reactivity, cortisol recovery, and relative cortisol output). Baseline cortisol was defined as Sample A. Cortisol reactivity was calculated by subtracting baseline cortisol (Sample A) from the anticipated peak in cortisol (Sample C) and including baseline (Sample A) as a covariate in the statistical model. Cortisol recovery was calculated by subtracting peak cortisol (Sample C) from recovered cortisol (Sample D) and including peak cortisol (Sample C) as a covariate in the statistical model. Relative cortisol output (AUCb) was defined as the area under the curve from baseline (Sample A) to the final sample (Sample E). AUCb was calculated using a formula
derived by Pruessner et al. (2003), which uses the trapezoidal rule to estimate area under the curve.

\[
AUC_b = \left( \frac{(Sample\ B+Sample\ A)\cdot 10}{2} \right) + \left( \frac{(Sample\ C+Sample\ B)\cdot 10}{2} \right) + \left( \frac{(Sample\ D+Sample\ C)\cdot 10}{2} \right) + \left( \frac{(Sample\ E+Sample\ D)\cdot 40}{2} \right) - [Sample\ A \cdot (10 + 10 + 40 + 40)]
\]

First, a 2 (FKBP5) X 2 (CA) X 5 (cortisol samples) repeated measures Analysis of Covariance (RM-ANCOVA), controlling for relevant demographic covariates, was performed as an omnibus test to identify group differences in the cortisol response to the TSST. Second, I conducted 2 (FKBP5) X 2 (CA) factorial ANCOVAs, controlling for demographic covariates, to assess separate cortisol parameters (i.e., baseline cortisol, cortisol reactivity, cortisol recovery, and AUCb) as a method of following-up the omnibus RM-ANCOVA. Third, to determine the effect of different CA characteristics on the relation of FKBP5 and CA on the cortisol response, additional factorial ANCOVAs were conducted in which the CA variable was re-operationalyzed as each of the specific forms of adversity (i.e., witness to domestic discord/violence, peer-perpetrated bullying, physical, sexual, and emotional abuse [i.e., antipathy, indifference/neglect, or psychological abuse]), and with the threshold of adversity shifted to include at least ‘some’ abuse (i.e., CECA ratings of 3-some to 1-marked).

All statistical models used the default Type III sum of squares procedure in SPSS. This method accounts for intercorrelations among independent variables and unequal cell sizes. Significant interactions of FKBP5 and CA in the factorial ANCOVA models were followed up with simple main effects analyses. Partial \( \eta^2 \) is reported for the statistical parameters in the main analyses as an index of effect size (\( \eta^2_p \)). Partial \( \eta^2 \) values of .04, .25, and .64 correspond to small,
medium, and large effects as recommended by Ferguson (2009). Estimated marginal means are reported in all figures.

**Missing Data, Data Imputation, & Removal of Outliers**

As outlined above, participants who were missing genetic data or who did not complete the childhood adversity interview were excluded from the statistical analyses, as were participants who were missing more than one cortisol sample. For the remaining participants, any additional missing data were replaced using the multiple imputation function in SPSS software. Ten imputed datasets were generated and a final dataset was created with the mean imputed value inserted to replace missing values. Exclusion of cases missing one or more of SES (n = 1), BDI score (n = 2), AA score (n = 7), Tanner score (n = 11), Sample B (n = 2), and Sample E (n = 6) would have resulted in loss of over 25% of the sample and a significant loss of power. Tabachnick and Fidell (2007, pg. 72) state that multiple imputation is the most respectable method for dealing with missing data. Further, given that data were missing at random and comprised fewer than 5% of the total data points, multiple imputation is a more than adequate method of treating missing data according to recommended guidelines.

Prior to exclusion of the six participants with outlying cortisol values (i.e., +/- 3 – 6 SD above the mean), the cortisol distributions were positively skewed (skewness for the five cortisol sample distributions [Samples A to E] ranged from 2.35 to 3.06); however, with the outliers excluded, the data more closely approximated a normal distribution (skewness ranged from .904 to 1.50).
Chapter 3

Results

Preliminary Univariate Analyses

Univariate analyses were conducted to identify potential demographic and clinical characteristics to include as covariates in the main models. Demographic and clinical characteristics are presented in Table 1.

**FKBP5 & Childhood Adversity.** Those with versus without the risk allele of the FKBP5 gene did not differ significantly on any of the demographic or clinical characteristics listed in Table 1 (all $p$s > .32). Those with versus without childhood adversity (CA) did not differ significantly in terms of age ($t[88] = .51, p = .614$), Tanner scores ($t[88] = 1.49, p = .14$), sex ($\chi^2[1] = 1.55, p = .213$), or ethnicity ($\chi^2[2] = 3.20, p = .202$). However, those with CA had significantly lower socioeconomic status (SES), ($t[88] = 3.20, p = .002$), higher BDI scores ($t[88] = 3.83, p < .001$), and marginally higher AA scores ($t[88] = 1.92, p = .058$) than those without. Those with versus without CA were not differentially distributed across the FKBP5 genotypes $\chi^2(2) = 1.81, p = .179$.

**Cortisol Parameters.** Higher cortisol concentrations at samples A, B, D, and E were significantly associated with older age and higher Tanner scores, $rs(N = 90) > .25, ps < .017$. Sample C and AUCb were not significantly associated with any of the demographic or clinical characteristics, $rs(N = 90) < .20, ps > .06$.

Based on the above univariate analyses, all models were conducted including age, SES, BDI scores, and AA scores as covariates. Given the strong correlation between age and Tanner scores ($r[N = 90] = .51, p < .001$), only age was included as a covariate. Excluding the covariates
(i.e., age and SES) from the models outlined below did not change the pattern of results for any of the models (i.e., repeated measures analyses and analyses of baseline (Sample A), and relative cortisol output (AUCb). Therefore, for ease of interpretation, parsimonious models are presented with only AA and BDI scores included as covariates to control for symptoms of depression and anxiety psychopathology as outlined in the hypotheses.

**Goal 1: The Relation of FKBP5 and Childhood Adversity to the Overall Cortisol Response to the TSST**

*Within Group Cortisol Output Over Time.* The five cortisol concentrations (µg/dL) collected over the course of the TSST protocol were analyzed in a 2 (presence vs. absence of at least one FKBP5 risk allele) X 2 (presence vs. absence of severe CA) X 5 (Cortisol samples) repeated measures Analysis of Covariance (RM-ANCOVA), with BDI score, and AA score entered as covariates. Given that Mauchly’s Test of Sphericity was violated (Mauchly’s $W[9] = .11, p < .001$), the results are presented with the Greenhouse-Geisser correction.

The 3-way interaction of FKBP5, CA, and Cortisol was marginally significant, $F(4, 336) = 2.66, p = .063, \eta_p^2 = .03$. The 2-way CA X Cortisol interaction was also marginally significant for the risk genotype but not for the non-risk genotype, $F(4, 336) = 2.22, p = .067, \eta_p^2 = .03$ and $F(4, 336) = 1.35, p = .250, \eta_p^2 = .02$, respectively.

Contrary to my hypothesis of a more reactive cortisol response, participants with at least one risk allele and CA showed a significant linear decrease in their cortisol over time, $F(1, 84) = 4.15, p = .045, \eta_p^2 = .05$. In contrast, none of the polynomial models fit the cortisol trajectories for participants with the risk genotype and no CA well; however, there was a modest trend for the
typical quadratic response such that cortisol peaked following the stressor and then decreased, 
\( F(1, 84) = 2.76, p = .101, \eta^2_p = .03 \) (see Figure 3).

Upon examination of the cortisol curves depicted in Figure 3, I conducted univariate ANOVAs to statistically test specific features of the general trends in cortisol output over time. Specifically, I tested differences in baseline cortisol (Sample A) and differences in the relative cortisol output over time, controlling for differences in baseline (AUCb).

Figure 3. Estimated marginal means for cortisol concentrations at each sample point stratified by FKBP5 genotype (Risk = TT/TC [top] and Non-Risk = CC [bottom]) and CA. BDI and AA scores were included as covariates when calculating marginal means. Error bars denote standard error. CA = Childhood Adversity; BDI = Beck Depression Inventory score; AA = Anxious Arousal subscale score from the Mood and Anxiety Symptom Questionnaire
Goal 2: FKBP5 and Childhood Adversity in Relation to Specific Indices of the Cortisol Response to the TSST

Between Group Differences in Baseline Cortisol. I analyzed differences in baseline cortisol by conducting a 2 (presence vs. absence of at least one FKBP5 risk allele) X 2 (presence vs. absence of severe CA) factorial ANCOVA on Sample A cortisol concentrations, controlling for the effects of AA and BDI score. The 2-way interaction of FKBP5 and CA was not significant, $F(1, 84) = .48, p = .492, \eta^2_p = .01$. However, the main effect of FKBP5 was at trend, such that individuals with the risk allele had higher baseline cortisol ($M_{adj} = .15, SE = .01$) than those without the risk allele ($M_{adj} = .12, SE = .01$), $F(1, 84) = 3.69, p = .058$. The partial $\eta^2_p = .04$ indicated this effect was small (Figure 4). There was no main effect of CA, $F(1, 84) = .04, p = .838, \eta^2_p < .01$.

Figure 4. Estimated marginal means for baseline cortisol stratified by FKBP5 genotype (Risk = TT/TC and Non-Risk = CC) and CA. BDI and AA scores were included as covariates when calculating marginal means. Error bars denote standard error. CA = Childhood Adversity; BDI = Beck Depression Inventory score; AA = Anxious Arousal subscale score from the Mood and Anxiety Symptom Questionnaire.
**Between Group Differences in Relative Cortisol Output.** I analyzed differences in cortisol output relative to baseline (AUCb) by conducting a 2 (presence vs. absence of at least one FKBP5 risk allele) X 2 (presence vs. absence of severe CA) factorial ANCOVA, controlling for the effects of relevant covariates. The main effects of FKBP5 and CA on AUCb were not significant, \( F(1, 84) = 1.39, p = .243, \eta_p^2 = .02, \) and \( F(1, 84) = .153, p = .696, \eta_p^2 < .01. \) However, the FKBP5 x CA interaction was significant, \( F(1, 84) = 6.11, p = .015, \eta_p^2 = .07. \) For participants with at least one risk allele, those with severe CA had lower cortisol output than those without severe CA, \( F(1, 84) = 3.65, p = .059, \eta_p^2 < .04; \) however, this effect was at trend. There was no significant difference between CA groups for participants with the non-risk genotype \( F(1, 84) = 2.39, p = .126, \eta_p^2 < .03 \) (see Figure 5).

![Figure 5](image-url)

**Figure 5.** Estimated marginal means for cortisol output relative to baseline (AUCb) stratified by FKBP5 genotype (Risk = TT/TC and Non-Risk = CC) and CA. BDI and AA scores were included as covariates when calculating marginal means. Error bars denote standard error. CA = Childhood Adversity; BDI = Beck Depression Inventory score; AA = Anxious Arousal subscale score from the Mood and Anxiety Symptom Questionnaire.
To assess whether the above relation held controlling for clinician rated depression and anxiety diagnoses versus self-reported symptoms, I re-ran the analysis with BDI and AA scores substituted for a single variable defined as the presence versus absence of any depressive disorder or anxiety disorder diagnoses. The main effects of FKBP5 and CA on AUCb remained nonsignificant, $F(1, 85) = .94, p = .335, \eta_p^2 = .01$, and $F(1, 85) = .378, p = .540, \eta_p^2 < .01$, respectively. Consistent with the models including BDI score and AA score, the FKBP5 x CA interaction was significant, $F(1, 85) = 4.78, p = .032, \eta_p^2 = .05$. For participants with at least one risk allele, those with severe CA had lower cortisol output than those without severe CA, $F(1, 85) = 3.36, p = .070, \eta_p^2 = .04$; this effect was also at trend. For the non-risk genotype, there was no significant difference between CA groups, $F(1, 85) = 1.36, p = .246, \eta_p^2 = .02$.

**Goal 3: Effect of FKBP5 and Specific Childhood Adversity Characteristics on the Cortisol Response to the TSST**

*Form of Childhood Adversity.* To determine whether the above group differences in cortisol output (AUCb) were specific to any particular forms of adversity, I ran three additional models isolating three specific forms of adversity: emotional abuse (severe antipathy and/or indifference and/or psychological abuse), witness to domestic discord and/or violence, and bullying (cell sizes were too low to permit specific analysis of physical or sexual abuse separately).

No significant main effects or interactions with the FKBP5 genotype emerged in the AUCb models when examining either emotional abuse or bullying separately (two-way interactions: $F(1, 84) = .09, p = .769, \eta_p^2 < .01$ and $F(1, 84) = .73, p = .394, \eta_p^2 = .01$, respectively).
When witnessing domestic discord/violence was examined separately, there was a main effect, such that participants who witnessed domestic discord/violence had lower cortisol output than those who did not, $F(1, 84) = 4.13, p = .045, \eta_p^2 = .05$. Further, the main effect of FKBP5 was marginally significant, such that the risk allele was associated with lower cortisol, $F(1, 84) = 3.12, p = .081, \eta_p^2 = .04$. Further, consistent with the overall CA model, these main effects were qualified by a 2-way interaction of FKBP5 and domestic discord/violence on AUCb, $F(1, 84) = 6.47, p = .013, \eta_p^2 = .07$. Simple main effects analyses showed that for participants with the risk genotype, those who witnessed domestic discord/violence had lower cortisol than those who did not, $F(1, 84) = 9.63, p = .003, \eta_p^2 = .10$; there was, however, no significant difference in cortisol output with or without the experience of domestic discord/violence for participants with the non-risk genotype, $F(1, 84) = .09, p = .763, \eta_p^2 < .01$.

**Severity of Childhood Adversity.** In the current sample, when CA was operationalized as the presence versus absence of severe CA, about a quarter of the sample fell in the CA group (24.4%). However, when defined to also include experiences rated 3-some, the proportion of the sample in the CA group rose to over half ($n = 51; 56.7\%$). To test whether less severe forms of CA could affect the cortisol response to the TSST, I re-ran the AUCb analysis with the more liberal CA variable. Neither a significant interaction nor main effects of FKBP5 or CA on AUCb emerged, controlling for BDI and AA scores, $F(1, 84) < 1.34, ps > .233, \eta_p^2 < .02$. 
Chapter 4

Discussion

The purpose of the current study was to examine the interaction of polymorphisms in the FKBP5 gene and childhood adversity on the cortisol response to a psychosocial stress task. More broadly, I was interested in determining whether dysregulation of the neurobiological stress response was consistent with what would be expected given a diathesis-stress model of pathophysiology. Previous research documented the interaction of these variables for psychopathology risk, as well as the direct effects of specific FKBP5 gene variants and childhood adversity on the cortisol response, independently. However, no studies had investigated how these two factors might work in concert to affect the cortisol response to stress, despite limited but compelling evidence for how the FKBP5 gene may interact with childhood adversity to effect change at the molecular level via epigenetic changes in gene expression. Although there is some evidence that the epigenetic modifications presumably responsible for these differences in gene expression alter the cellular negative feedback mechanism, the question remained whether these changes would translate to differences in overall cortisol output following activation of the complete neurobiological stress response including activation of higher order brain processes. That is, would there be observable differences in cortisol output following a psychosocial stress challenge, presumably mediated by these putative epigenetic mechanisms? Thus, the current study had three goals, the first of which was to identify whether there were group differences in the overall cortisol response trajectories based on individuals’ FKBP5 genotype at rs1360780 and their history of childhood adversity. Second, the current study sought to characterize these group differences by evaluating specific indices of the cortisol response. Finally, the third goal was to
investigate whether specific characteristics of childhood adversity might account for the relation of FKBP5 gene polymorphisms and childhood adversity on the cortisol response.

**FKBP5, Childhood Adversity, and the Cortisol Response**

In support of the first goal of this study, the cortisol response to the psychosocial stressor varied as a function of FKBP5 risk allele and childhood adversity status, such that a history of childhood adversity was associated with an abnormal cortisol response in individuals with the FKBP5 risk allele (i.e., TT or TC genotype) but not in individuals with the non-risk genotype (i.e., CC genotype). Controlling for depression and anxiety symptoms and psychopathology, individuals with the FKBP5 risk allele and a history of childhood adversity showed a distinct neuroendocrine response in which cortisol decreased relative to baseline (i.e., a negative cortisol response), rather than the normal pattern of peaking following provocation. These results provide support for a diathesis-stress model of neurobiological dysregulation such that only individuals with the risk genotype showed an abnormal cortisol response in the face of early adverse experience, whereas individuals with the non-risk genotype were resilient to neurobiological dysregulation even in the face of adversity. This interaction was specific to the cortisol response to stress; that is, it was neither present at rest (i.e., at baseline) nor confounded by baseline differences (Goal 2). Further, this relation was present only for the most severe childhood adversity, and may be especially strong for the experience of witnessing domestic discord/violence (Goal 3).

Notably, the current results should be interpreted while taking into consideration the inclusion of depression and anxiety symptomatology in the statistical analyses. By controlling for depression and anxiety symptoms, the results suggest that the negative cortisol response that
emerged in the FKBP5 risk allele and childhood adversity group was unlikely because these individuals were more anxious coming into the lab than the rest of the sample, and therefore had elevated cortisol. It was also not because they had more severe depression symptomatology. Both anxiety and depression have been associated with differences in the cortisol response (e.g., Harkness, Stewart, Wynne-Edwards, 2011; Yoon & Joormann, 2011; Young, Abelson & Cameron, 2004); thus the statistical control of these factors is important to be confident that observed effects are specific to FKBP5 variation and childhood adversity irrespective of current psychopathology. Although it was the case that a large portion of the participants in the FKBP5 risk allele and childhood adversity group had a diagnosis of a major depressive disorder (78%) or an anxiety disorder (44%), this is not surprising given that the FKBP5 gene and childhood adversity are associated with increased risk for depression and post traumatic stress disorder (Appel et al., 2011; Binder et al., 2008; Zimmerman et al., 2011). Given that depression and anxiety diagnoses were distributed across all four groups, and controlling for diagnostic status or symptoms did not change the pattern of results, it is unlikely depression or anxiety psychopathology accounted for the observed effects. Instead, these results suggest that the FKBP5 risk allele in combination with childhood adversity produce an underlying and undifferentiated vulnerability for psychopathology that exists independent of depression- or anxiety-specific pathology.

It is still possible, however, that the interaction of FKBP5 and childhood adversity varies as a function of depression or anxiety psychopathology. For example there is evidence that depression and anxiety differentially affect the interaction of FKBP5 and childhood adversity on gene expression, which, in theory, would lead to opposite patterns of cortisol release. In PTSD, molecular evidence for the interaction of FKBP5 and early adversity is associated with increased
gene expression, and thus, theoretically decreased cortisol (Sarapas et al., 2011), whereas the findings in depression are mixed between changes in gene expression leading to either excessive or decreased cortisol release (Binder et al., 2004; Menke et al., 2013). Although the current study was insufficiently powered to detect such a three-way effect, future research should explore the possibility that psychopathology further moderates the effect of FKBP5 and childhood adversity on the cortisol response. Nevertheless, the results of the current study provide evidence that even when controlling for the effects of psychopathology individuals with the FKBP5 risk allele and childhood adversity show dysregulation of the neurobiological stress response. From past research, there is consistent evidence that this response is dysregulated in individuals with depression and anxiety disorders (e.g., see Burke et al., 2005; Yoon & Yoorman, 2011; Young, Abelson & Cameron, 2004); although, the nature of dysregulation (i.e., the index of the cortisol response and direction of the effect) appears to vary as a function of clinical characteristics such as severity and comorbidity. Further, research has also demonstrated that specific FKBP5 polymorphisms and childhood adversity are risk factors for the onset of depression and anxiety (Appel el al., 2011; Binder et al., 2008; Zimmerman et al., 2011). Given this converging evidence, it is plausible that the unique negative cortisol response pattern observed in the current study is evidence of a common neuroendocrinological endophenotype that underlies risk for internalizing disorders. Thus, this study provides a first step toward understanding the role that variation in the FKBP5 gene and childhood adversity may play in conferring risk for stress-related psychopathology via changes in the cortisol response. A logical next step to extend the current study is to examine whether depressive disorders and anxiety disorders differentially affect the relation of FKBP5 variation
and childhood adversity on the cortisol response, or whether this mechanism is truly transdiagnostic.

**FKBP5, Childhood Adversity, & Cortisol Output Relative to Baseline**

Although the results of the current study are consistent with my hypothesis that individuals with the FKBP5 risk allele and childhood adversity would show a dysregulated cortisol response, the results begin to deviate from my hypotheses in terms of the cortisol indices that best characterize the differences in cortisol trajectories and the direction of these differences. In keeping with Klengel et al.’s (2012) study on the epigenetic modification of the FKBP5 gene by childhood adversity exposure, and consistent with a diathesis-stress model of neurobiological dysregulation, I hypothesized that childhood adversity would be associated with dysregulated cortisol output but only in those with the FKBP5 risk allele. The changes in gene structure observed by Klengel et al. were consistent with what would be expected to produce heightened cortisol output and only observable in response to stress. Thus, I also hypothesized that the FKBP5 risk allele and childhood adversity group would show greater cortisol output and that these changes would be specific to the recovery phase of the cortisol response. However, in the current study, the differences in cortisol trajectories emerged immediately following the stressor and were characterized by less cortisol output. That is, individuals with at least one FKBP5 risk allele and childhood adversity showed a negative cortisol response that corresponded to less total cortisol exposure relative to baseline. However, consistent with my hypothesis, baseline cortisol did not vary as a function of the interaction of FKBP5 and childhood adversity status.

One possible explanation for why the results of the current study were opposite in direction to what was hypothesized based on the Klengel et al. (2012) study is that other genes
involved in HPA axis function (e.g., CRH receptor gene [CRHR1] and GR gene) or other FKBP5 polymorphisms (e.g., rs4713916) may functionally override any differences expected based on the FKBP5 rs1360780 genotype. Alternatively, however, it is possible that these results reflect functional differences associated with variation at the rs1360780 SNP. Although the results of the current study were opposite in direction to what was hypothesized, they are not necessarily in direct opposition to those found by Klengel et al. Cortisol output following a psychosocial stressor is determined both by the duration of excitatory or inhibitory effects of higher order processes on the HPA axis, as well as by the molecular processes governing the positive and negative feedback within the HPA axis itself. The negative feedback mechanism is the regulatory process by which the neurobiological stress response “shuts down” the acute stress response. Typically, improper functioning of this system is associated with prolonged cortisol release, and therefore prolonged cortisol recovery. It is also possible that oversensitivity of this mechanism might lead to premature recovery and decreased cortisol output, as was observed in the FKBP5 and childhood adversity group. Furthermore, it is conceivable that higher order effects may trump dysregulation within the HPA axis itself. By assessing cortisol output following a psychosocial stress challenge, both higher order and HPA axis functioning contribute to final cortisol output. Importantly, the Klengel et al. experiments were done in the absence of higher order effects, and thus, provide insight into only one functional component of the neurobiological stress response. Given that the dysregulation of the cortisol response was present in the pre-recovery period and was negative in direction, the results of the current study can be taken altogether to suggest that higher order inhibitory processes exerted influence on the HPA axis prior to initiation of the negative feedback mechanism, thereby effectively turning off the cortisol response before it began. This explanation does not discount the possibility that structural and functional changes in
higher order inhibitory processes may in fact be adaptations to chronic overexposure of cortisol first initiated by the molecular changes observed by Klengel et al. Of course, prospective, longitudinal designs are required to test this hypothesis. Nonetheless, one possible explanation for why the results of the current study were opposite to what was hypothesized is that dysregulation co-occurred at both levels of functioning (i.e., in higher order brain processes and at the cellular level via epigenetic modification of FKBP5 gene structure).

The goal of the current study was to identify group differences in cortisol output as a function of genetic variation in the FKBP5 gene and childhood adversity status; it did not seek to determine the mechanisms underlying these differences. However, the process by which molecular changes to cellular processes at the level of the HPA axis may initiate changes in higher order inhibitory brain processes is consistent with the theory of allostatic overload (Danese & McEwen, 2012). This theory suggests that prolonged activation of one allostatic system, such as the HPA axis, leads to over-compensatory changes within this system as well as to other highly-integrated systems (i.e., neural and immune system), thereby leaving individuals more susceptible to the development of disease (e.g., depression or anxiety disorders). Thus, perhaps over time, prolonged cortisol exposure due to the epigenetic changes that arise as a function of FKBP5 and childhood adversity status at the molecular level leads to over-compensatory responses by higher order brain processes. In reaction to stress, these higher order brain processes may act to prematurely suppress the cortisol response, which manifests as a negative cortisol response. Although not explicitly investigated in this study, perhaps allostatic overload is a mechanism that facilitates dysregulation of the neurobiological stress response both at the level of the HPA axis and in higher order structures.
As discussed above, in the current study, it is likely that higher order neural processes exerting effects on HPA axis function are at least partially driving the differences in cortisol output. For example, the amygdala and medial prefrontal cortex (mPFC) have established anatomical links with the HPA axis and are an important part of the neurobiological stress response. Specifically, decreased amygdalar activation and increased mPFC activation are associated with decreased cortisol output (see Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009; Kern et al. 2008; Taylor et al., 2008). This pattern of activation is consistent with the direction of cortisol results found for the FKBP5 risk allele and childhood adversity group. The role of the mPFC, which is to monitor emotional state (Fredrikson, Wik, 1995) and to appraise the perception and judgment of others (Amadio & Frither, 2006), is interesting given the social-evaluative nature of the TSST stress task. However, the question remains of how these higher order processes interact with the HPA axis to produce the cortisol response to a psychosocial stressor. One recently studied method that could provide insight into this question is the combined dexamethasone/TSST paradigm (Andrews, D’Aguiar, & Pruessner, 2012). By administering dexamethasone prior to the administration of the TSST, differences in the cortisol response to the TSST may be interpreted as differences in higher order cognitive processes controlling for any HPA axis dysregulation effects. For example, if the same negative cortisol response pattern was observed substituting the dexamethasone/TSST for the TSST in the current study, it would confirm that higher order brain processes are at least, in part, responsible for the pattern of cortisol dysregulation.

Although the current study was the first to examine the interaction of the FKBP5 gene and childhood adversity on the neurobiological response to a psychosocial stressor, these results extend previous research examining the main effects of the FKBP5 gene and childhood adversity
on the cortisol response to stress, independently. The main finding of the current study was that individuals with the FKBP5 risk allele and childhood adversity had lower cortisol output such that they showed a negative pattern of cortisol release in comparison to individuals without such a history. One of the objectives of the current study was to attempt to reconcile the opposing patterns of results and inconsistent detection of significant effects in past research by examining the interaction of the FKBP5 rs1360780 polymorphism and childhood adversity. However, the ability to reconcile these differences and compare current results to past research was impeded by the modest sample size of the current study. Specifically, it was not feasible to perform all pairwise comparisons for the four groups (i.e., stratified by FKBP5 risk allele and childhood adversity status) due to the inflation of the type 1 error rate that occurs with additional comparisons. Thus, although the following discussion aims to integrate the current findings with past research, additional studies with sufficient power to compare across all groups are necessary to reconcile inconsistencies in this literature.

The results of the current study are most consistent with the research on the relation of childhood adversity and the cortisol response. Childhood adversity has generally been associated with blunted cortisol following psychosocial stress challenge (Carpenter et al., 2007; Lucas-Thompson, 2012; MacMillan et al., 2009; Ouellet-Morin et al., 2011). However, previous studies indexed the cortisol response by measuring “reactivity” but failed to control for baseline cortisol. Consequently, it is impossible to know whether blunted “reactivity” reflects differences in absolute peak cortisol or reflects relative change in cortisol. Further, none of the studies provide mean values to determine if blunted responses equate to less positive cortisol release or to negative cortisol release. It would appear from the graphs of the overall cortisol trajectories included in these studies, that their results are more consistent with findings of blunted, but
positive cortisol output, whereas the current study found *negative* cortisol release. What these results suggest is that the FKBP5 risk allele moderates the effect of childhood adversity on the cortisol response in a dramatic way. The only other study to have observed a negative pattern of cortisol release in relation to childhood adversity found this pattern in severely depressed adolescents regardless of childhood maltreatment history (Harkness, Stewart, & Wynne-Edwards, 2011). Thus, by examining polymorphisms of the FKBP5 gene as the moderator of this relation, the results of the current study suggest that the rs1360780 FKBP5 polymorphism confers risk for cortisol response dysregulation that is more consistent with an underlying vulnerability in common with severe depression. Although genetic risk in this study was defined as the presence versus absence of the FKBP5 risk T allele at rs1360780, this variable may have been capturing broader genetic risk comprised of multiple FKBP5 polymorphisms and even multiple genes. Importantly, the current study controlled for current depression and anxiety symptomatology yet still found the same negative cortisol response observed in the severely depressed group. Thus, taking the results of the current study and that of Harkness et al. (2011) altogether, it suggests that this negative pattern of cortisol release may be a biological marker for an endophenotype of depression characterized by severe episodes. Alternatively, it is possible that the negative pattern of cortisol release arises as a consequence of severe depression. That is, this response pattern may be a pathophysiological ‘scar’ incurred from having suffered from severe depression, rather than a vulnerability marker that exists prior to the first onset of the disorder. Consequently, as a residual symptom of severe depression, a negative pattern of responding may predispose individuals to depression relapse. However, to differentiate these hypotheses, longitudinal studies are needed to investigate the temporal onset of this cortisol response and whether it prospectively predicts depression onset and/or relapse. Further research is also necessary to identify whether
this cortisol response pattern is associated with particular symptom clusters or depressogenic behaviours. This research may help shed light on why this cortisol response pattern is common to individuals with severe depression as well as adolescents and young adults with a history of childhood adversity and the FKBP5 risk allele. Identifying clinical and behavioural correlates of this negative cortisol response pattern may provide insight into the clinical utility of the neurobiological stress response as a potential target for treatment intervention.

The current study also did not find evidence of an interaction of FKBP5 risk allele status and history of childhood adversity, nor a main effect of childhood adversity on baseline cortisol. These results are consistent with the null effect found by other studies that examined initial baseline cortisol differences prior to stress challenge in individuals with and without a history of childhood adversity (Carpenter et al., 2007; Lucas-Thompson, 2012; MacMillan et al., 2009; Ouellet-Morin et al., 2011). However, studies assessing basal cortisol functioning, specifically, have generally found higher cortisol in individuals with a history of childhood adversity (see Tarullo & Gunnar). The reason for this discrepancy is likely because these latter studies examined more complex indices of basal activity, such as diurnal variation in cortisol, or they failed to tease apart the effect of internalizing psychopathology. In the current study, the main effect of the FKBP5 gene was significant such that individuals with at least one risk allele at rs1360780 had higher baseline cortisol. Previous studies did not find baseline differences in cortisol as a function of the FKBP5 rs1360780 polymorphism (Ising et al., 2008; Mahon et al., 2012). Further, the results of the current study are inconsistent with what would be expected given the role of the FKBP5 gene in modulating the stress response (Klengel et al., 2012). It is possible that these results are evidence that the FKBP5 risk genotypes confers risk for a heightened state of the neurobiological activity even when the system is at rest, regardless of childhood adversity status.
However, this effect should be interpreted with caution given that it did not meet the conventional level of statistical significance and because baseline cortisol was assessed with only one sample; thus, it is a poor indicator of basal cortisol activity, which may vary across the day. Therefore, further evidence is required to determine whether the FKBP5 gene does, in fact, have a direct effect on basal cortisol. Nevertheless, the failure to find a significant interaction of FKBP5 risk allele status and history of childhood maltreatment strengthens the evidence that the negative pattern of cortisol release observed in the FKBP5 risk allele and childhood adversity group was not an artifact of differences in baseline cortisol among groups.

Previous studies on the main effect of the FKBP5 gene have had mixed success in detecting an effect of the risk allele at rs1360780 on cortisol release following stress challenge. Consistent with the current study, of the two studies examining the main effect of the FKBP5 rs1360780 polymorphism, neither found baseline effects (Ising et al., 2008; Mahon et al., 2012). However, whereas the study by Mahon et al. did not find differences in total cortisol output as a function of variation at rs1360780, Ising et al. did find differences in cortisol output. Specifically, homozygous carriers of the risk T allele at rs1360780 had greater cortisol output specific to the recovery phase than did heterozygous or non-risk allele carriers (Ising et al., 2008). Thus, the one study to find a main effect of the FKBP5 rs1360780 site found the opposite pattern of cortisol release than was found in the current study for the FKBP5 risk allele and childhood adversity group. However, these results were consistent with what would be hypothesized given the functional correlates of the FKBP5 rs1360780 polymorphism at the cellular level. Assuming that there is a main effect of variation at rs1360780 on cortisol output, why does the direction of this effect change when the FKBP5 genotype is examined in relation to childhood adversity? As is discussed above, this may be the effect of childhood adversity-dependent higher order brain
processes that suppress HPA axis activity, thereby producing a negative pattern of cortisol release as an adaptive response to initially higher cortisol. However, this precise mechanism was not evaluated in the current study and, thus, requires more targeted research.

Because of the paucity of research in this area and the complexity of the cortisol response and its measurement, the above discussion attempting to resolve these discrepancies is largely speculation. Nevertheless, what the current study tells us is that the main effects of the FKBP5 rs1360780 site and childhood adversity on the cortisol response are further qualified by an interactive effect. Specifically, the combination of these factors leads to a distinct neurobiological response to stress, which is characterized by decreasing cortisol following provocation. The detection of this interaction is evidence that genetic variation at FKBP5 moderates the effect of childhood adversity on the cortisol response. Taken from the opposite perspective, these results highlight the importance of considering the environment when assessing the effect of genetic factors such as FKBP5 polymorphisms on the cortisol response. For example, childhood adversity may counteract direct genetic effects of variation in the FKBP5 gene via changes in higher order brain processes. The negative pattern of cortisol output observed in this study is most consistent with the pattern of HPA axis responding shown in severely depressed adolescents (and adults; see Burke et al., 2005). These results suggest that identifying common vulnerability factors of depression and neurobiological dysregulation, such as FKBP5 polymorphisms, will be more informative than assessing the heterogeneous depression phenotype as a moderator of the effect of childhood adversity on the cortisol response.
FKBP5 & Specific Childhood Adversity Characteristics on the Cortisol Response

Because of the wide heterogeneity in the experience of childhood adversity, the third goal of this study was to examine how different characteristics of adversity affected the interaction of childhood adversity and the FKBP5 gene on cortisol output. Specifically, I examined whether the interaction effect held for each of the different forms of adversity, independently, and for when childhood adversity was inclusive of less severe forms of adverse experience (i.e., CECA ratings of at least 3-some). The following analyses were exploratory, and therefore caution should be taken when interpreting the results.

First, only the specific experience of witnessing domestic discord/violence emerged as a significant moderator of the effect of FKBP5 polymorphisms on cortisol response to the TSST. The absence of an effect for emotional abuse was surprising given the strong and preferential relation of emotional abuse with both cortisol output and depression status found in previous studies (Gibb, Butler & Beck, 2003; Carpenter et al., 2009). One characteristic that differentiates witnessing domestic discord/violence from the other forms of adversity is that the child is not the direct victim of the abuse. Thus, a potential explanation for why the interaction was present only when childhood adversity was defined in this way is that children who witness domestic discord/violence may internalize this form of adversity differently than do children who experience other forms of adversity. For example, children may not see themselves as victims of these experiences, and therefore they may not seek comfort or support from their parents for their distress. It is also possible that other characteristics that distinguish witnessing domestic discord/violence from other forms of abuse are responsible for this effect, such as the frequency, duration, or onset of adversity. Further research is needed to explore these characteristics to
determine why witnessing domestic discord/violence is implicated so strongly in the
dysregulation of the neurobiological stress response.

An alternative, but not mutually exclusive, explanation for the observed gene-
environment interaction of the FKBP5 gene and witnessing domestic discord/violence, on the
cortisol response is the presence of a gene-environment correlation. One type of gene-
environment correlation, known as the passive type, refers to circumstances where the child’s
genotype is correlated with their home environment. Because parents pass on their genes to their
biological children as well as create their home environment, it sets-up the possibility that
statistically detected gene-environment interactions are either completely or partially confounded
with gene-environment correlations. In the case of the FKBP5 gene, Bevilacqua et al. (2012)
found an association between aggression and the same polymorphism of the FKBP5 gene
implicated in the current study (i.e., rs1360780), such that individuals with the T risk allele and
childhood adversity showed more aggression and violent behavior. If parents with the FKBP5
risk allele at rs1360780 and history of childhood adversity also generate more domestic
discord/violence as a consequence of a predisposition for aggression, what statistically appears as
a gene-environment interaction (i.e., between child genotype and domestic discord/violence), may
in fact be a gene-environment correlation (i.e., between the parent genotype, passed onto the
child, and domestic discord/violence). Statistical models that test whether gene-environment
interactions exist even after controlling for gene-environment correlations require the use of twin
designs or much larger sample sizes to allow for more sophisticated statistical modeling. Future
studies employing either of these methods are required to be able to tease apart these effects.

Second, I found null effects for the interaction of childhood adversity and the FKBP5
gene on cortisol output when the threshold for adversity included less severe adversity, rated as at
least ‘some’. These results are important because they suggest that only when adversity reaches a severe threshold will it have pathophysiological effects. One explanation for why severe adversity is required for dysregulation in conjunction with FKBP5 genetic risk is because, in the short term, exposure to severe adversity produces greater and more prolonged cortisol responses. Cortisol concentrations may need to reach a certain threshold in order to initiate the epigenetic mechanisms of FKBP5 modification and potential neuronal remodeling in higher order brain structures that presumably lead to the development of a negative cortisol response. These results also have important implications for interventions aimed at normalizing dysregulated responding because they suggest that resources need only be focused on children who have experienced the most severe adversity. Further, these results provide encouraging news for identifying children at greatest risk for neurobiological dysregulation because severe adverse experience is the most likely to be reported to authorities.

**Gene-Environmental Interaction Model of Dysregulation of the Neurobiological Stress Response**

The current study examined the gene-environment interaction of variation in the FKBP5 gene and childhood adversity on the cortisol response, and was conceptualized according to a diathesis-stress framework for understanding dysregulation of the neurobiological stress response. Specifically, I hypothesized that the FKBP5 risk allele would leave individuals vulnerable to childhood adversity, but individuals without genetic vulnerability would be resilient to such adversity. A similar but competing framework for understanding gene-environment interactions is one of differential susceptibility (Belsky et al., 2009). In the differential susceptibility model, genetic variability confers the degree of responsivity an individual has to their environment –
whether positive or negative. Thus, the same genetic variant associated with increased risk for adverse outcomes in negative environments may be associated with more positive outcomes in highly supportive environments. In this way, genes can be thought of as “plasticity” genes rather than “vulnerability” genes. In the absence of measuring positive environmental exposure, the current study is unable to differentiate between these two developmental models. However, this competing differential susceptibility hypothesis highlights the importance of investigating positive dimensions of the environment, such as social support, in addition to the presence of negative experiences.

The differential susceptibility hypothesis also raises interesting questions about potential behavioural correlates of negative versus positive patterns of cortisol output. The purpose of the neurobiological stress response is to recruit physiological and cognitive resources to appropriately confront a stressor. Theoretically, a negative pattern of cortisol, as shown by those with the FKBP5 risk allele and childhood adversity, may correspond to an inability to recruit sufficient resources, and therefore to poor coping behaviour, whereas a positive cortisol response may correspond to better coping behaviour. In support of this hypothesis, individuals with lower cortisol output following pharmacological challenge had higher self-reported escape-avoidance behaviour than those with an average or elevated cortisol response (Hori et al., 2010). However, it is also possible that a negative cortisol response is an adaptive response that confers a behavioural and/or biological advantage in states of acute or prolonged stress. In the current study, patterns of cortisol release that did not follow the typical response pattern were conceptualized as ‘dysregulation’ of the neurobiological stress response and an indication of pathophysiology of this system; however, further research exploring the behavioural and physiological correlates of the negative response pattern are required to determine whether the
use of this terminology is accurate. Moreover, cortisol output following stress provocation is only one index of the complete physiological stress response. Further research on how other indices of the stress response such as the sympathetic nervous system and immune system may also aid in understanding the function of this negative cortisol response pattern.

**Strengths & Limitations**

Numerous papers have been published on the issue of false positive findings in science (e.g., Ioannidis, 2005; Simmons, Nelson, & Simonsohn, 2011). One area that has received considerable attention is candidate gene association and candidate gene-environment interaction studies in psychology and psychiatry (Duncan & Keller, 2011). Concerns about the replicability of these designs and the publication of false positive results have prompted journals to institute editorial policies about the methodology of candidate gene studies. For example, the Journal of Abnormal Child Psychology released an editorial policy in April 2013 that outlined seven standards used to evaluate and guide their editorial decision-making about such studies (Johnston, Lahey, & Matthys, 2013). Although these standards highlight several of the strengths of the current study, they also underscore the importance of not over interpreting the results. Each of these seven standards are discussed below as they pertain to the current study.

The first of these standards highlights the importance of using reliable measurement for the variables of interest. One reason past gene-environment studies have failed to replicate is because of the use of imprecise measures of the environment, such as self-report checklists (Monroe & Reid, 2008; Wong, Day, Luan, Chan, & Wareham, 2003). The current study used the Childhood Experience of Care and Abuse (CECA; Bifulco et al., 1994) interview and rating system to measure childhood adversity. Largely regarded as a gold-standard method of assessing childhood history, the CECA procedures are designed to probe for contextual information, which
allows adverse experiences to be graded for severity. Further, they are designed to safeguard against biases that can arise with retrospective reporting. In this study, I found that the effect of FKBP5 and childhood adversity on cortisol output held for severe (i.e., ratings of marked or moderate) adversity but not when the definition of adversity was loosened to include ratings of ‘some’. However, if the current study had used a checklist measure of adversity, it would have been unable to differentiate the severity of adversity, thereby compromising the validity of this construct and thereby limiting the ability of the study to detect a significant effect. Consequently, the use of the CECA for evaluating childhood experience is methodological strength of the current study.

The second of the candidate gene study standards is the selection of biologically and psychologically plausible genetic variants, environments, and phenotypes for the proposed gene-environment interaction. In the case of the current study, previous research had established the relation of the cortisol response with each of the FKBP5 gene and childhood adversity. Further, there was evidence of an epigenetic mechanism that might mediate this relation (Klengel et al., 2102), and each of these relations converged on the same genetic variant of the FKBP5 gene (i.e., rs1360780). Further, in contrast to gene-environment studies on broader psychiatric phenotypes, this study targeted a much narrower neurobiological phenotype by assessing the cortisol response. Although still polygenetically determined, cortisol output following a psychosocial stress task likely has fewer genetic determinants than does risk for psychopathology. Finally, an additional strength of the current study is the use of the Trier Social Stress Task, which is a well-established psychosocial stress paradigm, and an ecologically valid index of the cortisol output that might be expected in response to a real-world stressor.
The third standard for evaluating candidate gene studies pertains to population heterogeneity with respect to ethnicity. The current study employed a relatively homogenous sample of participants of European ancestry (approximately 85% of the sample), and no ethnic differences were detected across groups. Therefore, it is unlikely that population heterogeneity accounted for the observed effects. As a consequence of having little ethnical diversity, like many other studies, the generalizability of these findings to other ethnical populations is limited.

The fourth and fifth standards pertain to the statistical analysis of gene-environment interactions. The exploratory nature and small sample size of the currently study limited strict adherence to these guidelines. For example, in the statistical analyses a correction was not applied to the alpha value. Further, some of the reported results did not reach the traditional .05 level of statistical significance. Thus, this study was at an increased risk for making a type I error. In the absence of such correction procedures, the omnibus repeated measures analysis was used to limit the number of statistical models by first determining whether group differences existed, before identifying the most appropriate cortisol indices for follow-up tests. The sample size of the current study limited the power of the study below the recommended level of 80 percent. However, because of problems with the genetic samples, a large portion of the expected sample was lost. In the current study, it is possible limited power inhibited the ability to detect small but true effects in baseline cortisol or in the exploratory analyses of the effect of adversity characteristics. Nevertheless, the increased chance for type 1 error and limited power reinforce the degree of caution that should be taken when interpreting these results. Finally, as discussed earlier, the sample size precluded the use of statistical modeling that would allow for statistical control of gene-environment correlation. Thus, it is unclear whether a gene-environment correlation accounts for some or all of the detected effects.
The final two standards that candidate gene studies are encouraged to follow are the use of prospective designs and replication in an independent sample. Given the limitations of the available data, neither of these guidelines were feasible. An independent sample was unavailable to replicate these results and the sample was too small to halve for separate analysis. However, the call for prospective studies brings attention to a persistent gap in the neurobiological stress response literature. To date there are no prospective studies on the cortisol response before and after the onset of childhood adversity. These studies are needed to establish the causal relation of early adversity and abnormal patterns of responding. However, when considering the enormous cost of recruiting two samples of sufficient size, following these participants over time, the time-cost of using rigorous methods of assessing childhood adversity, and finding a developmentally appropriate but analogous index of neurobiological functioning, it is understandable that efforts to follow these seven guidelines have fallen short.

**Conclusion**

The current study examined the interaction of variation at the rs1360780 site of the FKBP5 gene and childhood adversity on the neurobiological response as indexed by cortisol output following a psychosocial stress task. This was the first study to show FKBP5 genotype-dependent group differences in cortisol output following a psychosocial stressor between individuals with and without a history of childhood adversity. However, contrary to the direction of my hypothesized effect, those with severe adversity had lower cortisol output following stress despite no differences in basal cortisol. Thus, individuals with the FKBP5 risk genotypes (i.e., TT/TC at rs1360780) and severe childhood adversity showed a negative pattern of cortisol release in which their cortisol output decreased following stress. This relation existed independent of
depression or anxiety psychopathology and was specific to the experience of severe adversity. The only type of childhood adversity for which the interaction with FKBP5 held when examined on its own was witnessing domestic discord/violence. Altogether, these results are consistent with a diathesis-stress model in which the FKBP5 risk allele leaves individuals more susceptible to neurobiological dysregulation following severe adverse experience. Moreover, these results highlight the importance of detailed measurement and characterization of environmental experience in terms of severity and type of adversity when assessing gene-environment interactions.

Given the limited research examining the interaction of the FKBP5 gene and childhood adversity at different levels of the neurobiological functioning (i.e., overall cortisol response or molecular processes), the implications of the current results for understanding the determinants of cortisol output in response to a stressor are purely speculative. Nevertheless, the current study raises interesting questions about how higher order brain processes might interact with molecular processes at the level of the HPA axis to affect final cortisol output following stress. One possible explanation for why the results of the current study are inconsistent with what might be expected given the limited molecular evidence is that the neurobiological stress response is a product of both HPA axis dysregulation and higher order neural processes. Replication and further investigation into the interaction of the FKBP5 gene and childhood adversity on stress responsivity in terms of functional differences in higher order neural processes and the molecular processes governing the neurobiological response is required to elucidate how these two factors affect different levels of neurobiological functioning.

Considerable research is also required to elucidate the clinical implications of these findings for depression and anxiety psychopathology. However, as personal genome sequencing
becomes relatively more affordable, and as the fields of pharmacogenetics and therapygenetics grow (i.e., study of the relationship between genetic variants and successful treatment response with drugs or psychotherapy, respectively), so does the potential for these results to have significant treatment and prevention implications. If higher order brain processes act as important determinants of the cortisol response by overriding dysregulation of the HPA axis, then these processes may be important therapeutic targets for normalizing the neurobiological stress response, and consequently depression and anxiety symptoms. For example, psychotherapy directed at higher order brain processes may prove to be equally effective as pharmacological treatments that target basic molecular processes in regulating cortisol output. In order to address these questions, adequately powered studies are required to test the interaction of FKBP5 and childhood adversity in clinical versus non-clinical populations, and to determine whether the unique pattern of responding observed in this study is clinically relevant in terms of behaviours such as negative thought patterns or the clinical course of psychopathology.
References


Vulnerability genes or plasticity genes?. *Molecular Psychiatry, 14*(8), 746-754.


Hollingshead, A. B. (1975). *Four factor index of social position*. Unpublished manuscript, Yale University, New Haven, CT.


Lavebratt, C., Aberg, E., Sjoholm, L. K., & Forsell, Y. (2010). Variations in FKBP5 and BDNF genes are suggestively associated with depression in a Swedish population-based cohort. *Journal of*


FKBP5 and CRHR1 with cortisol response to acute psychosocial stress in healthy adults.


Papiol, S., Arias, B., Gasto, C., Gutierrez, B., Catalan, R., & Fananas, L. (2007). Genetic variability at HPA axis in major depression and clinical response to antidepressant treatment. *Journal of*
Affective Disorders, 104(1-3), 83-90.


Tonmyr, L., & Bartholomew, S. (1998). International studies on the incidence and prevalence of


Appendix A

Sample Questions from the Childhood Experience of Care and Abuse Scale (CECA)

RELATIONSHIP WITH PARENT FIGURES

MOTHER

How well do you get on with your mother?
Are you close?

(AFFECTION)
Is she affectionate towards you?
How does she show it? Did you ever wish she were more affectionate?

(COMPAIONSHIP)
Does your mother spend much time with you?
Do you enjoy this time?
What sort of things do you do together? Are there any special activities or games?
Can you have a laugh together?

ANTIPATHY

Can she be hard to please?
IF YES: In what sort of way?
Is she very critical of you?
Is she ever cold and distant?
Do you ever feel she doesn't want you?
Does she ever say anything rejecting? What sort of thing?

Do you argue much with her? What about? How often?

Does she ever push/slap/hit you?
How often does this happen? (Skip to physical abuse section if necessary)

Was your relationship the same when you were younger? Has it changed at all over childhood?
IF YES: When was that? In what way did it change? Why do you think that was?

FATHER

What is the relationship with your father like?
Are you close to him? Is it a different relationship from that with your mother?

Is he affectionate towards you?
How does he show it? Do you ever wish he was more affectionate?

Does your father spend much time with you?
What sort of things do you do together? What about times when he isn't working?

ANTIPATHY

Is he hard to please?
IF YES: In what sort of way?
Is he ever very critical of you?
| RELATIONSHIP WITH SIBLINGS | How well do you get on with your brother(s) and sister(s)?
| Do you enjoy spending time with them? What sort of things do you do together? |
| FAVOURITISM | Are there any favourites in the family?  
| If YES: Who is that? In what way are they favoured? By mother or father?  
| Was that any different when you were younger? In what way? |
| SCAPEGOATING | Does any one of the children get picked on more than the others?  
| If YES: Who? In what way? Which parent does that? |
| PARENTAL INDIFFERENCE | Do you feel your parents always have time for you and take an interest?  
| Can you go to them if you are upset or unhappy? Are they usually helpful?  
| Is that the same for your mother and father?  
| If NOT: Which one takes more interest? In what way? |
| (BIRTHDAY) | Do you parents always remember your birthday?  
| Do you celebrate it in some way? |
| (MATERIAL CARE) | Do your parents take good care of your material needs?  
| For example washing your clothes and cooking your meals?  
| Are you expected to do any of that yourself now?  
| Do you always have enough to eat? |
| (SCHOOL) | Are your parents keen for you to do well at school?  
| Have they given you guidance in choosing courses?  
| Are they satisfied with your achievements?  
| Have they taken an interest in your choice of career? |
| (ILLNESS) | If you were ill and had to take time off school who would look after you?  
| Are your parents particularly caring if you were ill? Is that both of them?  
| Do you get any special treatment if you are ill? What sort of thing? |
CHANGES

Have your parents changed at all in the amount of interest they've shown in you since you were small?
IF YES: In what way?