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# The Effects of HPA Axis Genetic Variation and Early Life Stress on Cortisol Levels in Preschool Age Children and on Amygdala and Hippocampus Volumes, Reactivity, and Connectivity at School Age

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by
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# Abstract of Dissertation

Effects of HPA Axis Genetic Variation and Early Life Stress on Cortisol Levels in Preschool Age Children and on Amygdala and Hippocampus Volumes, Reactivity, and Connectivity at School Age

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Internalizing psychopathology has been linked to increased cortisol reactivity and alterations in limbic brain structure and function, yet the mechanisms underlying these alterations are unclear. One key hypothesis is that stress plays a major causal role in these mechanisms. Animal studies find that chronic stress or glucocorticoid administration lead to alterations in hippocampal and amygdala structure and function. Relatedly, life stress is a major risk factors for depression while candidate gene studies have related variation in hypothalamic-pituitary-adrenal (HPA) axis genes to increased prevalence and severity of depression. The present work tested the hypothesis that genetic profile scores combining variance across 10 single nucleotide polymorphisms from four HPA axis genes (*CRHR1, NR3C2, NR3C1, FKBP5*) and early life stress would predict increases in stress cortisol levels in preschool-age children as well as alterations in hippocampal and amygdala volumes,

reactivity, and resting state functional connectivity in these same children at school age. The current results indicate that (1) childhood stress exposure and genetic profile scores both predict stress cortisol, (2) these factors interact to predict volumetric alterations, partially mediated by cortisol, (3) life stress predicts left amygdala reactivity while genetic profile scores interact with sex and pubertal status to predict amygdala and hippocampus reactivity to negative emotional stimuli, and (4) these factors and their interaction predict weakened amygdala functional connectivity with subcortical and prefrontal regions. Overall, these findings suggest a key role for stress exposure, genetic risk, and cortisol in contributing to individual differences in amygdala and hippocampus structure and function typically associated with internalizing pathology.

# Chapter 1: Introduction

## 1.1 Why Study Depression?

Internalizing disorders, like major depressive disorder (MDD) and anxiety disorders, are among the most prevalent and disabling mental health conditions. Recent epidemiological results estimate the lifetime morbid risk (proportion of people who will eventually develop at disorder during their life) for a depressive episode at 29.9% and for any anxiety disorder at 41.7% (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012). Furthermore, these disorders have a relatively early onset, typically prior to or during one's twenties where the lifetime prevalence among 13-17-year-olds was 12.6% for a depressive episode and was 32.4% for anxiety disorders (Kessler et al., 2012). Nonetheless, depression can onset even earlier among children/adolescents (the three-month prevalence for depressive disorders among 9-16-year-olds is 2.2%, see Costello, Mustillo, Erkanli, Keeler, & Angold, 2003) and has been characterized in children as young as preschool-age (Luby, Heffelfinger, Mrakotsky, Brown, Hessler, Wallis, et al., 2009a; Luby, Si, Belden, Tandon, & Spitznagel, 2009). Importantly, early-onset MDD vastly increases the odds of later MDD and comorbidity (Costello et al., 2003; Luby, Si, Belden, Tandon, & Spitznagel, 2009), continuing negative trajectories of emotional development if intervention does not occur. Yet, while there is promising evidence from small-scale trials of novel interventions for early-onset pathology (e.g. parent-child intervention therapy; Luby, Lenze, & Tillman, 2012), the efficacy of common treatments for adult MDD are generally quite low. Particularly, remission rates from antidepressants are generally <30%,

while drop-out rates from such trials are high (Pigott, Leventhal, Alter, & Boren, 2010). Similarly low remission rates are observed for cognitive behavioral therapy and psychodynamic therapies, with 40% of patients seeking additional treatment afterwards (Driessen et al., 2013). Symptom heterogeneity within depression (or different subtypes), comorbidity, genetic risk (e.g. family history), the experience of stressful life events, and low social support all can contribute to ineffective treatment and to treatment resistance (~20-30% of patients fail to respond to at least one standard course of antidepressants; Fava & Davidson, 2005). Understanding the role of factors, like genetic risk and stress, in the etiological mechanisms underlying depression and related alterations in emotional function may be key to improved treatment and diagnostics.

## 1.2 Understanding Risk for Depression

**1.2.1 Environmental Risk**: A meta-analysis of twin studies suggested that the heritability of MDD is ~40% whereas the remaining ~60% of liability is likely due to individual-specific environmental factors (with little contribution of twins' shared environment, see Sullivan, Neale, & Kendler, 2000). The experience of stressful and traumatic life events, particularly in early life, has been cited as one of the foremost individual environmental factors contributing to MDD risk (Green et al., 2010; Kendler, Karkowski, & Prescott, 1999; Kendler et al., 1995; Kendler, Kuhn, & Prescott, 2004; Kessler & Magee, 2009). Further, much work has suggested that a vast majority of MDD onsets are preceded by a provoking stressor, both in adults (e.g. G. W. Brown, Bifulco, & Harris, 1987) and in adolescents (e.g. Williamson, Birmaher, Anderson, Al-Shabbout, & Ryan, 1995). In

addition, childhood adversity has been suggested to potentially leave 'scars' that increase the liability to MDD for up to 10 years after the experience (Kessler, Davis, & Kendler, 1997). A history of childhood adversity has also been shown to predict early onset of MDD, which in turn predicts more severe later symptomatology (Turner & Butler, 2003), i.e. acting as a potential mediator of effects of childhood adversity on later pathology. Additionally, early life stress may sensitize an individual to the effects of stressors in the future, i.e. increasing liability to MDD in response to adult stressors (e.g. Hammen, Henry, & Daley, 2000). This type of interaction between early and later stressors is also observed in children around their transition into puberty (Rudolph & Flynn, 2007).

**1.2.2 Genetic Risk:** Despite the ~40% heritability estimate for MDD indicating a relative large effect of additive genetic factors, large-scale genome-wide association studies (GWAS) have largely failed to implicate any consistent genetic loci in the etiology of MDD (Bosker et al., 2010; Kohli et al., 2011; Lewis et al., 2010; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2012; Muglia et al., 2010; Rietschel et al., 2010; Shyn et al., 2011; Sullivan et al., 2009; Wray et al., 2012). This again may be due to the heterogeneity of depression and/or the large influence of environmental factors, suggesting that examination of more proximal outcomes, i.e. endophenotypes, or gene x environment interactions may prove a more fruitful approach. On the other hand, candidate gene studies targeting specific systems of interest have made more headway in identifying potentially relevant genetic risk factors for depression. These studies have mainly focused on genes regulating major neurotransmitter systems (e.g. serotonin: Clarke, Flint, Attwood, & Munafò, 2010), neurotrophic factor function (e.g. BDNF: Brunoni, Lopes, & Fregni, 2008; Verhagen et al., 2010), or hypothalamic-pituitary-adrenal (HPA) axis

function. Genes regulating the HPA axis are of particular interest for the current work (i.e. *CRHR1*, *NR3C1*, *NR3C2*, *FKBP5*; for examples of prior work, see Binder et al., 2004; Z. Liu et al., 2006; Papiol et al., 2007; van Rossum et al., 2006; van West et al., 2005; D. Wasserman, Sokolowski, Rozanov, & Wasserman, 2007; D. Wasserman, Wasserman, Rozanov, & Sokolowski, 2009; Zobel et al., 2008), given that they may moderate an individual's response to environmental stressors, as discussed in more detail below. Prior work has linked common variants in these genes to alterations in cortisol functioning, depression incidence and severity, and/or other depression-related phenotypes. Table 2.1 summarizes this prior work. Some of these studies have suggested main effects of these genes while others have found them to moderate effects of environmental stress. This prior work and the function of these HPA axis genes are reviewed in more detail in section *1.3: HPA Axis Function and Genes of Interest*.

**1.2.3 Gene x Environment Interaction:** Interactions between genetic and environmental effects may be a key factor potentially contributing to the lack of replicable GWAS results for MDD. Specifically, GWAS studies examine main effects of genetic variants but generally do not explore variants as moderators of environmental risk. Particularly, recent work has found that while certain genetic polymorphisms do not exhibit a strong *main* effect on depression risk, they do exhibit differential effects in negative vs. adaptive environments. For example, Caspi et al., (2003) found that while having the short vs. long 5-HTTLPR allele had no effect on depression probability or severity in individuals with low stress exposure (few stressful life events or no childhood maltreatment), having 1-2 short alleles increased depression among those with higher stress exposure (for meta-analysis, see Karg, Burmeister, Shedden, & Sen, 2011). Reframing this, the long allele appeared

protective against the effects of stress exposure while the short allele conferred environmental sensitivity to the negative effects of stress, while the effects of these different alleles were not differentiable in low stress conditions.

Further, HPA axis genes likely play a role in moderating the effects of environmental stress. Particularly, one could hypothesize that genes affecting HPA axis reactivity/regulation could potentiate/blunt the neuroendocrine and subsequent downstream effects of stress exposure, but might have little differential effect by genotype in low stress environments. Several studies have already noted interactions between the HPA axis genes of interest here and life stress. For example, several *CRHR1* single nucleotide polymorphisms (SNPs) have been suggested to moderate effects of childhood maltreatment on adult depression (Bradley et al., 2008; Heim et al., 2009; Laucht et al., 2013; Polanczyk et al., 2009) and to moderate effects of life stress on depression and suicidality (Wasserman et al., 2007; Ben-Efraim et al., 2011). Similarly, an *FKBP5* polymorphism has been suggested to moderate effects of childhood maltreatment on depression (Appel et al., 2011; Zimmermann et al., 2011), PTSD (Binder, 2009), and suicidality (Roy et al., 2010).

Recent studies have begun to bridge this type of gene x environment interactions with neuroimaging techniques. Particularly, as noted above, much can be gained by examining endophenotypes, like brain structure/function, that lie closer to the effects of genes and/or environmental factors than more complex (and heterogeneous) phenotypes, like psychopathology. Thus, neuroimaging studies examining genetic or environmental effects on the brain or imaging gene x environment interactions often aim to elucidate the mechanisms underlying a particular system of disruptions in psychopathology, e.g.

emotional function or reward sensitivity. This type of work has examined the effects of particular SNPs or genes on brain outcomes and more recently has examined polygenic effects, as examined here. Polygenic approaches that combine variance across SNPs from multiple genes may be very powerful in studying the effects of a particular biological system of interest on the brain and psychopathology risk and can better capture the polygenic nature of psychopathology and other complex traits. Yet, this work still faces many challenges. While polygenic approaches will capture more variance in neural outcomes than any one SNP alone (e.g. Nikolova et al., 2011), effect sizes are still expected to be relatively small as brain outcomes are complex in their genetic etiology. Thus, larger sample sizes are required to achieve adequate power, which becomes costly for neuroimaging and genetic analyses. In addition, this type of work is generally based on prior literature to build these polygenic scores, yet there is often weak prior evidence detailing the mechanistic function of a given SNP of interest. These strengths and limitations (and others) of imaging approaches to studying gene x environment interactions are reviewed in more detail by Bogdan, Pagliaccio, Baranger, & Hariri (*submitted*).

## 1.3 HPA Axis Function and Genes of Interest

Figure 1.1 schematizes the central pathway by which the HPA axis is activated and several mechanisms for negative feedback. Stress information is conveyed to the paraventricular zone (PVN) of the hypothalamus via projections from the amygdala, prefrontal cortex (PFC), and other regions, which induces release of corticotropin-releasing hormone (CRH). CRH binds to CRH type 1 and type 2 receptors in the pituitary, prompting

release of adrenocorticotropic hormone (ACTH). ACTH binds to melanocortin receptors in the adrenal glands prompting the release of cortisol into the bloodstream. Cortisol then has widespread effects on the brain and the body activating other stress-related responses and modulating brain reactivity. Cortisol release also induces negative feedback on the HPA axis directly and indirectly via brain regions, like the hippocampus. The roles of the four genes of interest here – *CRHR1, NR3C2, NR3C1,* and *FKBP5* – in the activation and regulation of the HPA axis are summarized below.

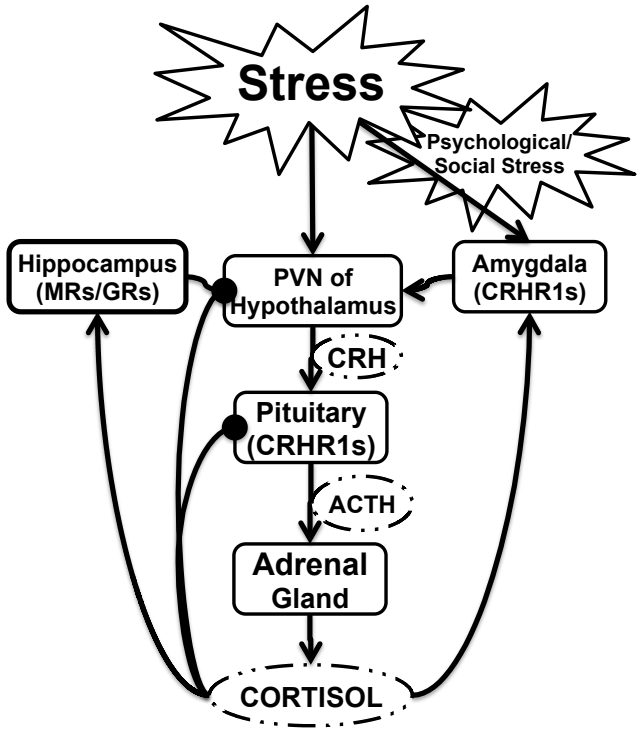


**Figure 1.1: Schematic Diagram of HPA Axis Activation and Regulation**
This figure outlines the core hypothalamic-pituitary-adrenal axis mechanism by which environmental stress leads to cortisol release and several paths by which cortisol exerts negative feedback on the HPA axis. Arrowheads denote activating paths; paths with round ends denote negative feedback/inhibitory paths. Hormones are denoted in dotted and dashed ovals.

**1.3.1 *CRHR1****:*** The *CRHR1* gene codes for the corticotropin releasing hormone receptor 1. This receptor is mainly localized to the pituitary (Van Pett et al., 2000), which serves an activating node in the HPA axis as noted above. Here, CRHR1 binds CRH, which is released from the PVN of the hypothalamus. *CRHR1* knockout mice show dysfunctional activation of the HPA axis and significantly decreased anxiety behavior suggesting the functional importance of *CRHR1* for normal HPA reactivity and in anxiety (G. W. Smith et al., 1998). It is also important to note that *CRHR1* is expressed in other brain regions, like the anterior cingulate, prefrontal cortex, amygdala, and hippocampus (Aguilera, 2004). Conditional knockout of *CRHR1* in the forebrain of post-natal mice, including the amygdala and hippocampus, leads to significantly reduced anxiety behaviors despite intact basal HPA activity. However, when these mice were exposed to stress, over-activity of the HPA axis became apparent, indicating a role for extra-pituitary *CRHR1* in stress reactivity (Müller et al., 2003).

In human studies, SNPs on this gene have been associated with increased pharmacologically-induced cortisol response in interaction with a history of childhood maltreatment (Tyrka et al., 2009). *CRHR1* (and *CRH* and CRH Binding Protein) SNPs have shown main effects and epistatic interactions predicting increases in cortisol reactivity in young children (Sheikh, Kryski, Smith, Hayden, & Singh, 2013). Finally, CRHR1 SNPs have been associated with increased depression severity and suicidality (in males with low stress exposure (D. Wasserman et al., 2007; 2009), depression status (three SNP haplotype (Z. Liu et al., 2006), and antidepressant response (three SNP haplotype in patients with high anxiety, see Licinio et al., 2004; Z. Liu et al., 2007).

**1.3.2 *NR3C2:*** The *NR3C2* gene codes for the mineralocorticoid receptor (MR), which serves as a high affinity, low capacity receptor for cortisol. MRs are highly expressed in the hippocampus where they have been suggested to help establish tonic inhibition of the HPA axis. Even at basal cortisol levels, MRs likely have their low capacity for cortisol filled, i.e. binding levels don't depend largely on stress-induced increases in cortisol (de Kloet, Joëls, & Holsboer, 2005; Jacobson & Sapolsky, 1991; Reul & de Kloet, 1985). Chronic corticosteroid administration in mice induces downregulation of MRs in the hippocampus and hypothalamus and depressive-like behavior, but administration of an MR antagonist can confer antidepressant-like effects (Wu et al., 2012). MRs also mediate stress-induced changes in glutamate-signaling in CA1 of the hippocampus (Karst et al., 2005), likely inducing alterations in hippocampal reactivity. Additionally, MRs can impact the stress system outside of the hippocampus. For example, overexpression of MRs in the mouse forebrain decreases anxiety-related behaviors (Rozeboom, Akil, & Seasholtz, 2007), again underscoring the role of MRs in normal and pathological stress function.

Human studies have mainly focused on one SNP, rs5522. The minor allele of rs5522 (functional missense polymorphism - MR I180V) has been associated with increased cortisol reactivity to stressors (DeRijk et al., 2006) and with increased depressive symptomology (Kuningas et al., 2007). This SNP has further been suggested to moderate the effect of childhood adversity on amygdala reactivity, where val carriers showed elevated amygdala reactivity and adversity predicted greater reactivity among iso carriers (Bogdan, Williamson, & Hariri, 2012).

**1.3.3 *NR3C1:*** The *NR3C1* gene codes for the glucocorticoid receptor (GR). Like MRs, GRs are highly expressed in the hippocampus, but are also expressed more

pervasively throughout the brain than MRs. GRs have a low affinity but high capacity for cortisol, which allows them to play a more dynamic role in HPA axis regulation by facilitating negative feedback at higher levels of cortisol (de Kloet et al., 2005; Jacobson & Sapolsky, 1991; Reul & de Kloet, 1985). It is also worth noting that the environment can have a large impact on both MRs and GRs in the brain. For example, early life stress in primates can alter MR and GR mRNA expression in the hippocampus (Arabadzisz et al., 2010) and an enriched environment can restore normal expression patterns and lessen depressive-like symptoms in chronically stressed rats (Zhang et al., 2011). In human studies, several *NR3C1* SNPs have been associated with altered cortisol reactivity to social stressors (sex x genotype interactions, see Kumsta et al., 2007) and increased depression status/severity (Szczepankiewicz et al., 2011; van West et al., 2005).

**1.3.4 *FKBP5:*** *FKBP5* codes for the FK506 binding protein 51, which serves as a co-chaperone protein in mature GR complexes. FKBP5 has been suggested to modulate GR sensitivity to cortisol. Specifically, when FKBP5 is bound to the GR complex, the receptor shows lower affinity for cortisol and less efficient translocation to the nucleus. GR activation can induce *FKBP5* mRNA and protein expression. Higher levels of FKBP5 confer GR resistance and keep the GR in a low affinity state for longer (Binder, 2009).

In human work, a functional variant in the *FKBP5* gene has been identified (rs1360780) where minor allele homozygotes show greater FKBP5 production and less effective cortisol negative feedback on the HPA axis (Binder, 2009). This may be driven by a change in the structural configuration of *FKBP5* where a long-range enhancer region is located closer to the transcription start site in T allele carriers. Homozygotes also show increased experience of MDD episodes but faster recovery with antidepressant treatment

(Binder et al., 2004) as well as increased/prolonged cortisol responses (Ising et al., 2008; Velders et al., 2011). FKBP5 has also been shown to moderate the effects of childhood adversity on amygdala reactivity to emotional stimuli, where rs1360780 minor allele carriers showed stronger positive associations between adversity and reactivity vs. major allele homozygotes (White et al., 2012).

## 1.4 Cortisol Alterations

The unique and interacting effects of HPA axis genetic variants and environmental stress on depression are well complemented by findings of stress system dysregulation in depression. Particularly, up to 90% of MDD patients can be characterized by increased cortisol response to administration of dexamethasone and CRH; HPA axis dysregulation is most likely due to impaired feedback inhibition (Heuser, Yassouridis, & Holsboer, 1994). Dysregulation has also been observed in people with a high familial risk for MDD, indicating that HPA dysregulation or stress sensitivity may relate to genetic vulnerability to depression (Modell et al., 1998). Additionally, MDD patients who respond to treatment (including antidepressants, lithium, electroconvulsive therapy, and transcranial magnetic stimulation) show a normalization of HPA axis abnormalities, i.e. a decrease in hyper-responsivity to stress (McKay & Zakzanis, 2010). This has lead to the idea that HPA normalization may be a common pathway of antidepressant effects (Holsboer & Barden, 1996). The importance of the stress system is again underscored by the finding that persistent dysregulation after treatment is associated with a high risk for early relapse (Ribeiro, Tandon, & Greden, 1993).

Further, potential risk factors for MDD have been related to alterations in cortisol functioning. Particularly, environmental stress has been shown to induce HPA axis dysregulation (e.g. Carpenter et al., 2007; Lovallo, Farag, Sorocco, Cohoon, & Vincent, 2012; Ouellet-Morin et al., 2011) and the HPA axis genes of interest here have been related to altered cortisol function in a variety of samples (e.g. Binder et al., 2004; DeRijk et al., 2006; Heim et al., 2009; Menke et al., 2013; Rosmond et al., 2000; Tyrka et al., 2009; Velders et al., 2011). Alterations in cortisol reactivity/regulation are likely an integral step in the mechanism linking stress-related risk factors to depressive pathology.

## 1.5 Neural Change

While cortisol has many downstream cellular and systems-level effects (de Kloet et al., 2005), its impact on the brain is of key interest to understanding psychopathology. Particularly, cortisol and brain structure/function may serve as core reciprocal steps linking risk to pathology, where cortisol impacts certain brain structures/functions and variation in these structures contribute to cortisol reactivity/regulation. Of relevance to the current work, cortisol is tightly tied to the hippocampus and amygdala; both are involved in the reactivity/regulation of the HPA axis, both are impacted by cortisol, and both show structural and functional alterations related to depression and stress. The roles of the amygdala and hippocampus in the HPA axis and their structural alteration in depression are discussed below. Amygdala and hippocampus function and amygdala functional connectivity have also been linked to stress and depression; these relationships are reviewed more in Chapters 3 and 4, respectively.

**1.5.1 Hippocampus**: Depression has been associated with bilateral hippocampal volume decreases (~8-10%), which are generally shown to correlate with the number of MDD episodes experienced (Campbell & MacQueen, 2004; Videbech & Ravnkilde, 2004). Stress dysregulation is a major candidate hypothesis for the mechanism of volumetric loss in depression. Particularly, while the hippocampus is a major source of inhibition on stress circuitry in the brain (i.e. hippocampal MRs regulating basal HPA axis activity and GRs contributing to cortisol negative feedback), the hippocampus is also particularly vulnerable to the neurotoxic effects of cortisol (Jacobson & Sapolsky, 1991). Prolonged, elevated cortisol levels can cause damage to the hippocampus, which may be observed as volumetric loss in humans.

Consistent with this hypothesis, animal studies have consistently shown chronic stress and corticosteroid administration to reduce dendritic branching and length of hippocampal CA3 pyramidal neurons (e.g. Conrad, LeDoux, Magariños, & McEwen, 1999; Magariños, McEwen, Flügge, & Fuchs, 1996; McKittrick et al., 2000; Watanabe, Gould, & McEwen, 1992) as well as to potentially cause cell death in the CA3 subfield and to impair neurogenesis in the dentate gyrus with prolonged stress exposure (Reagan & McEwen, 1997; Sapolsky, 2000). It has been suggested that these cortisol effects may damage the hippocampus via a glutamate/NMDA-mediated mechanism of excitotoxicity (Magariños et al., 1996; McEwen, 1997; Reagan & McEwen, 1997). Relatedly, stress and cortisol have also been suggested to decrease glutamate regulation by astrocytes or to lead to glial loss (e.g. Campbell & MacQueen, 2004; Cotter, Pariante, & Everall, 2001; Sapolsky, 2000). However, one recent study importantly showed that stress-induced loss of CA1 dendritic spines and

decreases in spine length accounted for gray matter volume loss observed by MRI in mice, rather than neuronal or glial loss (Kassem et al., 2012).

It is also important to note that to observe relatively large changes in regional brain volume, it is likely that a variety of mechanisms are at play (e.g. including differences in neurotrophic factors or factors influencing NMDA receptors or glutamate excitability, which could moderate excitotoxic effects). Nonetheless, a stress-/cortisol-mediated mechanism of change in limbic structure is highly supported by the animal literature and related human literature, e.g. high endogenous levels of cortisol across several years are associated with smaller hippocampal volumes in older adults (Lupien et al., 2005). Additionally, patients with Cushing's syndrome who exhibit hypercortisolemia tend to show reductions in hippocampal volume, where 24-hour mean cortisol levels were negatively correlated with hippocampal volumes (Starkman, Gebarski, Berent, & Schteingart, 1992). Importantly, treatment of Cushing's can lead to enlargement of the hippocampus, associated with reductions in cortisol levels (Starkman et al., 1999). Further, the experience of life stress has been associated with small hippocampal volumes, and particularly in people with or at-risk for depression (e.g. Carballedo et al., 2012; Frodl, Reinhold, Koutsouleris, Reiser, & Meisenzahl, 2010; U. Rao et al., 2010; Vythilingam et al., 2002). Importantly, positive environmental factors, like maternal support in early childhood may predict larger hippocampus volumes (Luby et al., 2012a). Given the role of the hippocampus in the regulation of the HPA axis, it is also important to note that these stress/cortisol effects on the hippocampus may also impact responsivity to future stressors. For example, animal studies have shown that chronic psychosocial stress can downregulate expression of MR and GR mRNA in the hippocampus (though this varied by

sub-region for MR mRNA; Meyer, van Kampen, Isovich, Flügge, & Fuchs, 2001) whereas increased maternal care was related to increased hippocampal GR mRNA and improved HPA axis regulation (D. Liu, 1997). There is also some evidence from human studies that smaller hippocampal volumes may be a risk factor for developing PTSD after trauma exposure (Gilbertson et al., 2002). Understanding the role of stress and cortisol in determining brain structure may help explain, at least in part, the underlying neural alterations observed in depression.

**1.5.2 Amygdala**: The amygdala is also a key structure in the pathology of depression. Elevated amygdala activation during emotional tasks has been clearly shown in depressed adults, adolescents and children (e.g. Beesdo et al., 2009; D, Gaffrey, Botteron, Belden, & Luby, 2012; Gaffrey et al., 2011; Pagliaccio et al., 2011; Surguladze et al., 2005; Yang et al., 2010). Relationships between stress, depression, and amygdala reactivity are discussed further in Chapter 3. Yet, human imaging studies on amygdala volume have been quite mixed in their findings regarding a relationship with stress and depression (Hamilton, Siemer, & Gotlib, 2008). Some studies indicate that amygdala volumes may be enlarged in depression (Frodl et al., 2002; 2004; Lange & Irle, 1999; Saleh et al., 2012; Van Eijndhoven et al., 2009; Vassilopoulou et al., 2012), while other studies find no difference in volume (Bremner et al., 2000; Frodl et al., 2004; Munn et al., 2007; Tamburo et al., 2009) or decreases in volume (Hastings, Parsey, Oquendo, Arango, & Mann, 2004; Sheline, Gado, & Price, 1998; Sheline, Sanghavi, Mintun, & Gado, 1999; Siegle, Konecky, Thase, & Carter, 2003) with a recent meta-analysis finding decreased volume in the left amygdala (Sacher et al., 2012). Additionally, some studies associate stress with enlarged amygdala volumes (Holzel et al., 2010; Tottenham et al., 2010) while another associated cortisol

administration with smaller amygdala volumes (E. S. Brown, Woolston, & Frol, 2008). Furthermore, experience of frequent positive maternal behaviors may attenuate volumetric increases of the amygdala across development (Whittle et al., 2013).

The animal literature tends to indicate that chronic stress is associated with increased amygdala volume and dendritic length/arborization, though there may be some differences based on the type of stressor (Cui, Sakamoto, Higashi, & Kawata, 2008; Vyas, Mitra, Rao, & Chattarji, 2002; Vyas, Jadhav, & Chattarji, 2006). This is critical given the amygdala's role as a mediator of HPA axis activation due to psychological or social stressors (Herman & Cullinan, 1997). Psychological stress can increase activation of the amygdala CRH system; amygdala activity can also be enhanced by glucocorticoid administration contributing to leading to a positive feedback loops with the HPA axis (Makino, Hashimoto, & Gold, 2002). CRH in the amygdala likely contributes to altered reactivity of the stress-system with stress exposure and depression. Relatedly, overexpression of CRH in the amygdala has been shown to decrease negative feedback on the HPA system and to increase symptoms of anxiety and depression (Keen-Rhinehart et al., 2009). Again, understanding the effects of stress and cortisol on amygdala structure may help to elucidate mixed findings in depression in human studies. Interestingly, CRHR1-expressing neurons in the amygdala can be modulated by circulating glucocorticoid, which can increase the excitability of limbic cells (de Kloet et al., 2005); this is of potential interest to studies relating amygdala function to stress exposure and depression in humans. Particularly, the potential interacting effects of HPA axis genetic variation and stress exposure on amygdala and hippocampus structure/function in humans have yet to be fully elucidated.

# 1.6 Development

**1.6.1 Why Study Development?**: The brain (Giedd et al., 1999; 1996), cortisol function (Shirtcliff et al., 2011; Watamura, Donzella, Kertes, & Gunnar, 2004), and emotional skills (e.g. identification of facial emotion, see Thomas, De Bellis, Graham, & LaBar, 2007) all show rapid development across childhood and into adolescence, making this a window of potential vulnerability. Particularly, rapid neuronal growth and wiring during childhood have been suggested to allow for a period of increased sensitivity to both positive and negative environmental factors (Andersen, 2003) where the development of cortico-limbic circuitry is likely of particular relevance (Andersen & Teicher, 2008). As the amygdala, hippocampus, and PFC are all developing rapidly over childhood (Giedd et al., 1996; 1999), early life (and prenatal) stress can have large, long-lasting effects on the brain, though this may vary across development and by region (Lupien, McEwen, Gunnar, & Heim, 2009).

**1.6.2 Early Life Stress**: Much focus has been given to the effects of *early* life stress on depression. For example, childhood adversity has been associated with increased odds of developing depression as an adult (Kessler et al., 1997; Molnar, Buka, & Kessler, 2001; Young, Abelson, Curtis, & Nesse, 1997). Epidemiological studies tend to find little specificity for effects of different types of stressors, rather there is often strong inter-correlation between the experience of different types of childhood adversities, like abuse, poverty, parental divorce, and parental psychopathology. Nonetheless, as noted previously, the experience of childhood adversity/stressors can have a long lasting impact on risk for psychopathology, increasing the odds for up to ten years (Kessler et al., 1997), likely by

altering normative developmental trajectories. Furthermore, while the experience of stressors as an adult can greatly increase the odds of a depressive episode in the following months (Kendler et al., 1999), early childhood adversity has been suggested to sensitize individuals to the later stressors, i.e. less severe stressors can precipitate depressive onset in those with childhood exposure (Harkness, Bruce, & Lumley, 2006). Additionally, it has been suggested that depressed patients with and without childhood trauma exposure may respond differentially to psychotherapy vs. pharmacological therapies (Nemeroff et al., 2003), again indicating that understanding the mechanisms relating stress to depression may be critical to furthering our understanding and treatment of depression.

Most animal studies have focused on the effects of early stressors on the *adult* brain, mirrored by human studies of the impact of childhood maltreatment/trauma. Yet, Several studies have explored the effect of stress across the course of development, for example, early life stress has been shown to cause delayed (i.e., only apparent later in development, see Andersen & Teicher, 2004; Isgor, Kabbaj, Akil, & Watson, 2004), but prolonged/lasting effects on hippocampal structure in rodents (Meaney, Aitken, Bhatnagar, & Sapolsky, 1991). This is paralleled by studies in humans, which typically find that early life stress leads to hippocampal changes apparent in adulthood but not childhood (e.g. Woon & Hedges, 2008). On the other hand, amygdala volume differences may be apparent in childhood when hippocampal changes are not (e.g. Tottenham et al., 2010). This has led to theorizing about temporal discrepancies in the development of these two regions in terms of structure, involvement in HPA axis function, stress sensitivity/vulnerability, etc. as well as potential interactions, whereby stress-induced amygdala dysfunction may precede hippocampal alterations (Tottenham, 2009). Nonetheless, investigations of the effects of

stress must occur across development to truly understand its effects. Further, studying HPA axis genetic factors may be key to understanding this, i.e. individuals predisposed for high HPA reactivity may be primed to show these stress-related neural alterations earlier in childhood.

**1.6.3 Childhood-Onset Depression**: Depression has been shown to affect pre-pubertal children (Carlson & Cantwell, 1980; Puig-Antich, Blau, Marx, Greenhill, & Chambers, 1978; Ryan et al., 1987) and even children as young as preschool-age (3-5 years old) (Luby, 2009; Luby, Heffelfinger, Mrakotsky, Brown, Hessler, Wallis, et al., 2009a; Luby, Si, Belden, Tandon, & Spitznagel, 2009b). Early onset depression has been estimated to affect ~3% of children (Fleming & Offord, 1990). Importantly, preschool-onset major depressive disorder (PO-MDD) is strongly predictive of later depression (more so than predicting other disorders), suggesting that PO-MDD exhibits continuity with or increase risk for later MDD rather than acting as a nonspecific precursor to general psychopathology (Luby, Si, Belden, Tandon, & Spitznagel, 2009b).

Importantly, both a family history of affective disorders and of early experiences of stressful life events have been cited as potential risk factors for PO-MDD. Both variables predict increased depression severity at a 6-month follow-up, with stressful life events partially mediating the relationship between family history and depressive severity (Luby, Belden, & Spitznagel, 2006). This is particularly salient for the proposed work, as it indicates a major role for genetic factors (at least partially indexed by family history) and for stress-related environmental factors. Also, children with PO-MDD show over-active cortisol responses to laboratory stress tests (frustration and separation) as compared to control children (Luby et al., 2003), further suggesting a role for stress-system alterations

in the development of PO-MDD. This type of alteration in cortisol (and other biological measures) has been observed across childhood, adolescent, and adult depression with some observed inconsistencies that may be due to normative developmental differences or methodological differences (Kaufman, Martin, King, & Charney, 2001).

## 1.7 Aims of the Current Work

While much of the literature points to important effects of stress-related factors, like environmental stressors and HPA axis genetic variants, on depression, relatively little human work has been done to elucidate the specific underlying mechanisms. Thus, the goal of the current work was to test a focused set of hypotheses regarding these mechanisms, particularly concerning the effects of HPA axis genetic variation and early life stress on cortisol reactivity and the structure and function of the amygdala and hippocampus in children. First, we created HPA axis genetic profile scores, based on prior literature, to sum variance across multiple SNPs of interest (Chapter 2). Then, we tested the hypotheses that elevated profile scores and/or early experience of life stress would predict elevated cortisol reactivity and alterations in amygdala and hippocampus volume (Chapter 2). Next, we extended this to test the hypothesis that these stress-related risk factors would predicted potentiated amygdala and hippocampal reactivity to negative emotional stimuli (Chapter 3). The final hypothesis for this work was that these stress-related risk factors would predict alterations in amygdala connectivity, specifically, reduced positive connectivity between the amygdala and subcortical regions and reduced negative connectivity between the amygdala and PFC (Chapter 4).

# Chapter 2: Stress-System Genes and Life Stress Predict Cortisol Levels and Amygdala and Hippocampal Volumes in Children

Reference: Pagliaccio, D., Luby, J. L., Bogdan, R., Agrawal, A., Gaffrey, M. S., Belden, A. C., Botteron, K. N., Harms, M. P., and Barch, D. M. (2014). Stress-System Genes and Life Stress Predict Cortisol Levels and Amygdala and Hippocampal Volumes in Children. Neuropsychopharmacology. 39, 1245–1253. PMCID: PMC3957120

## 2.1 Abstract

Depression has been linked to increased cortisol reactivity and differences in limbic brain volumes, yet the mechanisms underlying these alterations are unclear. One main hypothesis is that stress causes these effects. This is supported by animal studies showing that chronic stress or glucocorticoid administration can lead to alterations in hippocampal and amygdala structures. Relatedly, life stress is cited as one of the major risk factors for depression and candidate gene studies have related variation in stress-system genes to increased prevalence and severity of depression. The present study tested the hypothesis that genetic profile scores combining variance across 10 single nucleotide polymorphisms from four stress-system genes (CRHR1, NR3C2, NR3C1, and FKBP5) and early life stress would predict increases in cortisol levels during laboratory stressors in 120 preschool-age children (3–5 years old), as well as hippocampal and amygdala volumes assessed with MRI in these same children at school age (7–12 years old). We found that stress-system genetic profile scores positively predicted cortisol levels while the number of stressful/traumatic life events experienced by 3–5 years old negatively predicted cortisol levels. The interaction of genetic profile scores and early life stress predicted left hippocampal and left amygdala volumes. Cortisol partially mediated the effects of genetic variation and life stress on limbic brain volumes, particularly on left amygdala volume. These results suggest that stress-related genetic and early environmental factors contribute to variation in stress cortisol reactivity and limbic brain volumes in children, phenotypes associated with depression in adulthood.

## 2.2 Introduction

Stress, particularly in early life, is one of the strongest predictors of major depressive disorder (MDD; Green et al, 2010; Kessler and Magee, 2009) making it critical to understand the neurobiological mechanisms underlying this association. Early stress exposure (e.g. Carpenter et al, 2007; Ouellet-Morin et al, 2011) and MDD (Heuser et al, 1994) are both associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, the regulatory system for stress/cortisol responsivity. Accumulating evidence from animal models (e.g. Conrad et al, 1999; Cui et al, 2008; Vyas et al, 2002; Watanabe et al, 1992) and humans (e.g. Brown et al, 2008; Campbell et al, 2004; Sacher et al, 2012; Videbech and Ravnkilde, 2004) has shown that stress exposure, HPA axis dysregulation, excessive corticosteroid levels, and depression relate to structural alterations in the hippocampus and amygdala, brain regions important in HPA axis regulation (Jacobson and Sapolsky, 1991; Lupien et al, 2009). Thus, differences in these structures are a promising mechanism linking stress to depression and may also arise from individual differences in HPA axis function. While environmental stress can induce HPA axis dysregulation (e.g. Carpenter et al, 2007; Lovallo et al, 2012; Ouellet-Morin et al, 2011), polymorphisms within genes coding for HPA axis proteins also relate to individual differences in stress responsivity, depression risk, and related phenotypes (Table 2.1). These polymorphisms may be important moderators of the effects of environmental stress on depression and brain structure (see Figure 2.1 for a schematic of these proposed mechanistic interactions).

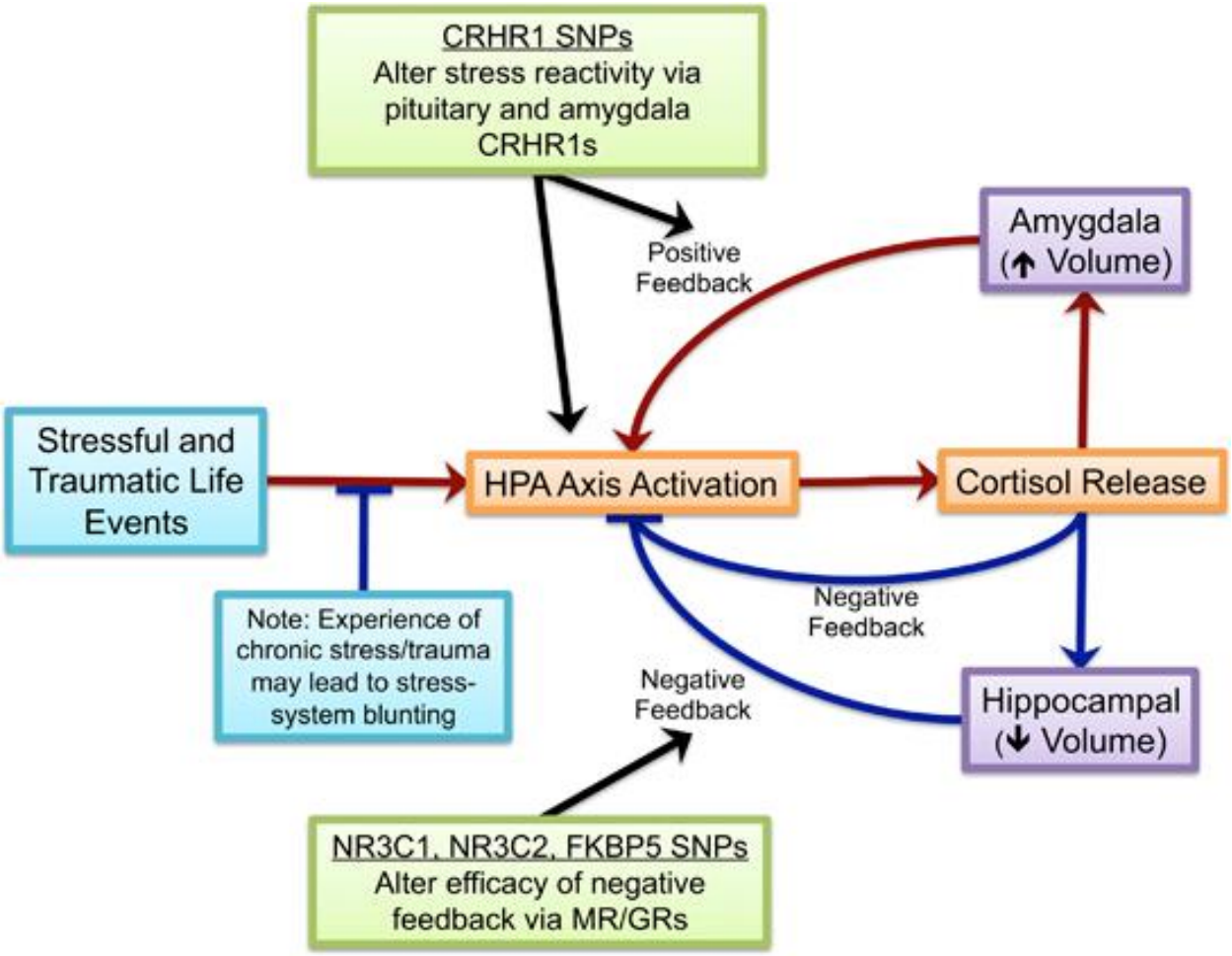


**Figure 2.1: Schematic of potential stress-related mechanisms of change in brain structure**
The experience of individual stressful and traumatic life events can activate the hypothalamic–pituitary–adrenal (HPA) axis, which prompts cortisol release having widespread effects on the brain and body. Notably, repeated activation of the HPA axis with life stress may in fact lead to blunting of the stress response to future stressors. Prolonged, elevated cortisol levels in animals are also shown to cause atrophy in the hippocampus and hypertrophy in the amygdala, largely through changes in dendritic remodeling. These structural changes may contribute to deficits in appropriate feedback onto the HPA axis. CRHR1 is expressed in the amygdala and pituitary, such that genetic variants may alter activation of the HPA axis and thus cortisol release. NR3C1, NR3C2, and FKBP5 are highly expressed in the hippocampus, where variants of these genes may alter cortisol negative feedback. Therefore, while stress-system genetic variants may alter the intrinsic reactivity and regulation of the HPA axis, changes in brain volume due to cortisol-mediated mechanism are likely to occur in interaction with the experience of environmental stressors.

**Table 2.1: Associations Between Selected HPA Axis SNPs, Stress Responsiveness, Brain, and Psychopathology**

| Gene | SNP | Associations |
|---|---|---|
| ***CRHR1*** 17q12-q22 | rs4792887 | Minor allele: increased depression and suicidality among male suicide attempters exposed to low stress (2;31)<br>Major allele: part of dose-dependent haplotype protective against MDD after CM (7) |
| | rs110402 | Minor allele: more seasonal and earlier onset MDD (23); Dex/CRH cortisol in interaction with CM (27); part of haplotype predicting higher depressive symptomology after CM (10;14;24)<br>Major allele: protective effect against depressive symptoms after CM (7;10;24); lower Dex/CRH cortisol response in men (10) |
| | rs242941 | Minor allele: more prevalent among MDD patients than controls (19)<br>Major allele: part of a haplotype associated with greater antidepressant treatment response in high anxiety MDD patients (17;18) |
| | rs242939 | Minor allele: more prevalent among MDD patients than controls (19), increased depression and suicidality among male suicide attempters exposed to low stress (32)<br>Major allele: part of a haplotype associated with greater antidepressant treatment response in high anxiety MDD patients (17;18) |
| | rs1876828 | Major allele: part of a haplotype associated with greater antidepressant treatment response in high anxiety MDD patients (17;18) |
| ***NR3C2*** 4q31.1 | rs5522 ^ | Minor allele (MR I180V): increased salivary and plasma cortisol to TSST (8); increased prevalence of depressive symptomology among older adults (13), increased amygdala reactivity at low childhood adversity (6) |
| **NRC31** 5q31.3 | rs41423247 | Major allele homozygotes: increased cortisol response to TSST in women but decreased in men (12); increased anticipatory cortisol to TSST (11), increased risk of MDD episode (28) |
| | rs10482605 | Major allele: increased in MDD patients compared to controls, potentially varying by ethnic population (29); part of a haplotype related to prevalence of childhood onset mood disorders (22) |
| | rs10052957 | Minor allele homozygotes: higher evening and total cortisol across the day (25); increased in MDD patients; decreased hippocampal volume (35) |
| ***FKBP5*** 6p21.31 | rs1360780 | Minor allele: increased FKBP5 protein levels (3); reduced total diurnal cortisol secretion (30); altered cortisol response after DST (4;5;21), TSST (5), and SSP (20); increased incidence of depression (15;16); interacts with CM to predict depression (1;34), PTSD (3), and suicide (26); increased MDD recurrence and more rapid response to antidepressant treatment (5); increased amygdala reactivity in the context of elevated childhood adversity (33), increased threat-bias, increased threat-related hippocampal activity, and morphological changes in the hippocampus (9) |

***Numbers in parentheses refer to reference list in Supplementary Table 2.16***
^ = rs5522 is an exonic, non-synonymous SNP, all other SNPs are intronic
CM = History of Childhood Maltreatment (emotional/physical abuse, trauma, or neglect)
DST = Dexamethasone Suppression Test; Dex/CRH = Dexamethasone/Corticotropin-Releasing Hormone Test
TSST = Trier Social Stress Test; SSP = Strange Situation Paradigm in Infants

The present study tested whether early life stressors and genetic variation within four HPA axis-related genes (CRHR1, NR3C2, NR3C1, and FKBP5) predict individual differences in HPA axis function and neural structure in children. Given the increase in depression risk in adolescence, we focused on school-age children to understand pathways contributing to this increasing risk. We adopted a polygenic approach by creating genetic profile scores, which additively combined 10 single nucleotide polymorphisms (SNPs), selected from the literature based on established association with HPA axis dysregulation, risk for depression, and/or related phenotypes (Table 2.1). We tested the hypotheses that genetic profile scores and early life stress exposure would predict: (1) increased cortisol responses to laboratory stressors in preschool-age children, (2) decreased hippocampal volume, and (3) increased amygdala volume in these same children at school age. Last, because HPA axis dysregulation is a putative mechanism by which stress leads to alterations in neural structure, we tested the hypothesis that (4) stress-related cortisol levels in early childhood would mediate the effects of genetic factors and early life stress on hippocampal and amygdala volumes later in childhood.

# 2.3 Materials and Methods

**2.3.1 Participants**: Data were analyzed from 120 children (58 females; 57.5% White, 30.0% African-American, and 12.5% of other or mixed race) enrolled in the Preschool Depression Study (PDS), a prospective longitudinal study of preschool-age children (N=306) conducted at the Washington University School of Medicine Early Emotional Development Program (WUSM EEDP) in St Louis.

For the PDS, 3–5-year-old children and their primary caregivers were recruited from daycares, preschools, and primary care sites in the St Louis area (see Luby et al (2009) for details), using the Preschool Feelings Checklist (Luby et al, 2004) to oversample for children with or at risk

for depression. Following an initial session during which psychopathology assessments, behavioral testing, and saliva collection occurred, children underwent annual clinical assessments and completed a neuroimaging session between the ages of 7 and 12 (current subsample mean age=10.38 years, SD=1.17 years). Parental written consent and child assent were obtained and the Institutional Review Board at Washington University approved all procedures.

Only children who met all inclusion criteria based on data quality and availability were included. Of the 306 children in the PDS, 168 completed the neuroimaging session. Forty-eight were excluded for missing, unusable, or poor quality structural imaging, genetic, or cortisol data, or for factors influencing cortisol (see Supplementary Materials), leaving a final sample size of N=120.

### 2.3.2 Psychopathology and Stress Assessment: Trained WUSM EEDP staff

conducted up to seven in-person assessments (current subsample mean=4.74 sessions, SD=1.01) with participants and their parents/guardians from study enrollment through the time of scan. Before children were 8, a reliable and age-appropriate semi-structured parent-report diagnostic interview was used to assess psychiatric symptoms, the Preschool-Age Psychiatric Assessment (PAPA; Egger et al, 2003). After age 8, the Childhood and Adolescent Psychiatric Assessment (CAPA; Angold and Costello, 2000) was used, which also includes child report. Interviews were audiotaped, reviewed for reliability, and calibrated for accuracy (Luby et al, 2009). Four diagnostic groups were created based on the PAPA/CAPA: Preschool-Onset MDD (N=45; MDD before age 6, note: the 2-week symptom duration criteria was relaxed as previously validated by Gaffrey et al (2011)), later MDD (N=16; MDD by the time of scan, but after age 6), other psychiatric conditions (N=28; no diagnosis of MDD ever, but another diagnosis by the time of scan), and healthy controls (N=31; no diagnoses through time of scan). For details, see Supplementary Table 2.1.

Analyses examined stressors experienced from birth through the baseline assessment when participants were 3–5 years old (current subsample mean age at baseline assessment=4.45 years, SD=0.77), at which time cortisol was collected. We summed the instances of stressful and traumatic

life events reported by parents during the PAPA. A full list of life events assessed and their frequency is presented in Supplementary Table 2.2.

### 2.3.3 Stress Induction, Cortisol Collection, and Analysis: Salivary

cortisol was collected three times during the baseline assessment; first upon arrival in the WUSM EEDP laboratory (1+ hours after the child's last meal to control for cortisol changes due to food/drink). As salivary cortisol levels are thought to indicate stress levels ~30 min prior to collection (Kirschbaum and Hellhammer, 1994), this first cortisol sample should represent time with the parent before the assessment. The second saliva collection occurred ~30 min after separation from the parent, during which time the child performed a variety of behavioral tasks. Following this collection, the child performed more behavioral tasks and several stress-/frustration-inducing episodes from the Laboratory Temperament Assessment Battery (LabTAB; Goldsmith et al, 1995, 2004; Supplementary Table 2.3). A final cortisol sample was taken after these stressful tasks. See Supplementary Materials and Suzuki et al (2013) for details.

As we had no specific hypotheses about differences between separation- or stressor-induced changes in cortisol, an area under the curve ground (AUC) value was used to capture variance across all three timepoints (C1, C2, and C3) using the time between collections (t1,2 and t2,3; in minutes) to yield a single summary variable ($AUC = \frac{(C_1+C_2)\times t_{1,2}}{2} + \frac{(C_2+C_3)\times t_{2,3}}{2}$, Pruessner et al, 2003). To control for circadian effects, the unstandardized residuals of AUC cortisol were saved from a linear regression with a binary time of day predictor (collection occurred either around 0900 hours or 1300 hours) and used for all subsequent analyses. See Supplementary Materials for additional validation of this approach.

### 2.3.4 MRI Acquisition and Volume Analysis: Structural images were

collected as part of a scan session that also included task-based and functional connectivity data. Imaging data were collected using a 3T TIM TRIO Siemens scanner. T1-weighted images were

acquired in the sagittal plane using an MPRAGE 3D sequence (TR=2400 ms, TE=3.16 ms, flip angle=8°, slab=176 mm, 176 slices, matrix size=256 × 256, field of view=256 mm, voxel size=1 × 1 × 1 mm).

FreeSurfer v4.5.0 (http://surfer.nmr.mgh.harvard.edu/, Fischl et al, 2002, 2004) was used to segment each participant's anatomical image, allowing estimation of left and right hippocampal and amygdala volumes and whole brain volume (WBV; total gray+cortical white matter volume). FreeSurfer was also used to segment and extract volumes for the hippocampal subfields (for details and validation against hand-tracing, see Van Leemput et al (2009)). This included volume estimates for the left and right presubiculum, CA1, CA2/3, fimbria, subiculum, CA4/dentate gyrus, and hippocampal fissure.

**2.3.5 Genetic Analysis**: DNA extracted from saliva was genotyped using standard high-throughput methods of the Sequenom Technology Core at Washington University (current subsample call rate=99.5%; Supplementary Table 2.4). PLINK v1.07 (Purcell et al, 2007) was used to assure that all SNP genotypes were in Hardy–Weinberg equilibrium (all ps>0.05) and to test whether allelic frequencies differed by ethnicity (Supplementary Table 2.4).

A recent study documented the utility of combining genetic variants in a biologically informed manner to study polygenic effects on the brain, whereas single polymorphisms alone were not significantly predictive (Nikolova et al, 2011). For the current study, we focused only on genes coding for integral HPA axis proteins and selected SNPs previously associated with increased cortisol, MDD prevalence/severity, and/or related phenotypes (Table 2.1). Fifteen SNPs were identified from the four genes of interest and were narrowed down to 10 to reduce linkage disequilibrium (LD; all pairwise r2<0.49; Supplementary Figure 2.2) using SNPSpD (Nyholt, 2004). Sum scores across these 10 SNPs were created, where higher genetic profile scores indicate more genotypes previously associated with increased cortisol, MDD prevalence/severity, and/or related phenotypes.

**2.3.6 Data Analysis**: We used hierarchical linear regressions in IBM SPSS Statistics v20 (Armonk, NY: IBM) to predict cortisol and regional brain volumes. Predictors were added in steps to understand their effects alone and controlling for covariates. Regressions first controlled for ethnicity and sex. Next, centered, continuous variables for genetic profile scores and life events were entered. Interactions between these factors and interactions with sex were then entered. Predictors were assessed with and without controlling for WBV to test the specificity of effects on limbic brain volumes. Relatedly, amygdala volumes were added as covariates in predicting hippocampal volumes and vice versa to assess specificity and the variance shared by these regions predicted by genetic and environmental factors. Finally, we controlled for diagnostic status to test if this accounted for any of the main/interaction effects. See Supplementary Materials for details.
We used the simple moderation model from the PROCESS tool for SPSS (Hayes, 2013) to parse significant interaction effects. Figures in the main text display simple slopes (split by sex or at mean±1 SD values of continuous moderators), at mean levels of the covariates; scatterplots of raw data are presented in the Supplementary Materials. PROCESS was also used to test for mediation effects. This regression-based approach estimates the indirect effect of an independent variable on a dependent variable via a mediator, equivalent to the difference between the total effect (not controlling for the mediator) and the direct effect of the independent variable (controlling for the mediator). To determine significance of the indirect effect, PROCESS uses bootstrapped confidence intervals (CIs; significant when not overlapping zero; Hayes, 2013). See Supplementary Materials for details.

# 2.4 Results

**2.4.1 Control Analyses**: The distributions of all variables of interest (Supplementary Figure 2.3) and differences by ethnicity, sex, and diagnostic status (Supplementary Table 2.5; which

were controlled for in the main analyses) are in the Supplementary Materials. There was no correlation between early life stress and genetic profile scores in the whole sample or in the sex or ethnicity subgroups (ps>0.5; Supplementary Figure 2.4). Age was significantly correlated with the number of life events (r(118)=0.29, p=0.001) but not with genetic profile scores, cortisol, or any brain volumes (all ps>0.10). The regression results presented below remained significant when controlling for age (data not shown).

### 2.4.2 Main Effects of Genetic Profile Scores and Life Events: AUC

cortisol levels were positively predicted by genetic profile scores (β=0.32, t=3.04, p=0.003) and negatively predicted by life events (β=−0.28, t=−3.00, p=0.003), even when controlling for all covariates. Together these factors accounted for 8% of the variance in cortisol beyond ethnicity and sex (R2 change p=0.01; Supplementary Table 2.6). There was a sex difference in the strength of the genetic effect (β=0.22, t=2.23, p=0.028). Genetic profile scores were a significant positive predictor of cortisol in females (β=0.484, t=3.290, p=0.001), while showing a positive but non-significant relationship in males (β=0.099, t=0.876, p=0.383; Figure 2.2 and Supplementary Figure 2.5). Genetic profile scores and stressful life events did not significantly predict hippocampal or amygdala volumes (Supplementary Tables 2.7–2.10).

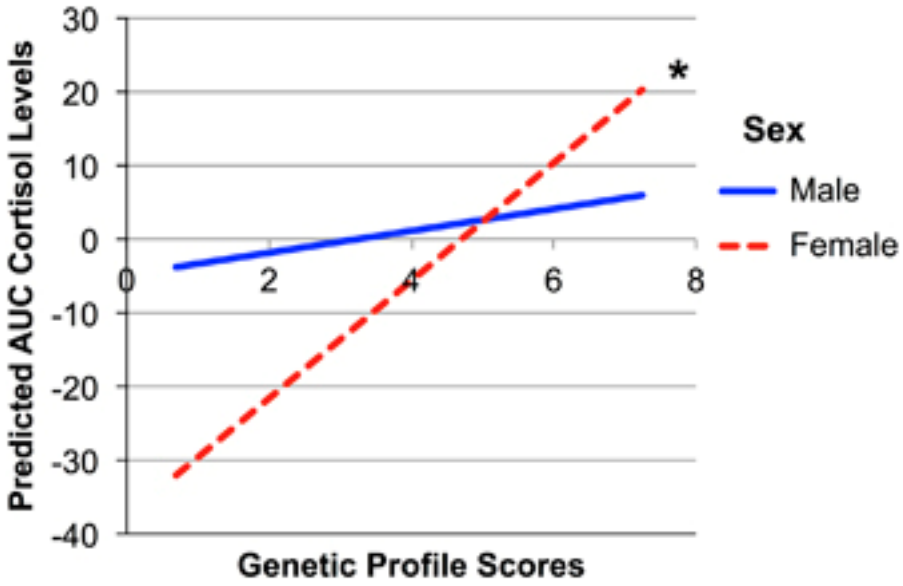


**Figure 2.2: Regression-predicted effects of genetic profile scores on cortisol split by sex**
The lines display the simple slopes of genetic profile scores predicting area under the curve (AUC) cortisol levels for males and females at mean values of all covariates (ethnicity, life events, genetic profile scores × life events, regional and WBV, and diagnostic status). Significant simple slopes are marked on the graph *$p$<0.05.

### 2.4.3 Genetic Profile Scores × Stressful Life Events Interactions: After accounting for main effects, cortisol, and WBV, the interaction between genetic profile scores and life events significantly predicted left hippocampal (β=0.21, t=2.83, p=0.04; Supplementary Table 2.7; Figure 2.3a and b) and left amygdala volume (β=0.16, t=2.10, p=0.04; Supplementary Table 2.9; Figure 2.3c and d). Post-hoc simple slope testing revealed that genetic profile scores negatively predicted left hippocampal volume in the context of few stressful life events (mean−1 SD). Stressful life events positively predicted left hippocampal volume in the context of high genetic profile scores (mean+1 SD). Note that as higher genetic profile scores but fewer life events predicted higher cortisol levels, these results may be consistent with a cortisol-related mechanism of hippocampal loss. Stressful life events negatively predicted left amygdala volume in the context of average to low (mean−1 SD) but not high (mean+1 SD) genetic profile scores. After controlling for hippocampal volume, this interaction was no longer significant, suggesting that it was related to shared variance between left amygdala and hippocampal volumes. These interaction effects were not significant for the right hippocampus or amygdala (ps>0.10; Supplementary Tables 2.8 and 2.10).

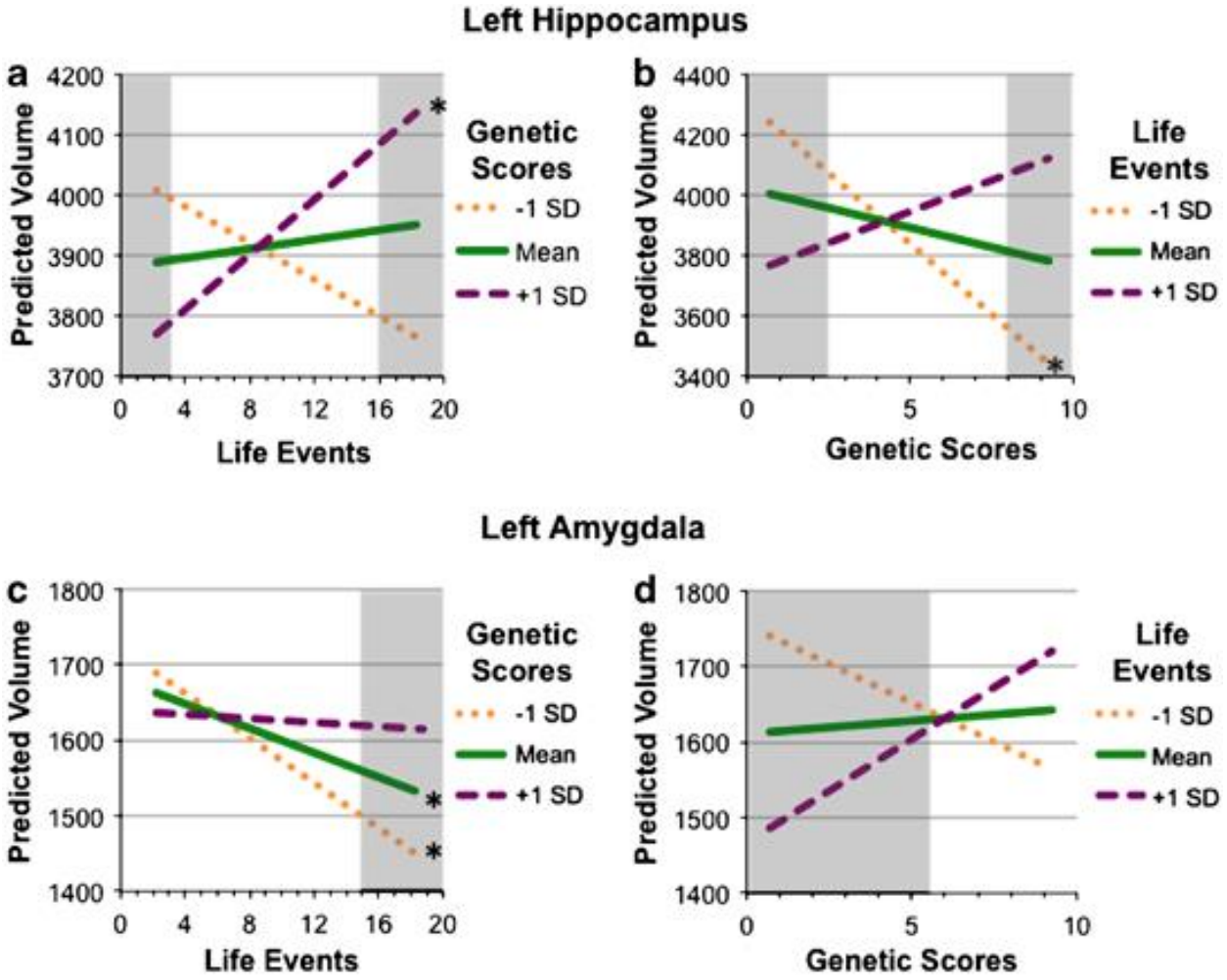


**Figure 2.3: The interaction of genetic profile scores and life events predicting left hippocampal and left amygdala volumes**

The lines display the simple slope of (a) life events at mean and mean±1 standard deviation values of genetic profile scores and of (b) genetic profile scores at mean and mean±1 standard deviation values of life events predicting left hippocampal volume ($mm^3$) events at mean values of covariates (ethnicity, sex, genetic profile scores × sex, life events × sex, genetic profile scores × life events × sex, cortisol, WBV, and diagnostic status). The equivalent simple slopes predicting left amygdala volumes are presented in panels (c) and (d). Gray shaded regions display the Johnson–Neyman results, which indicate the range of moderator values (genetic profile scores or life events) at which there is a significant relationship between the other predictor and brain volume (a: <3.3 and >15.7; b: <2.1 and >5.3; c: >14.4; d: <4.7). Significant simple slopes are marked on the graph * $p<0.05$.

**2.4.4 Follow-up Analyses on Hippocampal Subfields**: The animal literature shows that stress/corticosteroid administration impact the CA3 subfield and dentate gyrus of the hippocampus (e.g. Conrad et al, 1999; Gould et al, 1998; Pham et al, 2003; Watanabe et al, 1992). In the current data, while most of the left hippocampal subfields showed a trend towards a genetic profile score × life events interaction, the interaction significantly predicted CA2/3 and CA4/dentate gyrus volumes (both passed Bonferroni correction for seven multiple comparisons per hemisphere), accounting for ~5% of the variance in each subfield (Supplementary Table 2.11). On the right, this interaction only predicted presubiculum volume, but did not pass Bonferroni correction (Supplementary Table 2.12).

**2.4.4 Mediation Analyses**: As research suggests that chronic stress or glucocorticoid administration may lead to alterations in brain structure (e.g. Conrad et al, 1999; Cui et al, 2008; Vyas et al, 2002; Watanabe et al, 1992), we tested the hypothesis that individual differences in stress-related cortisol levels in early childhood would mediate the effects of genetic profile scores and early life stress on hippocampal and amygdala volumes in later childhood. We first tested this controlling for ethnicity, sex, interactions with sex, and diagnostic status (Supplementary Table 2.13), and then also controlling for WBV (Supplementary Table 2.14). Cortisol-mediated negative indirect effects of genetic profile scores and positive indirect effects of life events on left hippocampal (Figure 2.4a and b) and left amygdala volumes (Figure 2.4c and d). Higher genetic profile scores and fewer experiences of stressful life events each predicted smaller left hippocampal and amygdala volumes mediated by higher cortisol levels.

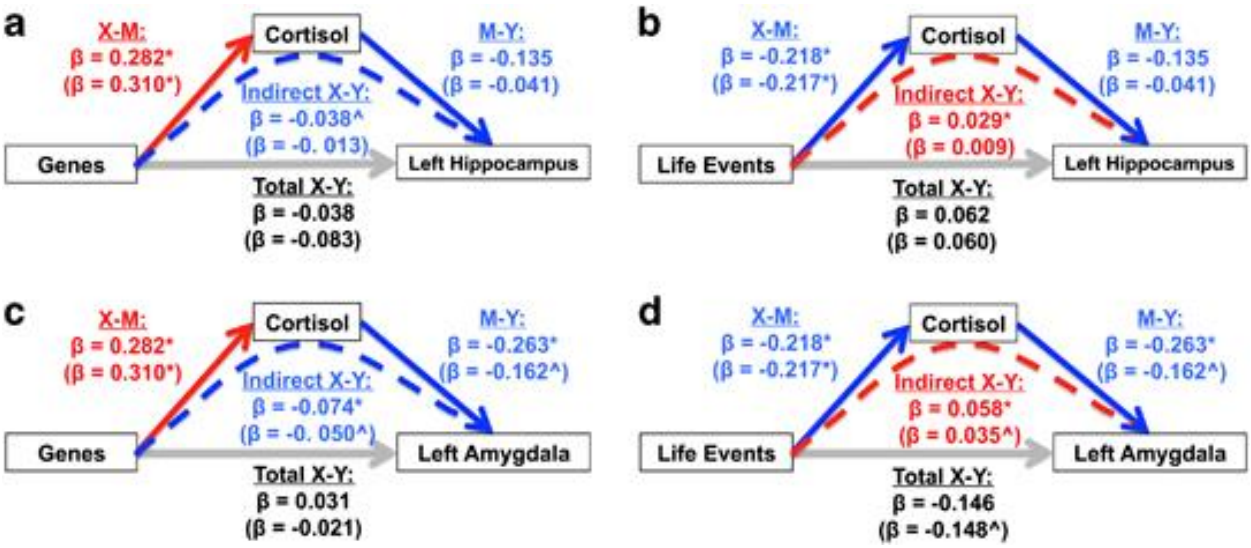


**Figure 2.4: Cortisol mediates the effects of genetic profile scores and life events on left hippocampal volume and left amygdala volumes**

The schematic diagrams represented the cortisol-mediated effects of genetic profile scores and life events on left hippocampal volume (a and b, respectively) and on left amygdala volume (c and d, respectively). Solid arrows represent unmediated effects (ie effects on the mediator or the total effect of the independent variables on cortisol or volume) while the dashed arrows represent the indirect effects via cortisol. Red arrows indicate positive relationships, blue arrows indicate negative relationships, and gray arrows indicate non-significant or near-zero total effects. Two standardized regression coefficients (β) are presented for each arrow, the top is the β coefficient from the regression model not controlling for WBV (Supplementary Table 2.13), while the bottom β in parentheses is the value from the regression model which does control for WBV (Supplementary Table 2.14). ^p<0.10, * p<0.05.

While the genetic profile score × life events interactions described in the previous sections appear specific to the left hippocampus and amygdala (i.e. controlling for WBV), the mediation results may indicate a more widespread cortisol effect on the brain. Specifically, cortisol was negatively correlated with WBV ($r(118)=-0.216$, $p=0.018$). After controlling for WBV, the mediation effects on left hippocampal volume were no longer significant, while indirect effects of genetic profile scores and life events on the left amygdala volume via cortisol remained trend-level significant (Supplementary Tables 2.13–2.14).

# 2.5 Discussion

Our results show that stress-system genetic profile scores and early life stress predict cortisol levels and interact to predict left hippocampal and amygdala volumes, putative phenotypes underlying associations between stress and depression.

## 2.5.1 Stress-system Genes and Life Events Predict Cortisol Reactivity

**Reactivity**: Genetic profile scores (higher scores indicating more SNPs associated with increased cortisol levels and/or depression) positively predicted cortisol levels during psychosocial stress in preschool-age children, providing validation for our polygenic approach. Sex moderated the effect of genetic profile scores on cortisol levels, such that the positive relationship between genetic profile score and cortisol was stronger in females than males. While the rates of depression did not differ by sex in this young sample (see Supplementary Materials), the literature shows a clear sex difference beginning in adolescence, with females twice as likely to develop MDD as males (e.g. Nolen-Hoeksema and Girgus, 1994). This stronger effect of genetic profile scores on cortisol among females may contribute to the sex differences in MDD prevalence, especially as genetic factors may be more influential in the etiology of MDD in females than in males (Kendler et al, 2001).

Congruous with the literature suggesting that life adversity results in blunted HPA axis responses to acute stress (Carpenter et al, 2007; Lovallo et al, 2012; Ouellet-Morin et al, 2011), we found that the number of stressful and traumatic life events experienced by preschool age negatively predicted cortisol levels. High or chronic levels of early life stress may induce stress-system 'burnout' or, relatedly, these children may have perceived the laboratory stressors as less stressful.

### 2.5.2 Stress-Related Effects on Hippocampal and Amygdala Volumes

**Volumes**: The interaction of genetic profile scores and early stressful life events predicted both left hippocampal and left amygdala volumes at school age. While life stress typically does not predict hippocampal volume in childhood (e.g., Woon and Hedges, 2008), exploring the genetic risk may be the key to detecting these differences early in development. However, the nature of the interactions between genetic profile scores and life events was somewhat atypical, as a diathesis–stress model would predict an additive interaction (e.g., Caspi et al, 2003), with smaller volumes associated with both higher genetic risk and more stressful life events. Instead, we found that a greater number of 'risk' SNPs predicted smaller left hippocampal volumes only in the presence of fewer stressful life events. This relationship suggests that the environment may set boundaries on the effects of genetic factors, with higher life stress over-riding genetic influences. Conversely, genetic risk may promote stress-related phenotypes and disorders, even in the absence of adversity/environmental provocation. Other studies have found conceptually analogous gene × environment interactions (e.g. Carballedo et al, 2013; Taylor et al, 2006). Within the left hippocampus, the interaction effect was most predictive of CA2/3 and CA4/dentate gyrus subfield volume. This is convergent with animal studies showing that chronic stress or corticosteroid administration reduce dendritic length and branching in hippocampal CA3 (e.g. Conrad et al, 1999; Watanabe et al, 1992) and impair neurogenesis in the dentate gyrus (e.g. Gould et al, 1998; Pham et al, 2003).

For the left amygdala, elevated stressful life events predicted decreased volume with lower, but not higher, genetic profile scores. In this case, the level of genetic risk appears to over-riding the environment's effects. Although the literature on amygdala volume with depression and stress is mixed, our findings are consistent with research indicating decreased amygdala volumes in depression (e.g. Keller et al., 2008; Sacher et al, 2012) and with cortisol administration (Brown et al, 2008). Controlling for hippocampal volume reduced the effect of the genetic profile score x life event interaction on the left amygdala volume, indicating that this interaction predicted variance shared between the left amygdala and hippocampus. This is particularly interesting given that both structures are thought to be affected by stress-/cortisol-mediated mechanisms, evident in the animal literature showing effects of chronic stress and corticosteroid administration on the hippocampus (Conrad et al, 1999; Pham et al, 2003; Watanabe et al, 1992) and amygdala (Cui et al, 2008; Vyas et al, 2002). Interestingly, the current effects are left-lateralized, consistent with meta-analytic work on amygdala volume in MDD (decreased left but not right volumes (Sacher et al., 2012)) though not with hippocampal meta-analyses that show bilateral effects (Campbell et al, 2004; Cole et al, 2011).

Consistent with a stress-/cortisol-mediated mechanism, our mediation analyses indicated that cortisol levels in early childhood may mediate the influence of genetic profile scores and stressful life events on limbic brain volumes. Interestingly, cortisol levels also negatively predicted WBV. Controlling for WBV, there were no longer unique cortisol-mediated effects on the left hippocampus, suggesting that effects on limbic volume may share mechanisms with broader cortisol effects on the whole brain. However, trend-level cortisol-mediated effects remained for left amygdala volume, suggesting some effects over and above those seen for WBV. The genetic profile scores × life events interactions did show specificity to both the left hippocampus and amygdala, as these effects were not accounted for by broader influences on the whole brain.

Though depression is associated with changes in hippocampal and/or amygdala volume, we found no diagnostic status effects in this sample of children. Hippocampal volume loss may relate to the burden of MDD illness, only becoming visible with a 2+ year history and 1+ episodes (McKinnon et al, 2009) where volume tends to decrease with increasing MDD duration/number of episodes (MacQueen et al, 2003; Videbech and Ravnkilde, 2004). Despite no association with diagnostic status in this young sample, it will be important to test whether stress-related factors account for later depression-related differences in brain volumes. Future work must also determine whether this genetically influenced stress-/cortisol-mediated pathway affects brain function as well as structure, especially given recent work showing an impact of childhood cortisol levels on adult functional connectivity (Burghy et al, 2012) and evidence of the importance of stressful life events and abnormal stress reactivity in the genesis and maintenance of depression (e.g. Kendler et al, 1999; Lopez-Duran et al, 2009).

**2.5.3 Limitations and Future Directions:** First, using single continuous summary values increases power by combining variance and reducing the number of tests performed. However, the use of a summed count of life events may not represent the true mechanisms by which risk is accumulated. Likewise, while the assumption of additive effects of SNPs across different genes might be considered a strong one and does not take into account potential epistatic effects, it is somewhat supported in the study of complex traits (Hill et al, 2008). As knowledge regarding the effects of single SNPs and life events on phenotypes of interest accumulates, more sophisticated weighting according to predicted effect sizes may be warranted. Our relatively small sample and insufficient priors regarding the magnitude of expected effects or epistatic relationships prevented us from adopting such methodology at this point. To aid this in the future, we have presented the effects of individual SNPs in the Supplement.

An additional concern is that while the FKBP5 variant is functional, the remaining SNPs are intronic. Therefore, even though we prioritized genes central to HPA axis activation/regulation and

only included SNPs with prior evidence for association with cortisol and/or depression, the functional significance of our polygenic score remains to be explored. Yet, it is likely that our SNPs tag functional variants (Supplementary Materials). Notably, different ethnic groups may have different underlying patterns of LD (Supplementary Figure 2.2). While self-reported ethnicity was included as a covariate in these analyses, our polygenic scores may be further refined as research accumulates on differential markers in Caucasian and non-Caucasian cohorts. Currently, we avoided excluding any subpopulation, as this would have considerably diminished power. Nevertheless, it should be noted that the significant effects of genetic profile scores, life events, and their interaction on cortisol and brain volumes were observed in the Caucasian subsample alone (N=69), either reaching significance or showing a pattern in the same direction as in the full sample (data not shown).

Finally, while genetic profile scores more efficiently approximate the polygenic underpinnings of our outcome measures (but see limitations above), it is possible that our finding reflects a false positive given the relatively modest sample size and potential for low priors (Duncan and Keller, 2011). Novel reports of interactions, such as ours, are particularly vulnerable, though the current study was based on a strong set of a priori hypotheses about biological mechanisms from human and animal studies. Thus, replication is necessary when an analogous cohort of young children with similar data is available. Future studies should build further to explore the role of stress-related genetic/environmental factors in the intergenerational transmission of depression and in understanding the specific and overlapping effects of stress on limbic and WBVs.

**2.5.4 Conclusions**: We found that more 'risk' variants in stress-system genes and lower levels of stressful life events from birth to the preschool period predicted higher cortisol levels during lab stressors in preschool-age children. The interaction of these factors predicted left amygdala and left hippocampal volumes in these same children at school age. Cortisol, which negatively correlated with limbic and WBVs, may serve as a mediator of the effects of genes and life

stress on limbic brain volumes. The findings elucidate the association between normal variation in the stress-system and limbic brain volumes in children. Although diagnostic status was not strongly associated with differences in limbic volumes, the results may be potential evidence for a stress-mediated mechanism underlying putative depression-related changes in brain structure. This will be important in understanding differences in the normative developmental trajectory of cortisol reactivity and limbic brain structure as well as differences related to stress and psychiatric disorders.

# 2.6 Supplementary Information

### 2.6.1 Preschool Depression Study Subsample Exclusion Criteria:

Three hundred and six children were enrolled in the PDS, 168 of whom completed the neuroimaging session. Of the 138 who did not complete the neuroimaging session, 67 refused to participate when asked, 9 repeatedly didn't show for appointments or canceled after having been scheduled, 13 were medical screen outs, 2 were deceased, and 8 had moved out of state. An additional 39 children were not scanned because the grant supporting the neuroimaging focused on healthy children and children with PO-MDD. Some children with other diagnoses were scanned through limited supplementary funds on a first asked-first completed basis. Of these 168 children who did participate in the neuroimaging session, 17 were excluded for missing, unusable, or poor quality structural data. Two additional children were excluded for missing all genetic data. Finally, 29 children were excluded for missing/incomplete cortisol data or for factors that can influence cortisol, including fever within 24 hours preceding collection, recent tooth loss, or use of steroid medications/inhalers.

### 2.6.2 Functional Role of Stress-System Genes of Interest in the HPA Axis:

***CRHR1****:* The *CRHR1* gene codes for the corticotropin releasing hormone receptor 1. *CRHR1* is mainly localized to the pituitary (Van Pett *et al*, 2000), which serves as an activating node in the HPA axis. Here, *CRHR1* binds corticotropin-releasing hormone (CRH) released from the periventricular nucleus of the hypothalamus. *CRHR1* knockout mice show dysfunctional activation of the HPA axis and significantly decreased anxiety behavior, again suggesting the importance of *CRHR1* for HPA function (Smith *et al*, 1998). It is also important to note that *CRHR1* is expressed in other brain regions, like the anterior cingulate, prefrontal cortex (PFC), amygdala, and hippocampus (Aguilera, 2004). Conditional knockout of *CRHR1* in the forebrain of post-natal mice, including the amygdala and hippocampus, leads to significantly reduced anxiety behaviors despite intact basal HPA activity. However, when the mice were exposed to stress, over-activity of the HPA axis became apparent, indicating a role for extra-pituitary *CRHR1* in stress (Müller *et al*, 2003). Human studies have indicated a potential role for variation in the *CRHR1* gene in HPA function and psychopathology. *CRHR1* (and CRH and CRH Binding Protein) SNPs have also been associated with cortisol reactivity in young children (Sheikh *et al*, 2013).

***NR3C2****:* The *NR3C2* gene codes for the mineralocorticoid receptor (MR), which serves as a high affinity, low capacity receptor for cortisol in the brain. It is highly expressed in the hippocampus where it has been suggested to help establish tonic inhibition of the HPA axis; with their low capacity for cortisol, MRs generally may be bound even at basal cortisol levels (de Kloet *et al*, 2005; Jacobson and Sapolsky, 1991; Reul and de Kloet, 1985). Chronic corticosteroid administration in mice induces depressive-like behavior and down-regulation of MRs in the hippocampus and hypothalamus, but administration of an MR antagonist can confer antidepressant-like effects (Wu *et al*, 2012). MRs also mediate stress-induced changes in glutamate-signaling in CA1 of the hippocampus (Karst *et al*, 2005). Additionally, MRs can impact the stress system outside of the hippocampus. For example, overexpression of MR in the mouse forebrain decreases anxiety-related behaviors (Rozeboom *et al*, 2007).

***NR3C1****:* Relatedly, the *NR3C1* gene codes for the glucocorticoid receptor (GR), which is also expressed in the hippocampus as well as more pervasively throughout the brain. GRs have a low affinity but high capacity for cortisol, which allows them to play a more dynamic role in HPA axis regulation by facilitating negative feedback at higher levels of cortisol (de Kloet *et al*, 2005; Jacobson and Sapolsky, 1991; Reul and de Kloet, 1985). It is also worth noting that the environment can have a large impact on both MRs and GRs in the brain. For example, early life stress in primates can alter MR and GR mRNA expression in the hippocampus (Arabadzisz *et al*, 2010) and an enriched environment can restore normal expression patterns and lessen depressive-like symptoms in chronically stressed rats (Zhang *et al*, 2011).

***FKBP5****: FKBP5* or FK506 binding protein 51 is part of mature GR complexes along with several other proteins and has been suggested to modulate GR sensitivity to cortisol. Specifically, when *FKBP5* is bound, the GR complex shows lower affinity for cortisol and less efficient translocation to the nucleus. GR activation can induce *FKBP5* mRNA and protein expression. A functional variant in the *FKBP5* gene has been identified (rs1360780) where minor allele homozygotes show greater *FKBP5* production and less effective cortisol negative feedback on the HPA axis (Binder, 2009).

### 2.6.3 Stress Induction and Cortisol Collection:

After consent, the child was then separated from their mother who moved to another room complete questionnaires. The child then performed a pattern construction task, the LabTAB Snack Delay episode (an inhibitory control test), a computerized face task, the LabTAB Transparent Box episode, a narratives task, a picture similarity task, the LabTAB Popping Bubbles task (an exuberance/joy induction), and part of the Berkley Puppet Interview (an age-appropriate interview about depression and anxiety states; (Ablow and Measelle, 1993). Another saliva sample (2) was taken at this point.

Following this sample and a short break, another set of tasks and LabTAB stressors were enacted. This included a guilt induction, the LabTAB I'm not sharing (toys) episode, the NEPSY -

Visual Attention task (Korkman et al, 1998), a facial emotion labeling task, the LabTAB Box Empty episode, the LabTAB Impossible Circles episode, the LabTAB No Candy Bars Left episode, the DAS - Naming Vocabulary test, the LabTAB Make the car go! episode, and 10 minutes of free play. At this point, the last saliva sample (3) was collected. Following this, the child received a small gift and was reunited with the mother.

Saliva samples were assayed for cortisol through the Washington University General Clinical Research Center using the Gamma Coat Cortisol Radioimmunoassay kit procedure (DiaSovin, Stillwater, Minn).

**2.6.4 Cortisol Data:** As described in the main text, we used an area under the curve ground (AUC) measure to summarize the variance across the three cortisol collections. As noted in the main text, we used the AUC value because we had no specific hypotheses about differences between separation- and stressor-induced differences in cortisol levels. The AUC value is also useful here because the children tended to have elevated cortisol levels at the first collection (pre-stress; see Figure 3.1), which may be a result of coming into an unfamiliar laboratory environment for an assessment. As such, cortisol at collection 1 would not serve as an appropriate baseline measure to compare the later collections against for a change score. The values from these three timepoints were all significantly correlated (cortisol 1 vs. 2: $r(118) = 0.574$, $p < 0.001$; cortisol 1 vs. 3: $r(118) = 0.428$, $p < 0.001$; cortisol 2 vs. 3: $r(118) = 0.662$, $p < 0.001$). Additionally, a repeated-measures ANOVA with cortisol collection timepoint as a repeated measure (3 levels) and time of day (A.M. or P.M.) as a between-subject factor revealed a significant main effect of collection timepoint (Greenhouse-Geisser correction for non-sphericity: $F(1.712,202) = 30.328$, $p < 0.001$) and of time of day ($F(1,118) = 6.304$, $p = 0.013$) but no significant interaction between timepoint and time of day (Greenhouse-Geisser correction for non-sphericity: $F(1.712,202) = 1.227$, $p = 0.291$), see Supplemental Figure 2.1. Thus, given that there was no significant interaction effect, we calculated the AUC across the three collections and then corrected for the main effect of time of day, as

described in the methods. As noted in Supplementary Table 2.4, these AUC cortisol levels accounting of time of day did not significantly differ by ethnicity or sex.

Additionally, using the same regression model as in the main analyses on AUC cortisol (Supplementary Table 2.6), we observe the same main effects of interest for each individual cortisol collection. All three time points are positively predicted by genetic scores (Cortisol 1: β= 0.28, t=2.60, p=0.01; Cortisol 2: β= 0.24, t=2.26, p=0.03; Cortisol 3: β= 0.31, t=2.98, p=0.004), are negatively predicted by life events (Cortisol 1: β= -0.15, t=-1.55, p=0.13; Cortisol 2: β= -0.32, t=-3.47, p<0.001; Cortisol 3: β= -0.25, t=-2.67, p=0.009), and are predicted by a genetic score x sex interaction (Cortisol 1: β= 0.29, t=2.90, p=0.005; Cortisol 2: β= 0.14, t=2.45, p=0.15; Cortisol 3: β= 0.24, t=2.52, p=0.01).

**2.6.5 Data Analysis - Regression Analyses:** The first step in each regression model included two binary variables for ethnicity (White or not, African American or not) referencing the three ethnicity groups assessed to control for ethnic (currently, ancestry informative markers are not available). The second step included main effects of genetic profile scores (note: these values were created with an averaging function that allows for missing data and scaled back to 0-10 for display purposes) and life events.

In predicting cortisol, the fifth step added regional brain volumes, to assess any relationships between cortisol and limbic brain volumes (note: although brain volumes were assessed after cortisol was collected, there is likely a high correlation between earlier and later brain volumes for each participant).

The seventh step in predicting regional brain volumes added amygdala volumes to predictions of hippocampal volumes and vice versa to examine effects of genetic and environmental factors shared between the hippocampus and amygdala. As stress-/cortisol-related mechanisms are proposed to underlie changes in both hippocampal and amygdala volume, this step will assess the degree of overlap in potential mechanism. Finally, in the last step, three binary variables were

added to test for differences due to the diagnostic groups (PO-MDD, Later MDD, Other Psychiatric, Healthy Controls).

**2.6.6 Data Analysis - PROCESS Models:** One benefit of the simple moderation model in PROCESS (PROCESS Model #1) is that it allows for probing of the interactions between continuous independent variables while accounting for covariates by extracting the effect of the independent variable at different values of the moderator. Additionally, it allows for visualization of the interaction at different values of the independent variables and mean values of the covariates (note: these visualizations present regression predicted values of the dependent variable at different values of the independent variables rather than raw data values). Finally, in addition to testing the moderation effect at specific values, PROCESS also utilizes the Johnson-Neyman technique to isolate the range(s) of moderator values at which there is a significant relationship between the other predictor and the outcome variable (Johnson and Fay, 1950). These regions of Johnson-Neyman significance are shaded gray in Figure 2.3 in the main text. The mediation model in PROCESS (PROCESS Model #4) uses a regression-based approach that estimates the total effect of the independent variable (X) on the dependent variable (Y) as the regression coefficient of X as a predictor of Y (controlling for any and all covariates, but not including the mediator in the model). The direct effect is the effect of X on Y independent of the effect of the mediator (M) on Y, estimated as the regression coefficient of X predicting Y in a regression model, including M as a predictor. The indirect effect, or the effect of X on Y via M, is estimated as the product of the effect of X on M and the effect of M on Y, controlling for X. The total effect of X on Y is the sum of the direct and indirect effects. The indirect effect is determined as significant at a given $\alpha$ level if the confidence interval does not include zero (with a null hypothesis that there is no indirect effect). This approach to mediation analysis is preferable to the traditional Sobel test because the bootstrapping procedure to determine significance does not assume a parametric sampling distribution of the indirect effect (which is important as this distribution is generally skewed; Preacher and Hayes, 2008; Zhao et al,

2010). Additionally, the bootstrapping mediation analysis increases power to detect indirect effects without increasing the Type 1 error rate (Preacher and Hayes, 2008).

Although genetic profile scores and life events predicted cortisol levels, neither showed a statistically significant main effect on hippocampal or amygdala volumes (though the left hippocampus and amygdala showed significant genetic profile score x life event interactions). While traditional mediation methods (i.e. Baron and Kenny, 1986) require a significant association between the dependent and independent variable, this has been found to be an unnecessary prerequisite for mediation (Hayes, 2009; Zhao et al, 2010). An independent variable may still exert an indirect effect on a dependent variable via the mediator even if the main effect is not statistically significant, for example, if two or more indirect effects exist and operate in opposite directions. On the other hand, a non-zero relationship must exist between the mediator (i.e., cortisol) and the dependent variable (regional brain volume) for simple mediation to be possible. While cortisol predicted left amygdala volumes (controlling for all other factors), it did not predict hippocampal volumes or right amygdala volume with all other factors in the model (ps>0.1; Supplementary Tables 2.7, 2.8, 2.10). However, there were significant zero-order correlations between cortisol and left hippocampal volume ($r(118)= -0.191$, $p=0.037$), left amygdala volume ($r(118)= -0.247$, $p=0.006$), and right amygdala volume ($r(118)= -0.195$, $p=0.033$). As such, the results in the main text, Figure 2.2, and Supplementary Tables 2.13 and 2.14 show the effects of genetic profile scores and life events on regional brain volumes mediated by cortisol, with and without controlling for whole brain volume.

In addition to the indirect mediation effects noted in the main text, genetic profile scores ($\beta= -0.039$, 90% CI [-0.109, 0.000]) and life events ($\beta=0.030$, 90% CI [0.000, 0.102]) both exerted a trend-level effect on right amygdala volume, which was no longer significant when controlling for WBV. Additionally, life events exerted a significant direct effect on left amygdala volume, which

remained significant even controlling for WBV (β= -0.183, t= -2.371, p=0.020), i.e. life events were still a significant predictor controlling for cortisol.

### 2.6.7 Effects of Ethnicity, Sex, and Diagnostic Status: Supplementary

Table 2.5 shows group differences by sex and ethnicity in the dependent and independent variables (genetic profile scores, life events, cortisol, regional and whole brain volumes). Males had larger whole brain volumes than females, but no other variable of interest differed by sex. All variables except cortisol showed ethnicity effects. Effects of sex and ethnicity are also observable in the regressions predicting cortisol and regional brain volume (Tables 2.6-2.10). In these regressions, a negative beta value for sex as a predictor indicated that values were larger for males than females and a negative beta value for "African American or Not" indicated that values for larger for non-African American than African American children. In all of these regressions, any significant effects of sex or ethnicity on regional brain volumes were no longer significant after WBV was added to the model, indicating that differences due to these factors were not specific to regional volumes. Finally, no effects of diagnostic group were found for any of the variables in Supplementary Table 2.4 (ANOVA by the four diagnostic groups, all ps > 0.05), except for one significant post-hoc test showing smaller right amygdala volumes in the Later MDD groups vs. the healthy control group (Tukey HSD, p = 0.025). None of the regression models showed any significant effects of diagnostic group. Adding diagnostic groups did not significant increase the R2 of any regression model and did not meaningfully change any of the effects of interest. There was also no significant difference in the distribution of diagnostic groups by sex ($\chi 2(3)= 0.921$, p = 0.820) or by ethnicity ($\chi 2(6)= 8.131$, p = 0.229).

### 2.6.8 Follow-up Analysis with CRHR1 Haplotype: Three of the SNPs used to

construct the genetic profile scores were assessed in previous studies as part of a haplotype. Specifically, rs242941, rs242939, rs1876828, three CRHR1 SNPs, formed a GAG haplotype that was associated with greater antidepressant treatment response in MDD patients with high anxiety

(Licinio et al, 2004; Liu et al, 2007). While the main analyses included all three SNPs independently in the genetic profile scores, we also tested whether our results would remain consistent when including these SNPs as a haplotype instead in the genetic profile scores to confirm that there was no bias in the genetic profile scores from summing across these 3 SNPs. Haplotypes were estimated statistically using PHASE 2.1.1 (Stephens and Donnelly, 2003; Stephens et al, 2001). Then, genetic profile scores were created summing across the remaining 7 SNPs and the GAG haplotype (coded as 0 = 2 GAG copies, 0.5 = 1 GAG copy, 1 = 0 GAG copies). Using these new scores, we performed the same analyses as in the main text. These results parallel those in the main text. Namely, the genetic profile scores (haplotype version) and genetic profile scores x sex interaction both predicted cortisol (ps < 0.05), the genetic profile scores x life events interaction predicted left hippocampal volume at all steps of the model (ps < 0.05), and the interaction predicted left amygdala volume when entered into the model at trend level significance (p = 0.065) and was not significant at later steps of the model when entering hippocampal volume and diagnostic status (ps > 0.20). Cortisol also significantly mediated the effects of these genetic profile scores on left hippocampal and amygdala volumes, when not accounting for WBV (ps < 0.05). Full details on these analyses are available upon request.

**2.6.9 LD Proxies:** Using SNAP (http://www.broadinstitute.org/mpg/snap/ldsearch.php), we searched for LD proxies of the 9 intronic SNPs. Using the 1000 Genomes pilot 1 data for the Caucasian (CEU) panel, at r2≥0.8, we identified putative proxies in the 3' gene untranslated region (3'UTR) for rs1876828 (four variants found in UTR mapping from 1000 Genomes to dbSNP as rs878887, rs878888, rs4525537 and rs4640231; CRHR1), for rs10482695 (rs6198, NR3C1), and for rs1360780 (rs3800373; FKBP5). As the 3' UTR is an essential regulatory region, the function of these LD proxies should be carefully examined in the future. In addition, a synonymous SNP, rs16940665 (mapping from 1000 Genomes to dbSNP), was also in high LD with rs1876828 (CRHR1), however its impact on gene modulation is unknown.

**Supplementary Table 2.1: Number of Children in Each Group with Diagnoses Through Time of Scan**

| | PO-MDD | Later MDD | Other Psychiatric | Healthy Control |
|---|---|---|---|---|
| **N** | 45 | 16 | 28 | 31 |
| **PO-MDD** [a] | 45 | 0 | 0 | 0 |
| **MDD** | 45 | 16 | 0 | 0 |
| **ADHD** | 22 | 7 | 9 | 0 |
| **Oppositional Defiant Disorder** | 24 | 6 | 13 | 0 |
| **Conduct Disorder** | 15 | 4 | 8 | 0 |
| **Generalized Anxiety Disorder** | 21 | 5 | 10 | 0 |
| **PTSD** | 8 | 0 | 1 | 0 |
| **Separation Anxiety Disorder** | 20 | 4 | 12 | 0 |
| **Mania** | 21 | 3 | 4 | 0 |
| **Dysthymia** | 9 | 4 | 2 | 0 |
| **OCD** | 7 | 3 | 4 | 0 |
| **Panic Attack** | 3 | 0 | 1 | 0 |
| **Panic With Agoraphobia** | 1 | 0 | 0 | 0 |
| **Panic Without Agoraphobia** | 0 | 0 | 1 | 0 |
| **Agoraphobia Without Panic** | 0 | 0 | 1 | 0 |
| **Social Phobia** | 10 | 5 | 5 | 0 |

*a: PO-MDD: MDD diagnosed before age 6, may have later MDD as well*

**Supplementary Table 2.2: Reported Instances of Each Type of Stressful and Traumatic Life Event Assessed**

| | Count of Participants by Reported # of Instances | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Stressful Life Events** | **Missing** | **Max** | **0** | **1** | **2** | **3** | **4** | **5** | **6+** |
| New Child in Home | 4 | 3 | 41 | 66 | 6 | 3 | 0 | 0 | 0 |
| Parental Separation | 18 | 3 | 77 | 23 | 1 | 1 | 0 | 0 | 0 |
| Parental Divorce | 22 | 1 | 90 | 8 | 0 | 0 | 0 | 0 | 0 |
| New Parental Figure | 20 | 2 | 79 | 19 | 2 | 0 | 0 | 0 | 0 |
| Moving House | 1 | 7 | 47 | 42 | 16 | 6 | 3 | 3 | 2 |
| Change Daycare/School | 13 | 6 | 52 | 42 | 4 | 5 | 2 | 1 | 1 |
| Lost Significant Person Through Moving | 1 | 2 | 99 | 19 | 1 | 0 | 0 | 0 | 0 |
| Death of Pet | 1 | 2 | 96 | 19 | 4 | 0 | 0 | 0 | 0 |
| Reduction in Standard of Living | 23 | 1 | 93 | 4 | 0 | 0 | 0 | 0 | 0 |
| Loss of Home Without Family Separation | 25 | 1 | 93 | 2 | 0 | 0 | 0 | 0 | 0 |
| Parental Arrest | 1 | 2 | 115 | 3 | 1 | 0 | 0 | 0 | 0 |
| Parental Hospitalization | 0 | 10 | 68 | 38 | 13 | 0 | 0 | 0 | 1 |
| Separation From Parent (1 week or more) | 5 | 10 | 79 | 24 | 6 | 3 | 0 | 1 | 2 |
| **Traumatic Life Events** | | | | | | | | | |
| Accident or Crash with Automobile, Plane, or Boat | 0 | 1 | 104 | 16 | 0 | 0 | 0 | 0 | 0 |
| Attacked by an Animal | 0 | 1 | 113 | 7 | 0 | 0 | 0 | 0 | 0 |
| Natural Disasters (flood,hurricane,tornado,earthquake) | 0 | 1 | 111 | 9 | 0 | 0 | 0 | 0 | 0 |
| Witnessed Another Person Being Threatened with Harm, Seriously Injured, or Killed | 0 | 1 | 111 | 9 | 0 | 0 | 0 | 0 | 0 |
| Physical Abuse | 21 | 1 | 94 | 5 | 0 | 0 | 0 | 0 | 0 |
| Sexual Abuse, Sexual Assault, or Rape | 24 | 2 | 93 | 2 | 1 | 0 | 0 | 0 | 0 |
| Accidental Burning, Poisoning, or Drowning | 2 | 2 | 102 | 14 | 2 | 0 | 0 | 0 | 0 |
| Hospitalization, Emergency Room Visit, or Invasive Medical Procedure | 17 | 7 | 36 | 45 | 12 | 8 | 0 | 1 | 1 |
| Death of Adult Loved One | 1 | 3 | 88 | 26 | 4 | 1 | 0 | 0 | 0 |
| Death of Sibling or Peer | 1 | 5 | 116 | 2 | 0 | 0 | 0 | 1 | 0 |

**Supplementary Table 2.3: Description of Laboratory Temperament Assessment Battery Stressors**

| LabTAB Episode | Description |
|---|---|
| Transparent Box | Attractive toy is locked in a transparent box that child cannot open. This task is designed to evoke frustration or anger by preventing the child from playing with the selected toy. The child will be able to see the object of desire (in this case a toy) through the clear plastic box but be unable to attain it because the box will be locked and the keys they try will not open it. |
| I'm Not Sharing Any Toys | Examiner takes more desirable toys than gives to child, leaving child with few desirable toys. |
| Box Empty | Child opens attractively wrapped gift expecting a toy and finds box empty. |
| Impossible Circles | Child is asked to draw perfect circles, but is always corrected for them not being perfect. |
| No Candy Bars Left | Child promised a preferred candy bar and then told that none are left and will have to take undesirable candy. |

**Supplementary Table 2.4: Single Nucleotide Polymorphism Data**

| Gene | SNP | Missing | Alleles | MAF | $Chi^2$ | Coding |
|---|---|---|---|---|---|---|
| *CRHR1* | rs4792887 | 0 | C>T | 0.154 | 14.970* AA>W | TT=1,CT=0.5,CC=0 |
| *CRHR1* | rs110402 | 0 | C>T | 0.379 | 0.678 | TT=1,CT=0,CC=0 |
| *CRHR1* | rs242941 | 1 | G>T | 0.382 | 11.470* AA>W | TT=1,GT=1,GG=0 |
| *CRHR1* | rs242939 | 0 | A>G | 0.125 | 12.740* AA>W | GG=1,AG=1,AA=0 |
| *CRHR1* | rs1876828 | 0 | G>A | 0.204 | 11.800* W>AA | GG=1,AG=1,AA=0 |
| *NR3C2* | rs5522 | 0 | A>G | 0.092 | 2.423 | GG=1,AG=1,AA=0 |
| NR3C1 | rs41423247 | 1 | G>C | 0.311 | 24.490* W>AA | GG=1,CG=1,CC=0 |
| NR3C1 | rs10482605 | 0 | T>C | 0.179 | 0.403 | TT=1,CT=0,CC=0 |
| NR3C1 | rs10052957 | 3 | G>A | 0.338 | 3.926 | AA=1,AG=0,GG=0 |
| *FKBP5* | rs1360780 | 4 | C>T | 0.336 | 6.567 | TT=1,CT=1,CC=0 |

*Missing = Number of participants from N=120 subsample missing a given SNP due to genotyping failure*
*Alleles = Alleles present in current sample (major>minor)*
*MAF = Minor allele frequency for current sample*
*$Chi^2$ = test of association between MAF and ethnicity (African American and White), * = Bonferroni corrected p-value < 0.05*
*Coding: Coding of each genotype based on previous literature for genetic profile score construction*
*Note: A SNP genotype was coded as 1 if it has been associated previously with increased cortisol reactivity, depression risk, and/or depression-related phenotypes and was coded as 0 if it has been associated with control or decreased cortisol levels, depression risk, and/or depression-related phenotypes. A genotype was coded as 0.5 if only allelic rather than specific genotype effects were found for cortisol reactivity, depression risk, and/or depression-related phenotypes. See Table 2.1 in the main text for relevant literature.*

**Supplementary Table 2.5: Distribution of Variables of Interest and Effects of Ethnicity and Sex**

| | | | | Effect of Ethnicity | | Effect of Sex | |
|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Range** | **F** | **Post-Hoc** | **t** | **Post-Hoc** |
| **Genetic Profile Scores** | 4.47 | 1.27 | 1.0 - 7.5 | 15.98*** | W<A=O | 1.53 | - |
| **Life Events** | 6.73 | 4.52 | 0 - 20 | 2.95 | W=A<O | 0.10 | - |
| **AUC Cortisol Levels (residuals)** | -0.54 | 19.15 | -33.4 - 53.5 | 0.62 | - | 0.19 | - |
| **Left Hippocampal Volume** | 3912 | 418 | 2779 - 4910 | 12.78*** | W>A | 1.52 | - |
| **Right Hippocampal Volume** | 4085 | 413 | 2965 - 5070 | 14.76*** | W=O>A | 1.27 | - |
| **Left Amygdala Volume** | 1628 | 192 | 1244 - 2199 | 7.60*** | W=O>A | 1.12 | - |
| **Right Amygdala Volume** | 1650 | 173 | 1280 - 2223 | 8.79*** | W=O>A | 1.21 | - |
| **Whole Brain Volume** | 1,205,221 | 110,761 | 907,427 - 1,481,621 | 9.93*** | W>O>A | 4.05*** | M>F |

*All values and stats for N=120 subsample used in all analyses*

*Effect of Ethnicity: ANOVA with three group (W = White, A = African American, O = Other)*

*Effect of Sex: Two-sample t-test by sex (M = males vs. F = female)*

**** p < 0.001*

**Supplementary Table 2.6: Regression Model Predicting Cortisol**

| Step | | Step 1 | | Step 2 | | Step 3 | | Step 4 | | Step 5 | | Step 6 | | Step 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | p | β | p | β | p | β | p | β | p | β | p | β | p |
| 1 | White | -0.04 | 0.77 | -0.05 | 0.75 | 0.02 | 0.89 | 0.02 | 0.92 | 0.03 | 0.85 | 0.05 | 0.71 | 0.06 | 0.68 |
| 1 | African American | 0.07 | 0.65 | -0.02 | 0.89 | 0.02 | 0.90 | 0.01 | 0.94 | -0.06 | 0.67 | -0.09 | 0.54 | -0.08 | 0.58 |
| 1 | Sex | -0.00 | 0.97 | 0.01 | 0.91 | 0.04 | 0.68 | 0.04 | 0.69 | -0.01 | 0.92 | -0.09 | 0.38 | -0.08 | 0.40 |
| 2 | Genetic Profile Score | | | 0.21 | 0.04 | **0.29** | **0.01** | **0.28** | **0.01** | **0.29** | **0.01** | **0.32** | **0.00** | **0.32** | **0.00** |
| 2 | Life Events | | | -0.21 | 0.02 | -0.22 | 0.02 | -0.22 | 0.02 | **-0.27** | **0.00** | **-0.27** | **0.00** | **-0.28** | **0.00** |
| 3 | Genes x Life Events | | | | | -0.02 | 0.81 | -0.02 | 0.84 | 0.06 | 0.49 | 0.06 | 0.52 | 0.06 | 0.50 |
| 3 | Genes x Sex | | | | | 0.17 | 0.07 | 0.17 | 0.08 | 0.19 | 0.05 | 0.21 | 0.02 | 0.22 | 0.03 |
| 3 | Life Events x Sex | | | | | 0.16 | 0.08 | 0.16 | 0.09 | 0.11 | 0.20 | 0.13 | 0.15 | 0.13 | 0.14 |
| 4 | Genes x Life Events x Sex | | | | | | | 0.05 | 0.58 | 0.04 | 0.65 | 0.02 | 0.81 | 0.02 | 0.81 |
| 5 | Left Hippocampal Volume | | | | | | | | | -0.25 | 0.12 | -0.23 | 0.15 | -0.22 | 0.16 |
| 5 | Right Hippocampal Volume | | | | | | | | | 0.28 | 0.09 | 0.35 | 0.03 | 0.36 | 0.04 |
| 5 | Left Amygdala Volume | | | | | | | | | -0.33 | 0.02 | -0.31 | 0.02 | -0.32 | 0.02 |
| 5 | Right Amygdala Volume | | | | | | | | | 0.02 | 0.90 | 0.10 | 0.46 | 0.12 | 0.41 |
| 6 | Whole Brain Volume | | | | | | | | | | | -0.28 | 0.04 | -0.28 | 0.04 |
| 7 | PO-MDD | | | | | | | | | | | | | 0.05 | 0.67 |
| 7 | Later MDD | | | | | | | | | | | | | 0.05 | 0.63 |
| 7 | Other Psychiatric | | | | | | | | | | | | | 0.04 | 0.69 |
| | Model $R^2$ | 0.01 | | 0.09 | | 0.14 | | 0.14 | | 0.24 | | 0.27 | | 0.27 | |
| | Model Adjusted $R^2$ | -0.02 | | 0.05 | | 0.08 | | 0.07 | | 0.14 | | 0.17 | | 0.15 | |
| | Model F | 0.41 | | 2.22 | | 2.29 | | 2.05 | | 2.51 | | 2.73 | | 2.21 | |
| | Model p | 0.75 | | 0.06 | | 0.03 | | 0.04 | | **0.01** | | **0.00** | | **0.01** | |
| | Change p | 0.75 | | **0.01** | | 0.08 | | 0.58 | | 0.02 | | 0.04 | | 0.96 | |

*Standardized beta and p values are presented for each predictor. For each step of the model, the $R^2$, adjusted $R^2$, F, and p value are presented, as well as the p value for change in $R^2$ at each step. Any step where a predictor, model, or change in $R^2$ is significant at p<0.05 is shaded gray and those significant at a Bonferroni corrected threshold of p<0.01 are bolded.*

**Supplementary Table 2.7: Regression Model Predicting Left Hippocampal Volume**

| | Step 1 | | Step 2 | | Step 3 | | Step 4 | | Step 5 | | Step 6 | | Step 7 | | Step 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Step** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** |
| **1 White** | 0.21 | 0.10 | 0.23 | 0.08 | 0.21 | 0.12 | 0.22 | 0.11 | 0.22 | 0.10 | 0.15 | 0.23 | 0.17 | 0.17 | 0.15 | 0.24 |
| **1 African American** | -0.27 | 0.04 | -0.26 | 0.05 | -0.24 | 0.06 | -0.24 | 0.07 | -0.23 | 0.07 | -0.08 | 0.50 | -0.04 | 0.75 | -0.04 | 0.73 |
| **1 Sex** | -0.20 | 0.02 | -0.20 | 0.02 | -0.20 | 0.02 | -0.20 | 0.02 | -0.20 | 0.02 | -0.02 | 0.78 | -0.03 | 0.71 | -0.03 | 0.70 |
| **2 Genetic Profile Scores** | | | 0.01 | 0.92 | -0.05 | 0.64 | -0.04 | 0.70 | 0.00 | 0.99 | -0.07 | 0.45 | -0.08 | 0.39 | -0.08 | 0.40 |
| **2 Life Events** | | | 0.06 | 0.46 | 0.05 | 0.54 | 0.05 | 0.56 | 0.02 | 0.82 | 0.04 | 0.60 | 0.09 | 0.29 | 0.10 | 0.23 |
| **3 Genes x Life Events** | | | | | **0.24** | **0.00** | **0.24** | **0.01** | **0.23** | **0.01** | **0.21** | **0.01** | 0.17 | 0.03 | 0.16 | 0.04 |
| **3 Genes x Sex** | | | | | -0.08 | 0.35 | -0.08 | 0.39 | -0.05 | 0.56 | -0.08 | 0.34 | -0.09 | 0.29 | -0.08 | 0.37 |
| **3 Life Events x Sex** | | | | | -0.02 | 0.81 | -0.02 | 0.84 | 0.00 | 0.96 | -0.01 | 0.92 | 0.02 | 0.77 | 0.01 | 0.86 |
| **4 Genes x Life Events x Sex** | | | | | | | -0.05 | 0.52 | -0.05 | 0.57 | -0.02 | 0.77 | -0.03 | 0.71 | -0.05 | 0.56 |
| **5 Cortisol** | | | | | | | | | -0.14 | 0.12 | -0.04 | 0.63 | 0.00 | 1.00 | 0.00 | 1.00 |
| **6 Whole Brain Volume** | | | | | | | | | | | **0.44** | **0.00** | **0.34** | **0.00** | **0.34** | **0.00** |
| **7 Left Amygdala Volume** | | | | | | | | | | | | | 0.25 | 0.04 | 0.25 | 0.04 |
| **7 Right Amygdala Volume** | | | | | | | | | | | | | -0.02 | 0.87 | -0.03 | 0.84 |
| **8 PO-MDD** | | | | | | | | | | | | | | | -0.09 | 0.38 |
| **8 Later MDD** | | | | | | | | | | | | | | | -0.03 | 0.76 |
| **8 Other Psychiatric** | | | | | | | | | | | | | | | -0.03 | 0.76 |
| **Model $R^2$** | 0.22 | | 0.22 | | 0.28 | | 0.28 | | 0.30 | | 0.42 | | 0.45 | | 0.46 | |
| **Model Adjusted $R^2$** | 0.20 | | 0.19 | | 0.23 | | 0.22 | | 0.24 | | 0.36 | | 0.39 | | 0.37 | |
| **Model F** | 10.80 | | 6.51 | | 5.39 | | 4.82 | | 4.65 | | 7.12 | | 6.74 | | 5.42 | |
| **Model p** | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | |
| **Change p** | **0.00** | | 0.76 | | 0.04 | | 0.52 | | 0.12 | | **0.00** | | 0.05 | | 0.85 | |

*Standardized beta and p values are presented for each predictor. For each step of the model, the $R^2$, adjusted $R^2$, F, and p value are presented, as well as the p value for change in $R^2$ at each step. Any step where a predictor, model, or change in $R^2$ is significant at p<0.05 is shaded gray and those significant at a Bonferroni corrected threshold of p<0.01 are bolded.*

**Supplementary Table 2.8: Regression Model Predicting Right Hippocampal Volume**

| | Step 1 | | Step 2 | | Step 3 | | Step 4 | | Step 5 | | Step 6 | | Step 7 | | Step 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Step** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** |
| **1 White** | 0.15 | 0.23 | 0.18 | 0.18 | 0.14 | 0.29 | 0.15 | 0.28 | 0.15 | 0.28 | 0.06 | 0.63 | 0.08 | 0.49 | 0.04 | 0.71 |
| **1 African American** | **-0.35** | **0.01** | **-0.35** | **0.01** | **-0.35** | **0.01** | **-0.35** | **0.01** | **-0.35** | **0.01** | -0.15 | 0.20 | -0.09 | 0.42 | -0.10 | 0.39 |
| **1 Sex** | -0.19 | 0.03 | -0.18 | 0.03 | -0.19 | 0.02 | -0.19 | 0.02 | -0.19 | 0.02 | 0.04 | 0.64 | 0.02 | 0.75 | 0.03 | 0.74 |
| **2 Genetic Profile Scores** | | | 0.04 | 0.65 | -0.02 | 0.86 | -0.01 | 0.89 | 0.00 | 1.00 | -0.10 | 0.28 | -0.10 | 0.26 | -0.09 | 0.29 |
| **2 Life Events** | | | 0.05 | 0.56 | 0.04 | 0.61 | 0.04 | 0.61 | 0.03 | 0.71 | 0.06 | 0.41 | 0.10 | 0.17 | 0.13 | 0.10 |
| **3 Genes x Life Events** | | | | | 0.14 | 0.10 | 0.13 | 0.11 | 0.13 | 0.11 | 0.11 | 0.14 | 0.07 | 0.31 | 0.06 | 0.41 |
| **3 Genes x Sex** | | | | | -0.12 | 0.18 | -0.12 | 0.20 | -0.11 | 0.24 | -0.14 | 0.07 | -0.14 | 0.08 | -0.11 | 0.17 |
| **3 Life Events x Sex** | | | | | -0.05 | 0.54 | -0.05 | 0.55 | -0.04 | 0.62 | -0.06 | 0.42 | -0.02 | 0.75 | -0.03 | 0.65 |
| **4 Genes x Life Events x Sex** | | | | | | | -0.03 | 0.74 | -0.03 | 0.76 | 0.01 | 0.93 | -0.01 | 0.92 | -0.05 | 0.47 |
| **5 Cortisol** | | | | | | | | | -0.05 | 0.60 | 0.08 | 0.29 | 0.12 | 0.12 | 0.12 | 0.12 |
| **6 Whole Brain Volume** | | | | | | | | | | | **0.58** | **0.00** | **0.42** | **0.00** | **0.42** | **0.00** |
| **7 Left Amygdala Volume** | | | | | | | | | | | | | 0.23 | 0.04 | 0.23 | 0.04 |
| **7 Right Amygdala Volume** | | | | | | | | | | | | | 0.08 | 0.44 | 0.09 | 0.42 |
| **8 PO-MDD** | | | | | | | | | | | | | | | -0.15 | 0.11 |
| **8 Later MDD** | | | | | | | | | | | | | | | -0.02 | 0.77 |
| **8 Other Psychiatric** | | | | | | | | | | | | | | | 0.01 | 0.87 |
| **Model $R^2$** | 0.24 | | 0.24 | | 0.27 | | 0.27 | | 0.27 | | 0.48 | | 0.53 | | 0.55 | |
| **Model Adjusted $R^2$** | 0.22 | | 0.21 | | 0.22 | | 0.21 | | 0.20 | | 0.43 | | 0.47 | | 0.48 | |
| **Model F** | 11.89 | | 7.16 | | 5.10 | | 4.51 | | 4.06 | | 9.11 | | 9.14 | | 7.72 | |
| **Model p** | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | |
| **Change p** | **0.00** | | 0.76 | | 0.22 | | 0.74 | | 0.60 | | **0.00** | | 0.01 | | 0.28 | |

*Standardized beta and p values are presented for each predictor. For each step of the model, the $R^2$, adjusted $R^2$, F, and p value are presented, as well as the p value for change in $R^2$ at each step. Any step where a predictor, model, or change in $R^2$ is significant at p<0.05 is shaded gray and those significant at a Bonferroni corrected threshold of p<0.01 are bolded.*

**Supplementary Table 2.9: Regression Model Predicting Left Amygdala Volume**

| | Step 1 | | Step 2 | | Step 3 | | Step 4 | | Step 5 | | Step 6 | | Step 7 | | Step 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Step** | **B** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** | **β** | **p** |
| **1 White** | 0.06 | 0.68 | 0.02 | 0.86 | 0.00 | 1.00 | 0.00 | 1.00 | 0.01 | 0.97 | -0.07 | 0.57 | -0.09 | 0.44 | -0.08 | 0.54 |
| **1 African American** | -0.32 | 0.02 | **-0.37** | **0.01** | **-0.35** | **0.01** | **-0.35** | **0.01** | **-0.35** | **0.01** | -0.19 | 0.13 | -0.14 | 0.23 | -0.14 | 0.26 |
| **1 Sex** | -0.16 | 0.07 | -0.16 | 0.08 | -0.16 | 0.07 | -0.16 | 0.07 | -0.15 | 0.07 | 0.04 | 0.67 | 0.03 | 0.74 | 0.03 | 0.74 |
| **2 Genetic Profile Scores** | | | 0.06 | 0.54 | 0.03 | 0.76 | 0.03 | 0.75 | 0.11 | 0.29 | 0.03 | 0.74 | 0.06 | 0.51 | 0.06 | 0.52 |
| **2 Life Events** | | | -0.15 | 0.09 | -0.14 | 0.10 | -0.14 | 0.10 | -0.20 | 0.02 | -0.18 | 0.02 | **-0.20** | **0.01** | **-0.21** | **0.01** |
| **3 Genes x Life Events** | | | | | 0.19 | 0.03 | 0.18 | 0.03 | 0.18 | 0.03 | 0.16 | 0.04 | 0.12 | 0.12 | 0.12 | 0.11 |
| **3 Genes x Sex** | | | | | 0.01 | 0.94 | 0.01 | 0.93 | 0.05 | 0.55 | 0.03 | 0.75 | 0.07 | 0.39 | 0.06 | 0.47 |
| **3 Life Events x Sex** | | | | | -0.16 | 0.07 | -0.16 | 0.07 | -0.12 | 0.17 | -0.13 | 0.09 | -0.11 | 0.13 | -0.11 | 0.17 |
| **4 Genes x Life Events x Sex** | | | | | | | -0.01 | 0.90 | 0.00 | 0.98 | 0.03 | 0.71 | 0.03 | 0.70 | 0.04 | 0.58 |
| **5 Cortisol** | | | | | | | | | **-0.27** | **0.00** | -0.16 | 0.05 | -0.18 | 0.03 | -0.18 | 0.03 |
| **6 Whole Brain Volume** | | | | | | | | | | | **0.47** | **0.00** | **0.29** | **0.01** | **0.29** | **0.01** |
| **7 Left Hippocampal Volume** | | | | | | | | | | | | | 0.06 | 0.65 | 0.05 | 0.69 |
| **7 Right Hippocampal Volume** | | | | | | | | | | | | | 0.27 | 0.06 | 0.28 | 0.05 |
| **8 PO-MDD** | | | | | | | | | | | | | | | 0.08 | 0.42 |
| **8 Later MDD** | | | | | | | | | | | | | | | 0.05 | 0.55 |
| **8 Other Psychiatric** | | | | | | | | | | | | | | | 0.01 | 0.88 |
| **Model $R^2$** | 0.14 | | 0.16 | | 0.22 | | 0.22 | | 0.28 | | 0.42 | | 0.47 | | 0.48 | |
| **Model Adjusted $R^2$** | 0.12 | | 0.13 | | 0.17 | | 0.16 | | 0.22 | | 0.36 | | 0.41 | | 0.40 | |
| **Model F** | 6.27 | | 4.45 | | 3.99 | | 3.51 | | 4.32 | | 7.16 | | 7.29 | | 5.86 | |
| **Model p** | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | |
| **Change p** | **0.00** | | 0.20 | | 0.04 | | 0.90 | | **0.00** | | **0.00** | | **0.01** | | 0.84 | |

*Standardized beta and p values are presented for each predictor. For each step of the model, the $R^2$, adjusted $R^2$, F, and p value are presented, as well as the p value for change in $R^2$ at each step. Any step where a predictor, model, or change in $R^2$ is significant at p<0.05 is shaded gray and those significant at a Bonferroni corrected threshold of p<0.01 are bolded*

**Supplementary Table 2.10: Regression Model Predicting Right Amygdala Volume**

| Step | | Step 1 | | Step 2 | | Step 3 | | Step 4 | | Step 5 | | Step 6 | | Step 7 | | Step 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | p | β | p | β | p | β | p | β | p | β | p | β | p | β | p |
| 1 | White | 0.06 | 0.66 | 0.08 | 0.57 | 0.04 | 0.80 | 0.03 | 0.83 | 0.03 | 0.82 | -0.06 | 0.62 | -0.07 | 0.58 | -0.06 | 0.61 |
| 1 | African American | **-0.34** | **0.01** | **-0.35** | **0.01** | **-0.37** | **0.01** | **-0.38** | **0.01** | **-0.38** | **0.01** | -0.17 | 0.16 | -0.13 | 0.27 | -0.14 | 0.24 |
| 1 | Sex | -0.17 | 0.51 | -0.17 | 0.06 | -0.18 | 0.04 | -0.18 | 0.04 | -0.18 | 0.05 | 0.06 | 0.48 | 0.05 | 0.58 | 0.03 | 0.69 |
| 2 | Genetic Profile Scores | | | 0.05 | 0.60 | 0.00 | 1.00 | -0.01 | 0.95 | 0.04 | 0.73 | -0.06 | 0.52 | -0.04 | 0.68 | -0.04 | 0.64 |
| 2 | Life Events | | | 0.02 | 0.86 | 0.02 | 0.84 | 0.02 | 0.83 | -0.02 | 0.87 | 0.02 | 0.85 | 0.00 | 1.00 | -0.01 | 0.91 |
| 3 | Genes x Life Events | | | | | 0.00 | 1.00 | 0.00 | 0.98 | 0.00 | 1.00 | -0.03 | 0.70 | -0.04 | 0.56 | -0.04 | 0.58 |
| 3 | Genes x Sex | | | | | -0.13 | 0.16 | -0.14 | 0.15 | -0.11 | 0.25 | -0.14 | 0.08 | -0.11 | 0.18 | -0.12 | 0.12 |
| 3 | Life Events x Sex | | | | | -0.08 | 0.36 | -0.08 | 0.35 | -0.06 | 0.51 | -0.07 | 0.33 | -0.06 | 0.44 | -0.07 | 0.38 |
| 4 | Genes x Life Events x Sex | | | | | | | 0.04 | 0.64 | 0.05 | 0.57 | 0.08 | 0.27 | 0.08 | 0.29 | 0.12 | 0.14 |
| 5 | Cortisol | | | | | | | | | -0.16 | 0.10 | -0.02 | 0.78 | -0.05 | 0.54 | -0.05 | 0.56 |
| 6 | Whole Brain Volume | | | | | | | | | | | **0.60** | **0.00** | **0.46** | **0.00** | **0.42** | **0.00** |
| 7 | Left Hippocampal Volume | | | | | | | | | | | | | -0.07 | 0.59 | -0.08 | 0.53 |
| 7 | Right Hippocampal Volume | | | | | | | | | | | | | 0.29 | 0.04 | 0.31 | 0.03 |
| 8 | PO-MDD | | | | | | | | | | | | | | | -0.02 | 0.80 |
| 8 | Later MDD | | | | | | | | | | | | | | | -0.15 | 0.08 |
| 8 | Other Psychiatric | | | | | | | | | | | | | | | -0.14 | 0.10 |
| | Model $R^2$ | 0.16 | | 0.16 | | 0.18 | | 0.19 | | 0.21 | | 0.43 | | 0.46 | | 0.49 | |
| | Model Adjusted $R^2$ | 0.14 | | 0.12 | | 0.12 | | 0.12 | | 0.13 | | 0.37 | | 0.39 | | 0.41 | |
| | Model F | 7.30 | | 4.38 | | 3.11 | | 2.77 | | 2.82 | | 7.36 | | 6.93 | | 6.08 | |
| | Model p | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | | **0.00** | |
| | Change p | **0.00** | | 0.86 | | 0.40 | | 0.64 | | 0.10 | | **0.00** | | 0.05 | | 0.16 | |

*Standardized beta and p values are presented for each predictor. For each step of the model, the $R^2$, adjusted $R^2$, F, and p value are presented, as well as the p value for change in $R^2$ at each step. Any step where a predictor, model, or change in $R^2$ is significant at p<0.05 is shaded gray and those significant at a Bonferroni corrected threshold of p<0.01 are bolded*

**Supplementary Table 2.11: Regression Models Predicting Left Hippocampus Subfields**

| | Full Volume | Presubiculum | CA1 | CA2/3 | Fimbria | Subiculum | DA4/DG | Fissure |
|---|---|---|---|---|---|---|---|---|
| **White** | 0.13 | 0.17 | 0.18 | 0.11 | -0.08 | 0.09 | 0.11 | 0.30* |
| **African American** | -0.09 | -0.14 | -0.03 | -0.16 | -0.44** | -0.24* | -0.21 | 0.03 |
| **Sex** | -0.02 | -0.16 | 0.08 | -0.05 | -0.16 | -0.08 | -0.06 | 0.01 |
| **Genetic Profile Scores** | -0.07 | -0.10 | 0.09 | 0.01 | 0.01 | 0.03 | 0.02 | -0.17 |
| **Life Events** | 0.05 | 0.06 | 0.09 | 0.03 | -0.10 | 0.03 | 0.03 | 0.07 |
| **Genes x Life Events** | **0.21**** | 0.14 | 0.14 | 0.23** | -0.14 | 0.11 | 0.22** | -0.04 |
| **Genes x Sex** | -0.07 | -0.02 | -0.03 | -0.03 | -0.04 | -0.04 | -0.03 | 0.02 |
| **Life Events x Sex** | -0.02 | 0.04 | -0.10 | -0.09 | -0.05 | -0.06 | -0.10 | -0.06 |
| **Genes x Life Events x Sex** | -0.04 | 0.07 | -0.10 | 0.01 | 0.07 | 0.05 | 0.05 | -0.09 |
| **Cortisol** | -0.04 | 0.07 | -0.01 | -0.12 | -0.11 | -0.02 | -0.07 | 0.12 |
| **Whole Brain Volume** | **0.44***** | 0.39*** | 0.48*** | 0.40*** | 0.17 | 0.47*** | 0.42*** | 0.08 |
| **PO-MDD** | -0.08 | -0.03 | -0.10 | 0.02 | -0.09 | -0.09 | 0.04 | 0.11 |
| **Later MDD** | -0.01 | -0.02 | -0.09 | 0.00 | -0.20* | -0.05 | 0.02 | -0.09 |
| **Other Psychiatric** | -0.02 | -0.05 | -0.08 | -0.07 | -0.06 | -0.05 | -0.03 | 0.02 |
| **Model $R^2$** | 0.42 | 0.43 | 0.42 | 0.44 | 0.34 | 0.49 | 0.47 | 0.22 |
| **Model Adjusted $R^2$** | 0.35 | 0.35 | 0.34 | 0.37 | 0.25 | 0.42 | 0.40 | 0.11 |
| **Model F** | **5.52***** | 5.46*** | 5.21*** | 5.81*** | 3.71*** | 6.99*** | 6.56*** | 2.03* |
| **Change in $R^2$ due to Genes x Life Events Interaction** | **0.04**** | 0.02 | 0.02 | 0.05** | 0.02 | 0.01 | 0.05** | 0.00 |

*Regression models were used to predict the full hippocampal volume estimates, as described in the main text, as well as 7 subfields. Standardized beta values from these regressions are presented. For each model, the $R^2$, adjusted $R^2$, model F are presented, as well as the change in $R^2$ due to adding the genes x life events interaction after all other variables in the model. Predictors of subfield volumes that were significant after Bonferroni correction (p<0.007) are shaded in gray.*

* p < 0.05; ** p < 0.01; *** p < 0.001

**Supplementary Table 2.12: Regression Models Predicting Right Hippocampus Subfields**

| | Full Volume | Presubiculum | CA1 | CA2/3 | Fimbria | Subiculum | DA4/DG | Fissure |
|---|---|---|---|---|---|---|---|---|
| **White** | 0.02 | 0.12 | 0.12 | 0.00 | -0.05 | -0.02 | -0.04 | -0.06 |
| **African American** | -0.16 | -0.07 | -0.12 | -0.18 | -0.50 | -0.32** | -0.23 | -0.25 |
| **Sex** | 0.04 | -0.14 | 0.05 | 0.06 | -0.19* | -0.08 | 0.09 | 0.15 |
| **Genetic Profile Scores** | -0.09 | -0.24** | 0.17 | 0.00 | -0.05 | -0.06 | 0.03 | -0.09 |
| **Life Events** | 0.09 | 0.01 | 0.16 | 0.11 | -0.04 | 0.03 | 0.14 | 0.22* |
| **Genes x Life Events** | 0.09 | 0.15* | 0.06 | 0.04 | 0.00 | 0.08 | 0.03 | -0.10 |
| **Genes x Sex** | -0.12 | -0.02 | -0.04 | -0.06 | -0.01 | -0.07 | -0.05 | -0.06 |
| **Life Events x Sex** | -0.07 | 0.06 | -0.10 | -0.13 | -0.03 | -0.01 | -0.16* | -0.13 |
| **Genes x Life Events x Sex** | -0.04 | 0.04 | -0.09 | -0.03 | 0.08 | 0.00 | -0.05 | -0.22* |
| **Cortisol** | 0.08 | 0.11 | -0.03 | 0.01 | 0.05 | 0.06 | 0.09 | 0.08 |
| **Whole Brain Volume** | **0.578*** ** | 0.46*** | 0.44*** | 0.57*** | 0.20* | 0.48*** | 0.57*** | 0.14 |
| **PO-MDD** | -0.14 | -0.05 | -0.13 | -0.07 | 0.02 | -0.10 | -0.07 | -0.10 |
| **Later MDD** | -0.03 | -0.12 | -0.07 | -0.02 | -0.14 | -0.02 | -0.03 | -0.14 |
| **Other Psychiatric** | 0.00 | 0.01 | -0.03 | -0.04 | -0.02 | 0.07 | 0.03 | -0.11 |
| **Model $R^2$** | 0.50 | 0.51 | 0.38 | 0.45 | 0.40 | 0.51 | 0.45 | 0.26 |
| **Model Adjusted $R^2$** | 0.43 | 0.45 | 0.30 | 0.38 | 0.32 | 0.45 | 0.38 | 0.16 |
| **Model F** | **7.399*** ** | 7.69*** | 4.47*** | 6.04*** | 4.90*** | 7.65*** | 6.02*** | 2.56** |
| **Change in $R^2$ due to Genes x Life Events Interaction** | 0.01 | 0.020* | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 |

*Regression models were used to predict the full hippocampal volume estimates, as described in the main text, as well as 7 subfields. Standardized beta values from these regressions are presented. For each model, the $R^2$, adjusted $R^2$, model F are presented, as well as the change in $R^2$ due to adding the genes x life events interaction after all other variables in the model. Predictors of subfield volumes that were significant after Bonferroni correction (p<0.007) are shaded in gray.*

* p < 0.05; ** p < 0.01; *** p < 0.001

**Supplementary Table 2.13: Results of Mediation Analyses Without Controlling for Whole Brain Volume**

| | Left Hippocampal Volume | | | Right Hippocampal Volume | | | Left Amygdala Volume | | | Right Amygdala Volume | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Without WBV** | **β** | **t / CI** | **p** | **β** | **t / CI** | **p** | **β** | **t / CI** | **p** | **β** | **t / CI** | **p** |
| **M - Y** | -0.135 | -1.536 | 0.127 | -0.044 | -0.497 | 0.620 | -0.263 | -2.964 | 0.004 | -0.140 | -1.537 | 0.127 |
| **X = Genetic Profile Scores** | | | | | | | | | | | | |
| **X - M** | 0.282 | 2.602 | 0.011 | 0.282 | 2.602 | 0.011 | 0.282 | 2.602 | 0.011 | 0.282 | 2.602 | 0.011 |
| **X - Y (total)** | -0.038 | -0.381 | 0.704 | -0.010 | -0.102 | 0.919 | 0.031 | 0.301 | 0.764 | -0.014 | -0.136 | 0.892 |
| **X - Y (direct)** | 0.000 | 0.003 | 0.997 | 0.002 | 0.023 | 0.982 | 0.105 | 1.025 | 0.308 | 0.025 | 0.242 | 0.809 |
| **X - Y (indirect)** | -0.038 | [-0.111, -0.001] (93%) 0.07 | | -0.012 | [-0.063, 0.019] (90%) 0.10 | | -0.074 | [-0.190, -0.007] (95%) 0.05 | | -0.039 | [-0.109, 0.000] (90%) 0.10 | |
| **X = Life Events** | | | | | | | | | | | | |
| **X - M** | -0.218 | -2.322 | 0.022 | -0.218 | -2.322 | 0.022 | -0.218 | -2.322 | 0.022 | -0.218 | -2.322 | 0.022 |
| **X - Y (total)** | 0.062 | 0.722 | 0.472 | 0.070 | 0.820 | 0.414 | -0.146 | -1.630 | 0.106 | 0.020 | 0.230 | 0.818 |
| **X - Y (direct)** | 0.033 | 0.373 | 0.710 | 0.061 | 0.689 | 0.492 | -0.203 | -2.297 | 0.024 | -0.010 | -0.111 | 0.912 |
| **X - Y (indirect)** | 0.029 | [0.000, 0.100] (95%) 0.05 | | 0.010 | [-0.015, 0.051] (90%) 0.10 | | 0.058 | [0.003, 0.154] (95%) 0.05 | | 0.030 | [0.000, 0.102] (90%) 0.10 | |

*Results from the PROCESS mediation models are presented using Genetic Profile Scores or Life Events as the independent variable (X), regional brain volumes as the dependent variables (Y), and cortisol as the mediator (M). These models controlled for ethnicity, sex, interactions with sex, and diagnostic status. The values for each model represent the regression coefficient (β), the t and p values for direct and total effects or the lower and upper confidence bounds (CI) and the corresponding confidence interval (≥90%; as marked). Effects significant at a trend-level or greater ($p < 0.1$) are shaded gray.*

**Supplementary Table 2.14: Results of Mediation Analyses Controlling for Whole Brain Volume**

| | Left Hippocampal Volume | | | Right Hippocampal Volume | | | Left Amygdala Volume | | | Right Amygdala Volume | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **With WBV** | **β** | **t / CI** | **p** | **β** | **t / CI** | **p** | **β** | **t / CI** | **p** | **β** | **t / CI** | **p** |
| **M - Y** | -0.041 | -0.490 | 0.625 | 0.079 | 1.027 | 0.307 | -0.162 | -1.958 | 0.053 | -0.018 | -0.228 | 0.820 |
| **X = Genetic Profile Scores** | | | | | | | | | | | | |
| **X - M** | 0.310 | 2.921 | 0.004 | 0.310 | 2.921 | 0.004 | 0.310 | 2.921 | 0.004 | 0.310 | 2.921 | 0.004 |
| **X - Y (total)** | -0.083 | -0.918 | 0.361 | -0.065 | -0.773 | 0.441 | -0.021 | -0.226 | 0.822 | -0.071 | -0.807 | 0.421 |
| **X - Y (direct)** | -0.070 | -0.746 | 0.457 | -0.090 | -1.024 | 0.308 | 0.030 | 0.314 | 0.754 | -0.065 | -0.711 | 0.479 |
| | | [-0.064, | (90%) | | [-0.008, | (90%) | | [-1.131, | (91%) | | [-0.057, | (90%) |
| **X - Y (indirect)** | -0.013 | 0.025] | 0.10 | 0.025 | 0.078] | 0.10 | -0.050 | -0.002] | 0.09 | -0.006 | 0.034] | 0.10 |
| **X = Life Events** | | | | | | | | | | | | |
| **X - M** | -0.217 | -2.371 | 0.020 | -0.217 | -2.371 | 0.020 | -0.217 | -2.371 | 0.020 | -0.217 | -2.371 | 0.020 |
| **X - Y (total)** | 0.060 | 0.773 | 0.441 | 0.068 | 0.932 | 0.354 | -0.148 | -1.872 | 0.064 | 0.018 | 0.238 | 0.812 |
| **X - Y (direct)** | 0.051 | 0.640 | 0.523 | 0.085 | 1.139 | 0.258 | -0.183 | -2.288 | 0.024 | 0.014 | 0.180 | 0.858 |
| | | [-0.017, | (90%) | | [-0.075, | (90%) | | [0.001, | (93%) | | [-0.026, | (90%) |
| **X - Y (indirect)** | 0.009 | 0.052] | 0.10 | -0.017 | 0.003] | 0.10 | 0.035 | 0.114] | 0.07 | 0.004 | 0.042] | 0.10 |

*Results from the PROCESS mediation models are presented using Genetic Profile Scores or Life Events as the independent variable (X), regional brain volumes as the dependent variables (Y), and cortisol as the mediator (M). These models controlled for ethnicity, sex, interactions with sex, diagnostic status, and whole brain volume. The values for each model represent the regression coefficient (β), the t and p values for direct and total effects or the lower and upper confidence bounds (CI) and the corresponding confidence interval (≥90%; as marked). Effects significant at a trend-level or greater (p < 0.1) are shaded gray.*

**Supplementary Table 2.15: Individual SNP Results**

| | | Predicting Cortisol | | | | | | Predicting Left Hippocampal Volume | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All | | Males | | Females | | SNP Effect | | Interaction with Life Events | |
| | | β | t | β | t | β | t | β | t | β | t |
| **CRHR1** | **rs4792887** | 0.293 | 0.794 | -0.35 | -0.639 | **1.005** | **2.059** | -0.179 | -0.559 | **1.061** | **3.197** |
| **CRHR1** | **rs110402** | 0.048 | 0.177 | 0.255 | 0.689 | -0.5 | -1.143 | -0.238 | -0.964 | 0.008 | 0.034 |
| **CRHR1** | **rs242941** | 0.186 | 0.927 | 0.086 | 0.287 | 0.306 | 1.121 | 0.133 | 0.747 | **0.386** | **2.158** |
| **CRHR1** | **rs242939** | 0.367 | 1.600 | 0.187 | 0.591 | **0.786** | **2.212** | 0.009 | 0.045 | **0.381** | **2.103** |
| **CRHR1** | **rs1876828** | -0.494 | -1.062 | -0.113 | -0.193 | -1.631 | -1.789 | 0.293 | 0.611 | 0.235 | 0.403 |
| **NR3C2** | **rs5522** | -0.121 | -0.483 | -0.066 | -0.167 | -0.094 | -0.292 | 0.150 | 0.670 | -0.103 | -0.441 |
| **NR3C1** | **rs41423247** | 0.176 | 0.587 | 0.312 | 0.547 | 0.083 | 0.239 | -0.222 | -0.799 | 0.033 | 0.136 |
| **NR3C1** | **rs10482605** | 0.177 | 0.899 | 0.019 | 0.063 | 0.355 | 1.37 | 0.191 | 1.075 | 0.294 | 1.516 |
| **NR3C1** | **rs10052957** | 0.074 | 0.216 | 0.04 | 0.08 | 0.031 | 0.063 | 0.048 | 0.160 | -0.330 | -1.444 |
| **FKBP5** | **rs1360780** | 0.246 | 1.250 | 0.232 | 0.75 | 0.296 | 1.165 | -0.109 | -0.628 | **0.331** | **2.005** |

*These results are provided to aid in the creation of better informed genetic profile scores in future research.*
*These values represent the regression coefficient and t-statistic for each SNP or interaction term from separate regressions run testing each SNP individually (i.e. these values were* not *extracted from a single regression including all SNPs as predictors). The regression models predicting cortisol each included the SNP of interest and two binary predictors controlling for ethnicity (White or not; African American or not). These models were performed across the whole sample (All) or split by gender (Males; Females). The regression models predicting left hippocampal volume included the same ethnicity predictors, sex, life events, the SNP of interest, and a term for the interaction between the SNP of interest and life events (both mean centered). Thus, the SNP Effect results are the coefficient and corresponding t-statistic for each SNP, at means levels of life events and the Interaction with Life Events values are for the interaction term as a predictor.*
*Bolded values are significant at p<0.05, but note that all individual SNP results should be interpreted with caution due to the sample size and number of multiple comparisons presented here and again, these results are provided to aid in the creation of better informed genetic profile scores in the future.*

**Supplementary Table 2.16: References for Main Text Table 2.1**


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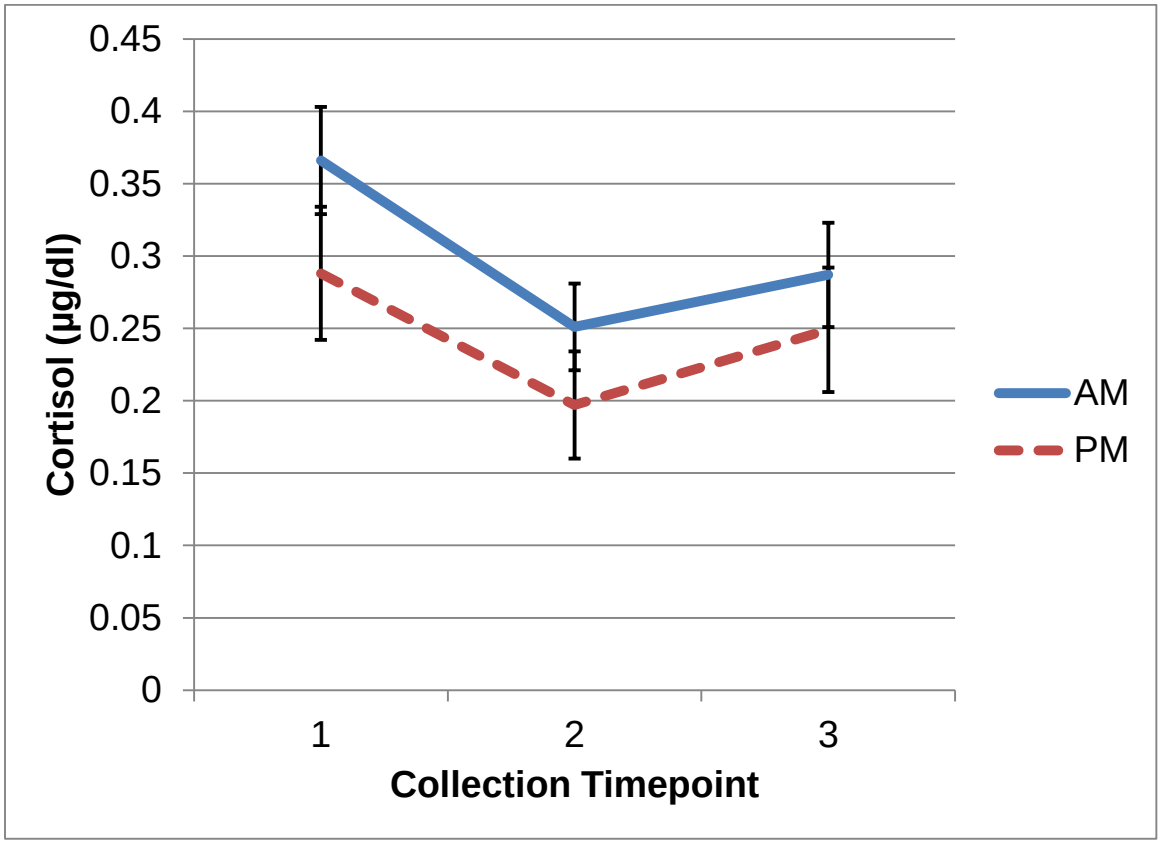
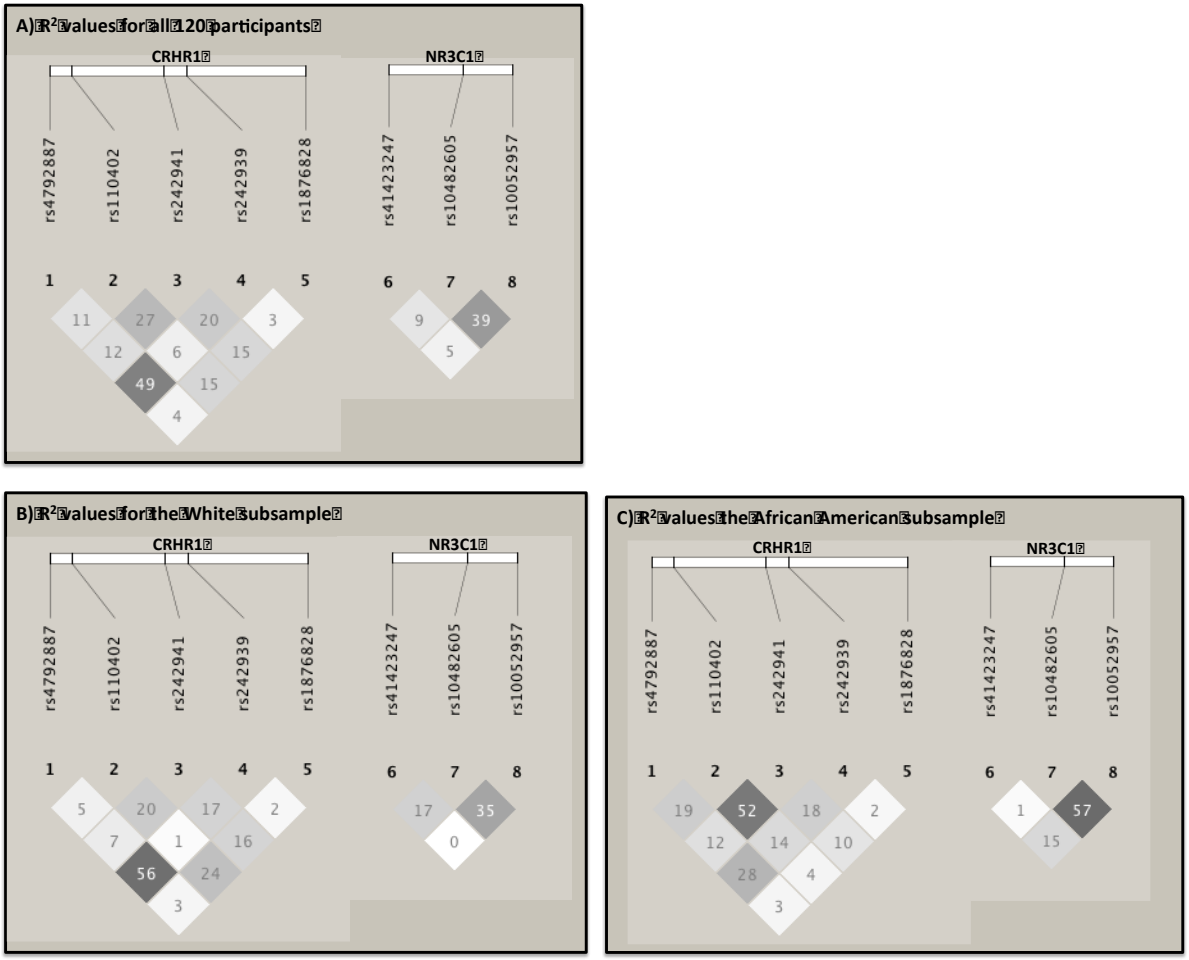
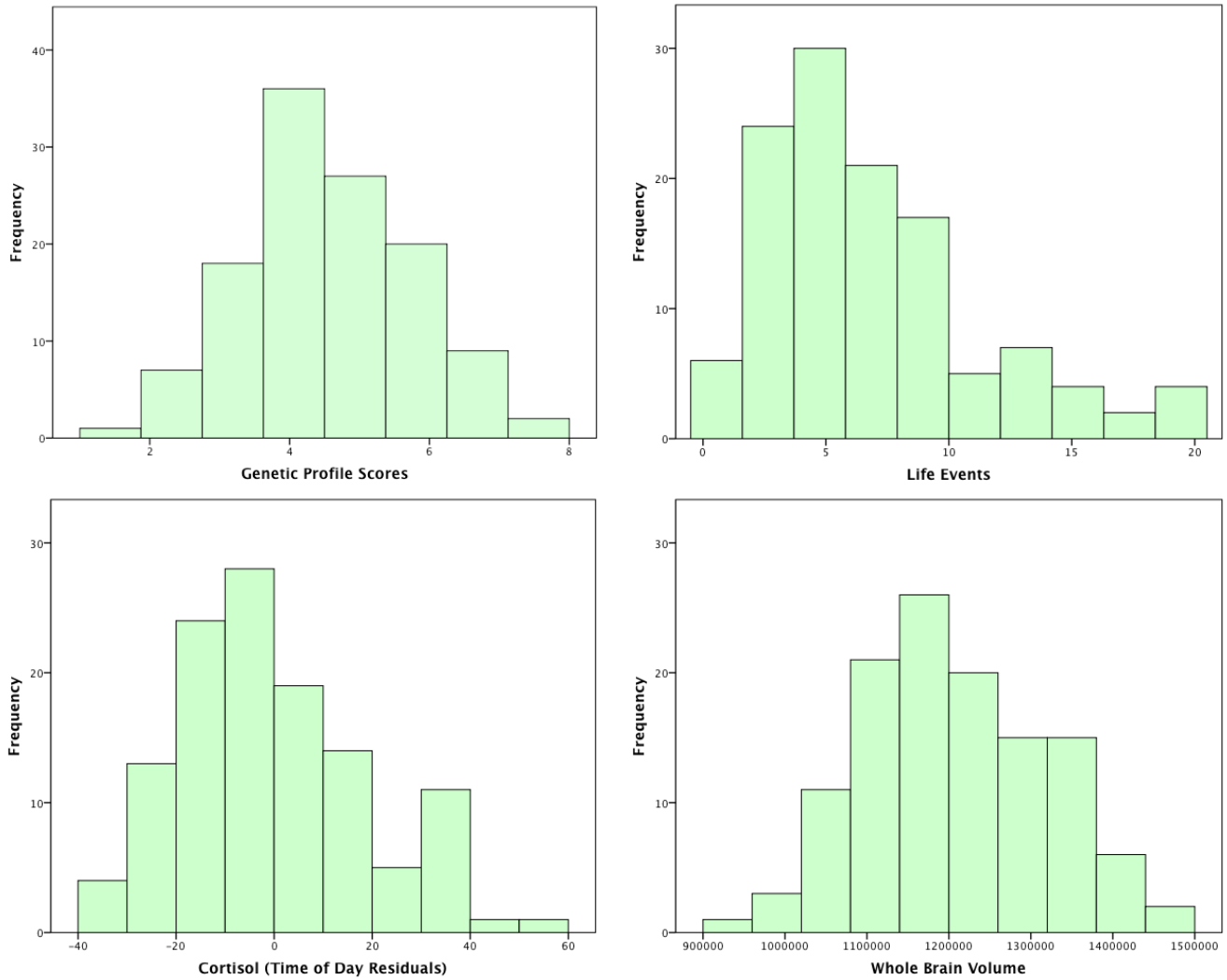



**Supplementary Figure 2.1: Mean Values of Cortisol at Each Timepoint for Morning and Afternoon Collections**
The values represent the mean saliva cortisol levels (µg/dl) at each of three collection timepoints during the baseline session split for the subsample of children assessed in the morning (AM) and in the afternoon (PM). Error bars represent 95% confidence intervals.

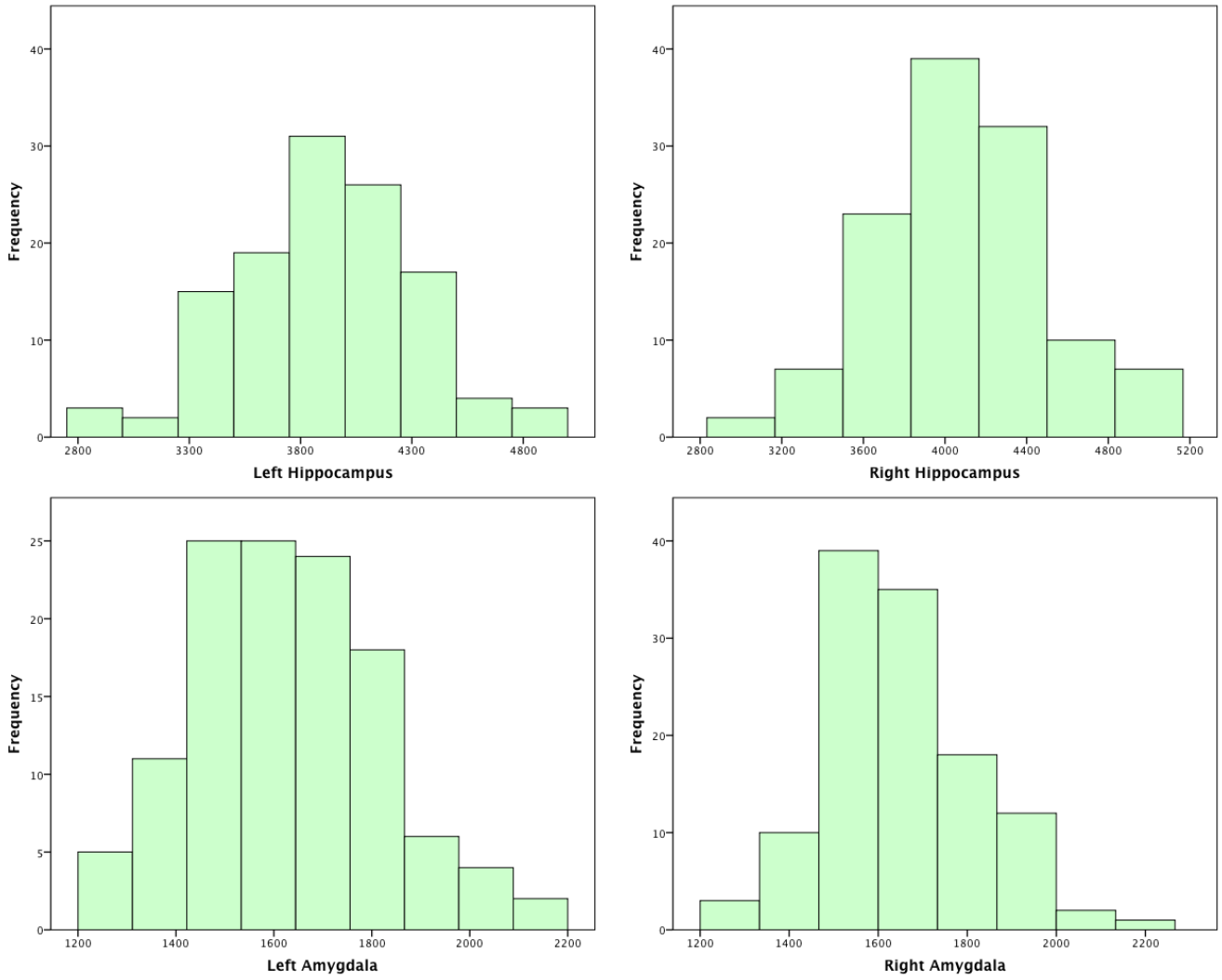



**Supplementary Figure 2.2: Linkage Disequilibrium Plots**

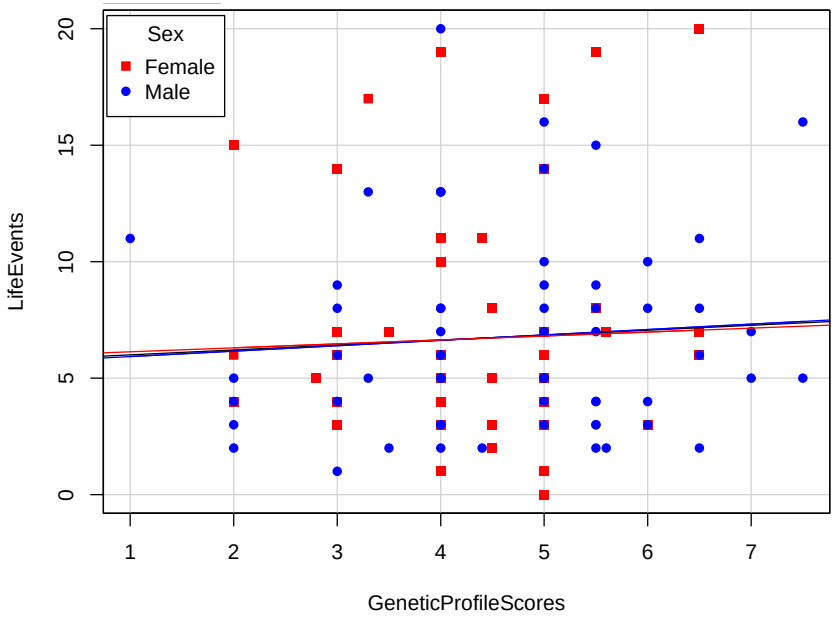
The plots were created using Haploview (Barrett et al, 2005) and display the pair-wise r2 values within the CRHR1 and NR3C1 SNPs for the A) whole sample used in the main analyses, B) the White subsample, and C) the African American subsample.

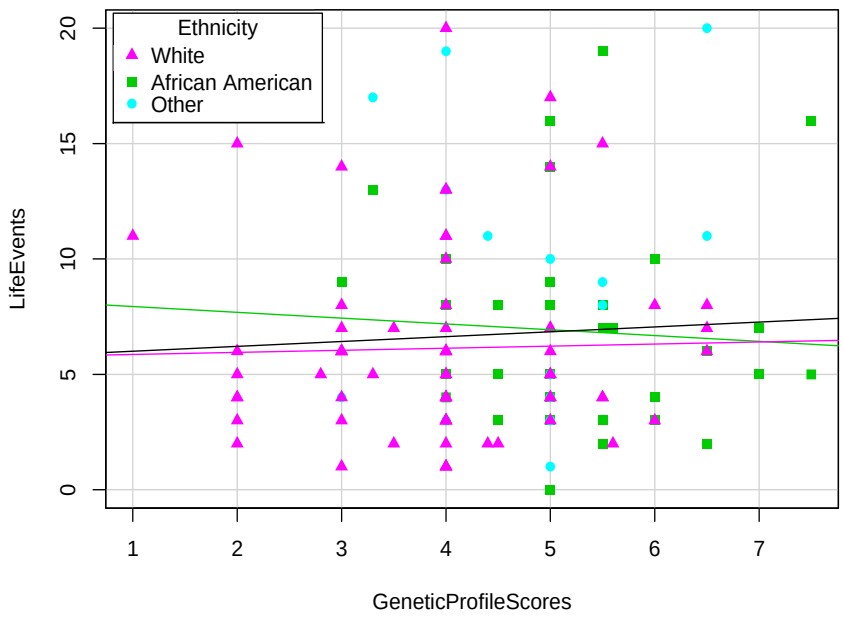



**Supplementary Figure 2.3: Histogram Plots**




**Supplementary Figure 2.3: Histogram Plots - continued**

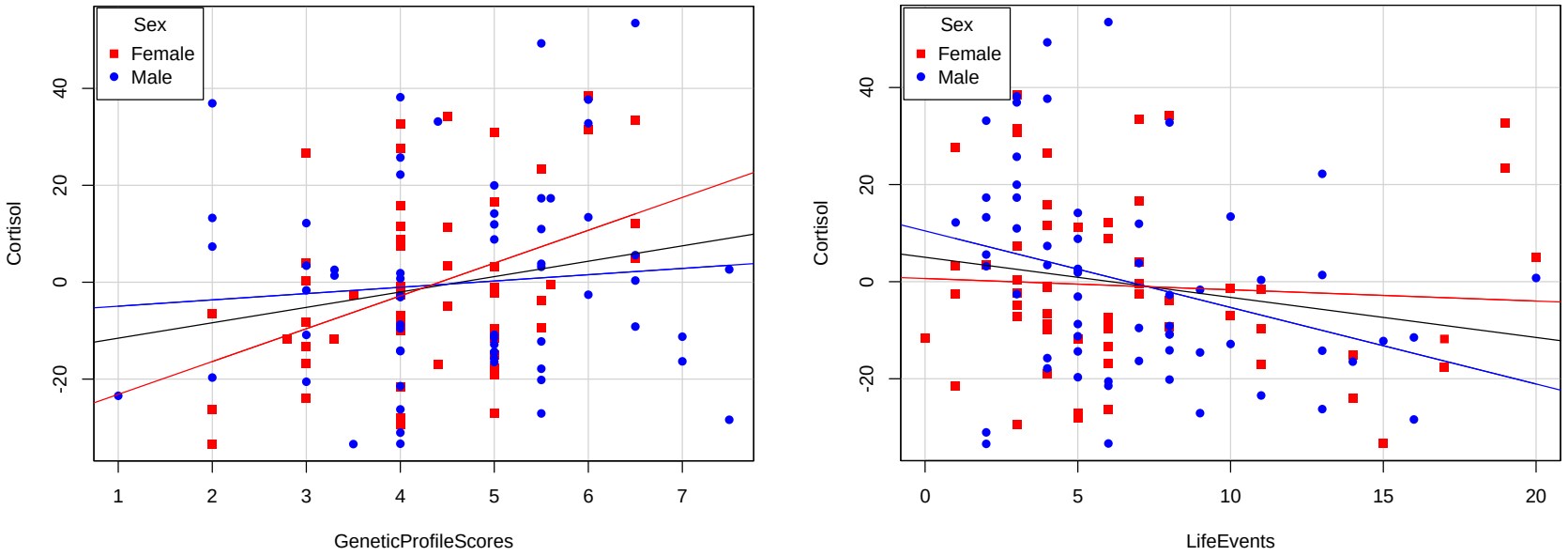


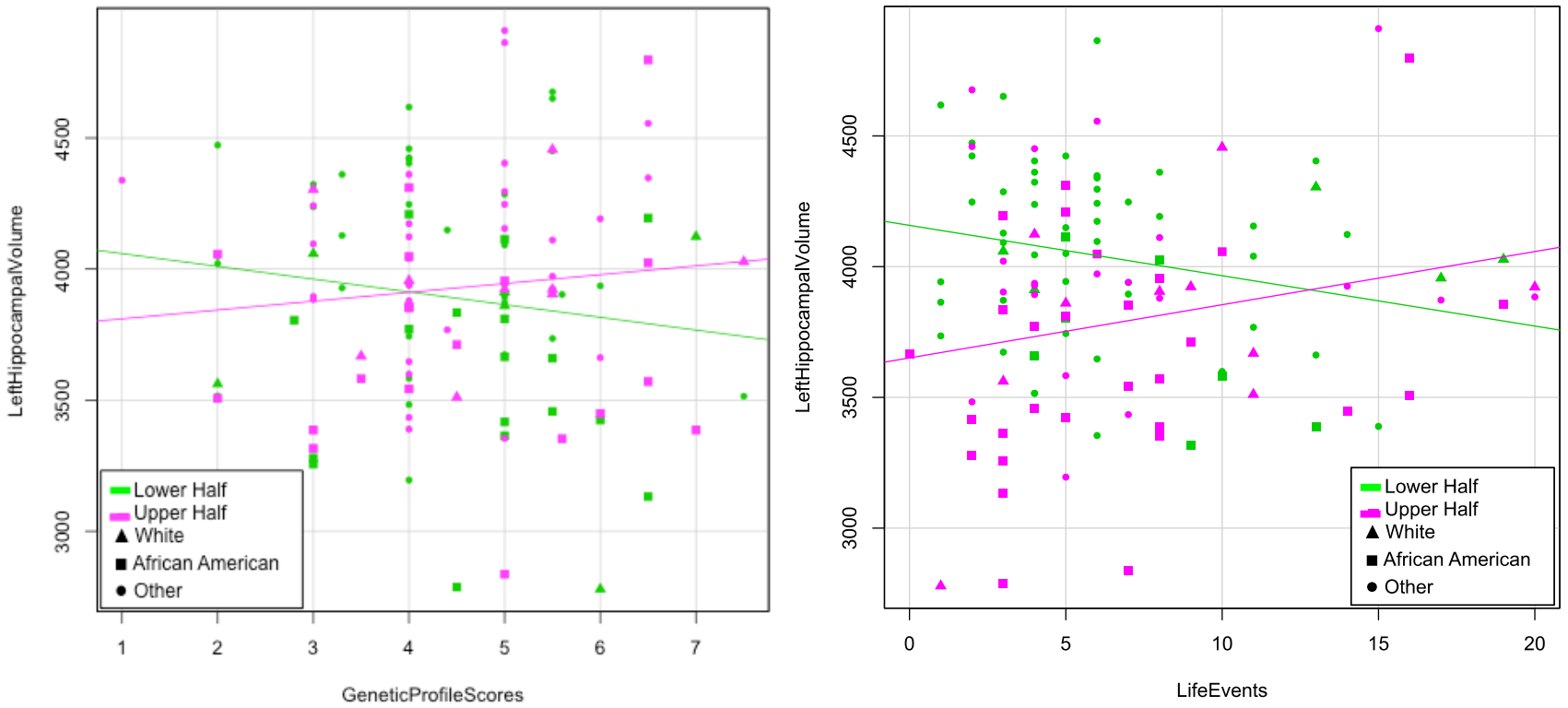

Whole Sample: (r(118)=0.059, p=0.519)
Male Subsample (r(60)=0.079, p=0.543)
Female Subsample (r(56)=0.037, p=0.781)

White Subsample (r(67)=0.026, p=0.832)
African American subsamples (r(34)= -0.062, p=0.718)

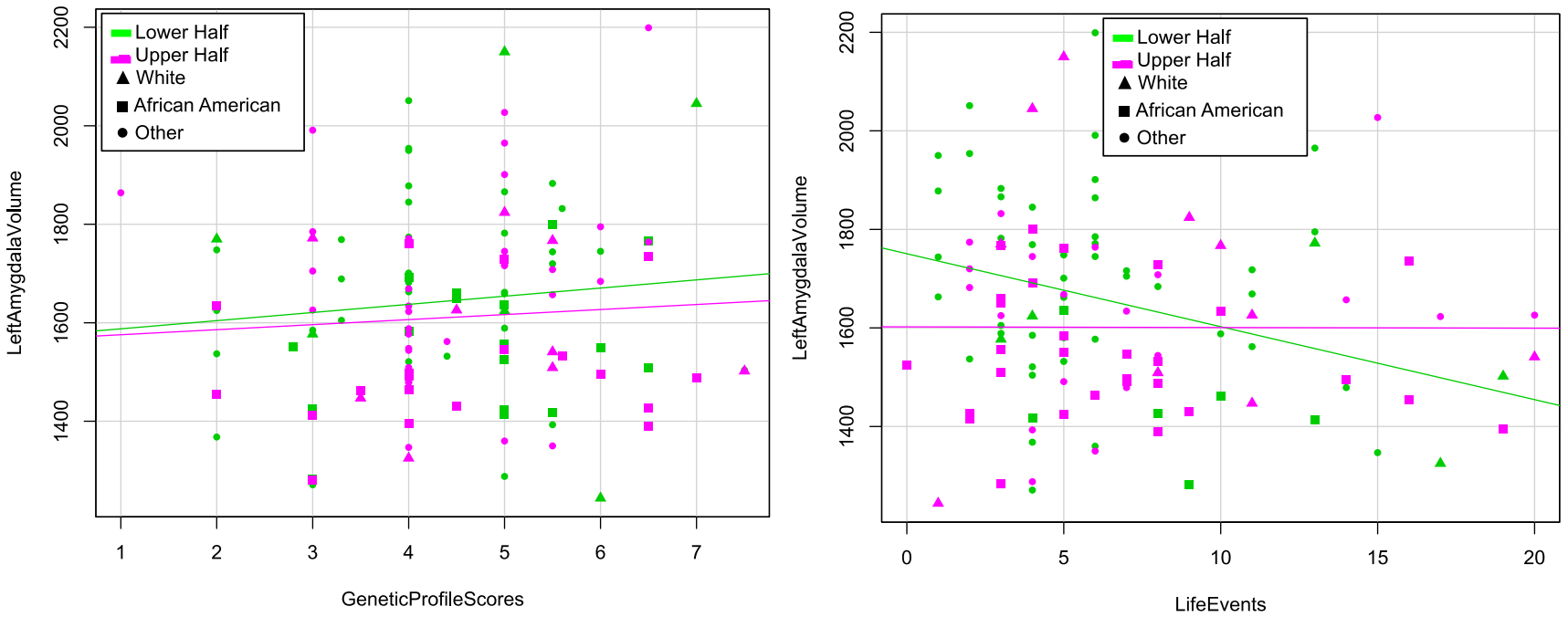
**Supplementary Figure 2.4: Genetic Profile Scores – Life Events Correlation**
The graphs display the raw Genetic Profile Scores plotted against the raw number of Life Events reported split by sex (left) or by ethnicity (right) with trend lines plotted for each subgroup. Correlation statistics are presented below each graph.




**Supplementary Figure 2.5: Genetic Profile Scores and Life Events Predicting Cortisol by Gender, Raw Data**
The graphs display the raw Genetic Profile Scores (left) or Life Events (right) variables plotted against AUC cortisol split by sex with trend lines plotted for each sex and for the whole sample (black line).




**Supplementary Figure 2.6: Genetic Profile Scores x Life Events Predicting Left Hippocampal Volume, Median Split, Raw Data**
The graphs display the raw Genetic Profile Scores (left) predicting raw Left Hippocampal Volume with trend lines displaying the relationship in the upper and lower halves of Life Events values by median split. The right side graph display the raw Life Events variable predicting raw Left Hippocampal Volume with trend lines displaying the relationship in the upper and lower halves of Genetic Profile Score values by median split. Shapes represent participant ethnicity.




**Supplementary Figure 2.7: Genetic Profile Scores x Life Events Predicting Left Amygdala Volume, Median Split, Raw Data**
The graphs display the raw Genetic Profile Scores (left) predicting raw Left Amygdala Volume with trend lines displaying the relationship in the upper and lower halves of Life Events values by median split. The right side graph display the raw Life Events variable predicting raw Left Amygdala Volume with trend lines displaying the relationship in the upper and lower halves of Genetic Profile Score values by median split. Shapes represent participant ethnicity.

# Chapter 3: Stress System Genetic Variation, Pubertal Development, and Sex Interact to Predict Amygdala and Hippocampus Responses to Negative Emotional Faces in School-Age Children

Reference: Pagliaccio, D., Luby, J. L., Bogdan, R., Agrawal, A., Gaffrey, M. S., Belden, A. C., Botteron, K. N., Harms, M. P., and Barch, D. M. (2015). Stress System Genetic Variation, Pubertal Development, and Sex Interact to Predict Amygdala and Hippocampus Responses to Negative Emotional Faces in School-Age Children. Neuroimage. 109(0), 1–11. PMCID: PMC4340765

# 3.1 Abstract

Accumulating evidence suggests a role for stress exposure, particularly during early life, and for variation in genes involved in stress response pathways in neural responsivity to emotional stimuli. Understanding how individual differences in these factors predict differences in emotional responsivity may be important for understanding both normative emotional development and for understanding the mechanisms underlying internalizing disorders, like anxiety and depression, that have often been related to increased amygdala and hippocampus responses to negatively valenced emotional stimuli. The present study examined whether stress exposure and genetic profile scores (10 single nucleotide polymorphisms within four hypothalamic–pituitary–adrenal axis genes: CRHR1, NR3C2, NR3C1, and FKBP5) predict individual differences in amygdala and hippocampus responses to fearful vs. neutral faces in school-age children (7–12 year olds; N = 107). Experience of more stressful and traumatic life events predicted greater left amygdala responses to negative emotional stimuli. Genetic profile scores interacted with sex and pubertal status to predict amygdala and hippocampus responses. Specifically, genetic profile scores were a stronger predictor of amygdala and hippocampus responses among pubertal vs. prepubertal children where they positively predicted responses to fearful faces among pubertal girls and positively predicted responses to neutral faces among pubertal boys. The current results suggest that genetic and environmental stress-related factors may be important in normative individual differences in responsivity to negative emotional stimuli, a potential mechanism underlying internalizing disorders. Further, sex and pubertal development may be key moderators of the effects of stress-system genetic

variation on amygdala and hippocampus responsivity, potentially relating to sex differences in stress-related psychopathology.

## 3.2 Introduction

Stress exposure has been shown to predict elevated threat-related amygdala reactivity across development (Bogdan et al., 2012, Ganzel et al., 2013, Grant et al., 2011 and Tottenham et al., 2011). Further, it has been suggested that the timing of stress along the developmental trajectory can greatly alter its influence (Tottenham and Sheridan, 2009), where early life stress can have long lasting and potentially irreversible effects on amygdala function and development (Cohen et al., 2013). Moreover, effects of life stress on amygdala reactivity may be moderated by stress-related genetic variants (e.g., Bogdan et al., 2012 and White et al., 2012). Importantly, heightened amygdala and hippocampus response to threat-related stimuli has also been observed in children, adolescents, and adults with depression (e.g. Barch et al., 2012, Beesdo et al., 2009, Bishop et al., 2004, Etkin et al., 2004, Ewbank et al., 2009, Gaffrey et al., 2011, Thomas et al., 2001b and Yang et al., 2010). Amygdala hyper-responsivity is similarly present in unaffected children at risk for depression (based on parental history of depression; Monk et al., 2008), suggesting that these differences may precede the development of psychopathology and that genetic risk and/or early environmental factors may play a key role. Given this and the prominent relationships between stress and depression (e.g. Green et al., 2010 and Kessler and Magee, 2009), understanding the relationship between individual differences in stress-related factors and differences in neural responsivity to emotional stimuli can be highly

informative both of normative emotional development and of the mechanisms underlying alteration in disorders.

We have shown previously that a profile score across ten single nucleotide polymorphism (SNPs) on four genes (CRHR1, NR3C2, NR3C1, FKBP5) integrally involved in the hypothalamic–pituitary–adrenal (HPA) axis and the experience of early life stress are related to cortisol reactivity and amygdala and hippocampus structure in school-age children (Pagliaccio et al., 2013a). SNPs on these genes of interest have been previously related to increased depression prevalence (e.g. CRHR1: Liu et al., 2006; NR3C2: Kuningas et al., 2007; NR3C1: van West et al., 2005; FKBP5: Lavebratt et al., 2010) and altered cortisol reactivity (e.g. CRHR1: Tyrka et al., 2009; NR3C2: DeRijk et al., 2006; NR3C1: Ising et al., 2008; FKBP5: Menke et al., 2013), as well as other related phenotypes, like suicidality (e.g. CRHR1: Wasserman et al., 2007; FKBP5: Roy et al., 2010) or antidepressant treatment response (e.g. CRHR1: Licinio et al., 2004; FKBP5: Binder et al., 2004). Further details and background are provided in Pagliaccio et al., 2013a. However, effects of sex and pubertal development may be key to understanding the influence of stress on amygdala and hippocampus function in children. For example, sex may moderate cortisol reactivity to acute stressors (e.g. Kirschbaum et al., 1995 and Kirschbaum et al., 1992) and, as shown in animal studies, also moderates the effects of environmental stress and stress-system genes on the HPA axis and the limbic system (e.g. Bourke et al., 2013, Shors et al., 2001 and Zohar and Weinstock, 2011). In addition, stress reactivity has a particularly strong effect on emotion processing during puberty (e.g. Natsuaki et al., 2009) and the brain is particularly sensitive to the effects of environmental stressors during this period (e.g. Holder and Blaustein, 2013). Of note, females tend to begin puberty earlier than males (Carskadon and

Acebo, 1993) and sex differences in depression prevalence emerge during this transitional time (e.g. Angold et al., 1998 and Angold and Worthman, 1993).

The goal of the current study was to assess whether stress exposure and HPA axis genetic variation predict amygdala and hippocampus responses to negative emotional stimuli in school-age children. We hypothesized that having more HPA axis genetic 'risk' variants, indexed by higher genetic profile scores, and the experience of greater numbers of life stressors, and/or their interaction would predict greater fearful–neutral face activity in the amygdala and hippocampus. In a follow-up analysis, we tested how sex and pubertal status moderated effects of genetic factors, given their moderating roles in stress function and risk for depression. Overall, these analyses aimed to elucidate our understanding of how genetic variation and stress exposure influence individual differences in amygdala and hippocampal responsitivity to emotional stimuli in school-age children. Findings may guide future exploration of whether and how these factors underlie risk for internalizing psychopathology.

# 3.3 Materials and Methods

**3.3.1 Participants**: A subsample of participants (N = 168) enrolled in the prospective longitudinal Preschool Depression Study (PDS; total N = 306) completed neuroimaging sessions. The PDS is being conducted by the Washington University in St. Louis School of Medicine Early Emotional Development Program (WUSM EEDP) and its broad goal is to explore clinical and neural outcomes relating to preschool-onset depression. The details of the study have been published previously (see, Luby et al., 2009). Briefly, 3- to 5-year old children and their primary caregivers were recruited from the St. Louis metropolitan area. Children and caregivers each completed in-

depth clinical interviews annually and children participated in a neuroimaging session at 7–12 years of age. Parental written consent and child assent were obtained prior to study participation and the Institutional Review Board at Washington University approved all experimental procedures.

Of the 134 children who completed the Facial Emotional Processing Task, 12 were excluded due to excessive head motion (see fMRI pre-processing section below). An additional 15 participants who identified as ethnicities other than White or African American were removed from the current analysis to reduce population stratification. (However, the main results are highly consistent when retaining these children in the analysis). This left a final sample of 107 children for the current analyses.

**3.3.2 Diagnostic Assessments**: Trained WUSM EEDP staff conducted up to seven in-person assessments (current subsample M = 4.84, SD = 1.02 assessments) with participants and their parents/guardians from study enrollment through the time of scan. Before children were age 8, a reliable and age-appropriate semi-structured parent-report diagnostic interview was used to assess psychiatric symptoms, the Preschool-Age Psychiatric Assessment (PAPA; Egger et al., 2003). After age 8, the Childhood and Adolescent Psychiatric Assessment (CAPA; Angold and Costello, 2000) was used, which also includes child-report. Interviews were audiotaped, reviewed for reliability, and calibrated for accuracy (Luby et al., 2009). Data from the PAPA/CAPA were used to assess the child's experience of stressful and traumatic life events from birth through the scan session (a full list of events and their frequencies is reported in Supplementary Table 3.1). The life events factor used in the main analyses represents the sum number of instances of both stressful and traumatic events experienced through the time of scan. We had no a priori method for weighting individual events and counts of stressful versus traumatic events were highly correlated ($r(105) = .436$, $p < 0.001$). Thus, all events were summed equally for the primary results, though an analysis separating stressful and traumatic events is presented in the supplement. PAPA/CAPA data

was also used to assess whether children met criteria for relevant psychiatric disorders (Supplementary Table 3.2 details the number of children meeting criteria for depressive, anxiety, and/or externalizing disorders up to and at the time of scan). Pubertal status at the time of scan was assessed using child self-report on the Tanner Pubertal Staging Questionnaire (Tanner, 1955). As about half of the children were prepubertal (Stage 1 N = 55), children in the remaining stages were combined into a "pubertal" group (N = 51: Stage 2 N = 19, Stage 3 N = 25, Stage 4 N = 7, Stage 5 N = 0).

**3.3.3 Genetic Profile Scores (GPS)**: Extensive details on the rationale, methods, and limitations of our HPA axis genetic profile score (GPS) creation in this sample have been published previously (Pagliaccio et al., 2013a). Briefly, previous work has documented the utility of additively combining genetic variants to study their polygenic effects on brain structure and function, where single polymorphisms alone may not be significantly predictive (Nikolova et al, 2011). We created an additive genetic profile score from 10 SNPs within 4 integral HPA axis genes; higher scores indicate more alleles previously associated with increased cortisol, depression prevalence/severity, and/or related phenotypes (e.g. antidepressant treatment response, suicidality, etc.). These 10 SNPs were narrowed down from a larger set of 15 to reduce linkage disequilibrium (all pairwise r2 < 0.49). Unweighted sum scores were created from the 10 SNPs of interest. Indicative of their construct validity, higher GPS predict elevated cortisol reactivity to a stressor in PDS participants (Pagliaccio et al., 2013a). The variants of interest included SNPs from CRHR1 (rs4792887, rs110402, rs242941, rs242939, rs1876828), NR3C2 (rs5522), NR3C1 (rs41423247, rs10482605, rs10052957), and FKBP5 (rs1360780). For

more background on each SNP and linkage disequilibrium plots, see Pagliaccio et al. (2013a).

**3.3.4 Facial Emotion Processing Task**: Participants completed a neuroimaging battery including high-resolution structural, resting state, and functional task scans. Only data from the Facial Emotion Processing Task was used for the current analysis. Directly following a sad mood induction and elaboration as described below (Furman et al., 2011), children completed a facial emotion processing task during which they were shown a series of 90 neutral and emotional faces (45 stimuli during each of 2 task runs) and were asked to judge the gender of the face, responding via a fiber optic button box to indicate whether the face was male or female. This task was chosen as previous research has indicated that those with or at-risk for depression show more robust amygdala activity than healthy controls in response to viewing emotional faces when attention was not constrained to the emotional content of the images (Fales et al., 2008 and Monk et al., 2008). This task was also preferable to a passive viewing task as the active gender judgment helps to ensure engagement with the visual stimuli.

Face stimuli were drawn from the MacArthur Network Face Stimuli Set, a validated stimulus set containing images of 43 different actors from different ethnic backgrounds (Tottenham et al., 2009). Children saw faces with neutral, sad, angry, happy, and fearful expressions, equally distributed across task runs, from 10 of the individuals in this stimulus set. Each stimulus was presented for 2250 ms, followed by an inter-trial interval of 250 ms, 2750 ms, or 5250 ms (each occurring at equal frequency); each task run lasted 247.5 s.

One original goal of the PDS was to probe potential emotional biases relating to preschool-onset depression apparent with varying intensity of emotional facial

expressions. To this end, children viewed both full- and half-intensity emotional faces. However, as we did not have specific hypotheses about emotional face intensity in the current analysis, we collapsed across the full- and half-intensity faces for each emotion type to increase our power.

Prior to the Facial Emotion Processing task, children underwent a mood induction and elaboration paradigm. The methods and results of this prior task have been discussed previously (Pagliaccio et al., 2011). Briefly, children watched a short clip from the film, My Girl, intended to induce sad mood followed by a series of verbal prompts to have the children mentally elaborate on the induced mood. fMRI scanning was performed during the elaboration period. After the elaboration, children began the Facial Emotion Processing task. Given that the original goals of the PDS included exploring the effects of a history of preschool-onset major depression on the brain, the mood induction was of interest because previous work has shown that negative mood induction can reactivate affective processing biases (Scher et al., 2005) and amygdala responses to emotional stimuli (Ramel et al., 2007) specifically in patients with a history of depression. Of note however, there were no correlations between induction-related activity during the elaboration period and fearful–neutral face activity in the Facial Emotion Processing task in our regions of interest (all ps > 0.18). Induction success did not differ as a function of sex, ethnicity, GPS, or life events (all ps > 0.26). Furthermore, the main results described below held when controlling for diagnostic status, mood ratings following mood induction, and induction-related activity during elaboration (data not shown). Further, GPS did not significantly differ based on the presence of depressive, anxious, or externalizing disorders nor did they predict mood ratings (ps > 0.10).

**3.3.5 MRI Acquisition**: Structural and functional imaging data were collected using a 3.0 Tesla TIM TRIO Siemens whole body scanner at Washington University in St. Louis. T1-weighted structural images were acquired in the sagittal plane using an MPRAGE 3D sequence (TR = 2400 ms, TE = 3.16 ms, flip angle = 8°, slab = 176 mm, 176 slices, matrix size = 256 × 256, field of view (FOV) = 256 mm, voxel size = 1 × 1 × 1 mm; interslice skip = 0). Functional images were collected during the face processing task with a 12-channel head coil using a T2*-weighted gradient-echo echo-planar sequence in the axial plane (TR = 2500 ms, TE = 27 ms, flip angle = 90°, FOV = 256 mm, voxel size = 4 × 4 × 4 mm, interleaved slice acquisition, transverse axial alignment). T2-weighted images were collected for registration purposes using a 3D SPACE acquisition (TR = 3200 ms, TE = 497 ms, 160 slices, FOV = 256, voxel size = 1 × 1 × 1 mm).

**3.3.5 fMRI Pre-Processing**: Imaging data were preprocessed using the following steps: (1) correction for slice-dependent time shifts; (2) removal of first 4 images of each run to allow BOLD signal to reach steady state; (3) elimination of odd/even slice intensity differences due to interpolated acquisition; (4) realignment of data acquired from each participant within and across runs to compensate for rigid body motion (Ojemann et al., 1997); (5) image intensity normalization to a whole-brain mode value of 1000; (6) registration of the 3D structural volume (T1) to an atlas template (WU “711-2B”) in the Talairach coordinate system (Talairach and Tournoux, 1988) using a 12-parameter affine transform and re-sampling to 1 mm cubic representation (Buckner et al., 2004 and Ojemann et al., 1997); (7) co-registration of the 3D fMRI volume to the T2, and the T2 to the participant's structural image; (8) transformation of the fMRI data to 3 × 3 × 3 mm voxel

atlas space using a single affine 12-parameter transform; and (9) spatial smoothing using a 6 mm full-width half-maximum Gaussian filter.

Stringent data quality criteria were used for data inclusion in the current analyses. The signal-to-noise ratio (SNR: mean signal/standard deviation across each BOLD run, computed for each slice and then averaged across all slices) for each of the two task runs was calculated using in-house software following preprocessing. Only task runs with an SNR above 200 were included in the current analyses (mean SNR for included first runs: 536.778 ± 192.336, minimum = 202; mean SNR for included second runs: 493.634 ± 188.070, minimum = 216).

Additionally, we applied previously validated corrections for head motion, termed "motion scrubbing" (Siegel et al., 2013). The motion scrubbing procedure assesses frame-wise displacement based on the movement parameters used in pre-processing step 4. For any given frame (i.e. timepoint), this represents the differential head motion from the previous frame summing across linear (x,y,z) and rotational displacements (yaw, pitch, roll, where degrees of rotation are converted to millimeters of movement by calculating displacement on the surface of a sphere with a radius of 50 mm). A temporal mask removed any frame with a sum displacement greater than 0.9 mm from analysis. If > 40% of a participant's total number of frames or fearful or neutral face trial frames were censored due to motion, that participant was excluded from analysis (n = 12). Therefore, frames with high motion were censored allowing us to retain participants who otherwise would contribute poor quality data, while participants with excessive data loss due to motion/numbers of frames censored were excluded. Details on the validity and efficacy of this procedure for the Facial Emotion Processing Task data in a subsample of

psychiatrically healthy children from the PDS have been published previously (Pagliaccio et al., 2013b).

**3.3.6 fMRI Analysis**: Analysis of fMRI data was performed using in-house software (FIDL analysis package, http://www.nil.wustl.edu/labs/fidl/index.html; Ollinger et al., 2001). A voxel-wise general linear model (GLM) approach was used, which incorporated regressors for linear trend and baseline shifts. Only those trials on which the participant made a correct gender judgment were included in the analysis, though there were very few incorrect trials (mean error rate ~ 4%). We assumed a canonical SPM hemodynamic response for this analysis, which results in beta estimates of brain responses to each of the five face types (neutral, sad, happy, fear, angry). The primary contrast of interest in these analyses was response magnitude to fearful–neutral faces to specifically assess amygdala and hippocampus responses to fear/threat-related stimuli (i.e. fearful faces) by subtracting neutral faces, which are expected to not carry threat-related social signals (though they may in some contexts or for some people) but rather control for general activity to face stimuli. For follow-up analyses, we examined responses to sad–neutral faces to assess whether our predictors of interest related more generally to negative emotional stimuli.

We used FreeSurfer v5.1 (Fischl et al., 2002 and Fischl et al., 2004) to create anatomical region of interest (ROI) masks by segmenting each participant's T1 anatomical image and extracting bilateral amygdala and hippocampal segmentations. Each participant's ROIs were down-sampled to match the functional resolution of the atlas space (3 × 3 × 3 mm) and registered to the common atlas space. We extracted beta estimates of responses to each face type from each participant's four individually defined anatomical

ROIs (left and right amygdala and hippocampus) for subsequent data analysis. Supplementary Figure 3.1 shows a heat map of the overlap of individual subject ROIs in atlas space for illustration purposes.

**3.3.7 Statistical Analysis**: Outliers (more than three times the interquartile range away from the 25th or 75th percentile) were Winsorized before subsequent data analysis (1–3 outliers identified for life events and brain activity variables; main analyses remain when excluding these outliers instead). We used hierarchical linear regressions in IBM SPSS Statistics v20 (Armonk, NY: IBM Corp.) to explore the effects of interest predicting left and right amygdala and hippocampus activity (magnitude estimates). The first step in each regression included ethnicity (White vs. African American) and sex as predictors. Next, genetic profile scores and stressful/traumatic life events were added as predictors. Consistent with recent recommendations (Keller, 2013), interactions between the predictors of interest (i.e., GPS and stressful life events) and the covariates (i.e. ethnicity and sex) were added in the next step to better control for potential confounds. Finally, an interaction between genetic profile scores and life events was added. False discovery rate (FDR; Benjamini and Hochberg, 1995) correction was used to control for multiple comparisons for the 12 hypothesized tests (i.e., main effects of GPS and life events as well as their interaction across four brain regions) setting a maximum acceptable FDR of 0.05.

As noted in the results, we also pursued a follow-up analysis to explore a GPS × sex interaction that emerged in the above analyses. Specifically, we tested the hypothesis that pubertal status might further moderate this effect. To do this, we ran four regression models as above. The first step included ethnicity and sex. GPS were added in the second

step, followed by interactions between GPS and the demographic factors. Life events were not of interest for these analyses given that they did not interact with sex or GPS in the initial models. Pubertal status was added in the fourth step, followed by interactions between GPS and pubertal status and between pubertal status and demographic factors. Next, the three-way interaction of interest, GPS × pubertal status × sex, was added. Finally, we controlled for all other three-way interactions (GPS × pubertal status × ethnicity, GPS × sex × ethnicity, pubertal status × sex × ethnicity). FDR correction was used to control for multiple comparisons for the GPS × sex × pubertal status effect tested in all four regions. We used the moderated moderation model from the PROCESS tool for SPSS (Hayes, 2013) to parse significant 3-way interaction effects by isolating simple slopes.

Power calculations were performed using G*Power 3 (Faul et al., 2009 and Faul et al., 2007).

# 3.4 Results

### 3.4.1 Control Analyses/Demographic and Clinical factors: Table 3.1

shows the means and standard deviations or counts of demographic and brain activity variables. We tested for any potentially confounding effects of demographic factors on our variables of interest. As noted in Supplemental Table 3.3, there were no significant differences in the variables of interest by sex or pubertal status (all ps > 0.05). There were significant ethnic differences where African American children had significantly higher genetic profile scores and stressful/traumatic life events experience. Additionally, it is important to note that there was no significant correlation between genetic profile scores

and life events ($r(105) = 0.006$, $p = 0.952$). Finally, the percent of frames cut/retained from motion scrubbing did not correlate with activity in any of the four regions of interest (all ps > 0.38).

**Table 3.1: Demographic and Brain Activity Variables**

| | Mean | Std | Minimum | Maximum |
|---|---|---|---|---|
| **Genetic Profile Scores** | 4.466 | 1.312 | 1.0 | 7.5 |
| **Stressful and Traumatic Life Events** | 17.776 | 10.418 | 4 | 50 |
| **Age at Scan (months)** | 123.930 | 15.186 | 83 | 153 |
| **Fearful-Neutral Face Activity** | | | | |
| **Left Amygdala** | 0.029 | 0.227 | -0.620 | 0.570 |
| **Right Amygdala** | 0.034 | 0.260 | -0.820 | 0.710 |
| **Left Hippocampus** | 0.020 | 0.156 | -0.330 | 0.450 |
| **Right Hippocampus** | 0.001 | 0.157 | -0.440 | 0.370 |
| | **Counts for Each Subgroup** | | | |
| **Sex** | Female = 54, Male =53 | | | |
| **Ethnicity** | White = 67, African American = 40 | | | |
| **Pubertal Status** | Prepubertal = 55, Pubertal = 52 | | | |

*Demographic and Brain Activity Variables: Mean, standard deviation (std), minimum, and maximum values are presented for predictors of interest and age at scan as well as for outcomes of interest (Fearful-Neutral face magnitude estimates in the left and right amygdala and hippocampus. Counts of participants by sex, ethnicity and pubertal status are also presented.*

### 3.4.2 Regression Results Predicting Fearful–Neutral Face Activity:

Supplementary Tables 3.4–3.7 present all steps of the regression models predicting fearful–neutral face activity in the left and right amygdala and hippocampus. These results indicated that the number of stressful/traumatic life events experienced by the time of scan positively predicted fearful–neutral face activity in the left amygdala ($b = 0.008$, $\beta = 0.368$, $t = 3.800$, $p < 0.001$; FDR corrected $p = 0.003$; see Figure 3.1), but did not significantly predict activity in the right amygdala or left or right hippocampus (all ps and FDR corrected ps > 0.10). This effect of life events remained significant in a follow-up regression step controlling for age at scan (months) and age × sex and age × ethnicity interactions (life events: $b = 0.009$, $\beta = 0.406$, $t = 4.035$, $p < 0.001$). Further follow-up analysis examining the influence of stressful life events and traumatic life events as separate predictors is presented in Supplementary Table 3.8. Additional follow-up analyses examine the potential role of family income as a proxy of socio-economic status (Supplementary Table 3.9). Briefly, higher family income correlates with lower experience of stressful life events, but income does not predict left amygdala activity whereas life events continue to predict activity even controlling for income.

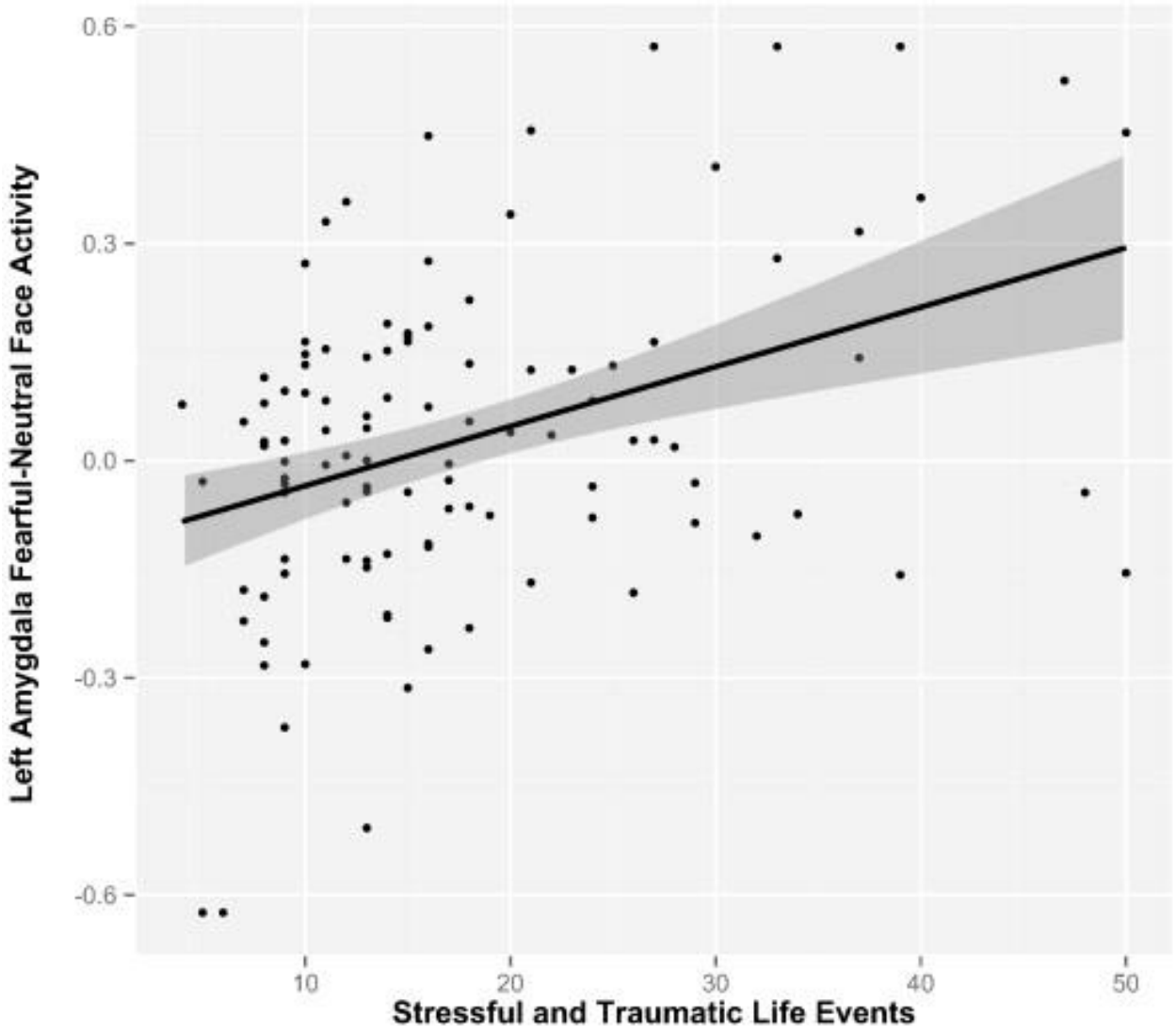



**Figure 3.1: Effect of life events experience on left amygdala activity.**
This figure displays the relationship between sum counts of stressful and traumatic life events experienced by the time of scan and fearful–neutral face activity in the left amygdala (difference in magnitude estimates for fearful face vs. neutral face contrasts). The shaded region represents the 95% confidence interval around the fit line.

In the main regression models, genetic profile scores did not significantly predict activity in any of the four regions (all ps and FDR corrected ps > 0.08). Across the four regions, the GPS × life events interaction only predicted left hippocampal activity (b = − 0.002, β = − 0.206, t = − 2.116, p = 0.037, FDR corrected p = 0.210), but this effect did not pass FDR correction for multiple comparisons. Finally, though not hypothesized, a GPS × sex interaction predicted left (b = 0.051, β = 0.426, t = 2.150, p = 0.034) and right (b = 0.051, β = 0.425, t = 2.145, p = 0.034) hippocampal activity in step 3 of each regression (Supplementary Tables 3.6–3.7). This interaction was also a trend-level predictor of left (b = 0.059, β = 0.342, t = 1.824, p = 0.071) and right (b = 0.071, β = 0.358, t = 1.777, p = 0.079) amygdala activity in regression step 3 (Supplementary Tables 3.4–3.5). Simple slope analyses for the left and right hippocampus indicated a positive relationship between genetic profile scores and activity among females (left: b = 0.067, β = 0.219, t = 2.368, p = 0.019; right: b = 0.062, β = 0.194, t = 2.176, p = 0.032) but a negative relationship among males (left: b = − 0.026, β = − 0.137, t = − 0.911, p = 0.364; right: b = − 0.020, β = − 0.194, t = − 0.688, p = 0.493).

**3.4.3 Exploratory Analysis with Pubertal Status**: Given that puberty is a key transitional period when sex differences in depression prevalence tend to develop (e.g. Angold et al., 1998 and Angold and Worthman, 1993), we conducted follow-up analyses to examine whether puberty further moderated the GPS × sex interaction predicting fearful–neutral activity reported above. The GPS × pubertal status × sex interaction predicted fearful–neutral face activity in the left and right amygdala and left and right hippocampus (Figure 3.2). This interaction effect passed FDR correction in all four regions (Table 3.2; all ps and FDR corrected ps < 0.04) and remained significant in the left amygdala (b = 0.177 β

= 1.022, t = 2.195, p = 0.031), left hippocampus (b = 0.116, β = 0.974, t = 2.092, p = 0.039), and right hippocampus (b = 0.128, β = 1.066, t = 2.291, p = 0.024), when further controlling for the other three-way interactions (GPS × pubertal status × ethnicity, GPS × sex × ethnicity, pubertal status × sex × ethnicity, none of which were significant predictors themselves [ps > 0.06]). The spatial extent of this interaction is displayed in Supplementary Figure 3.2.

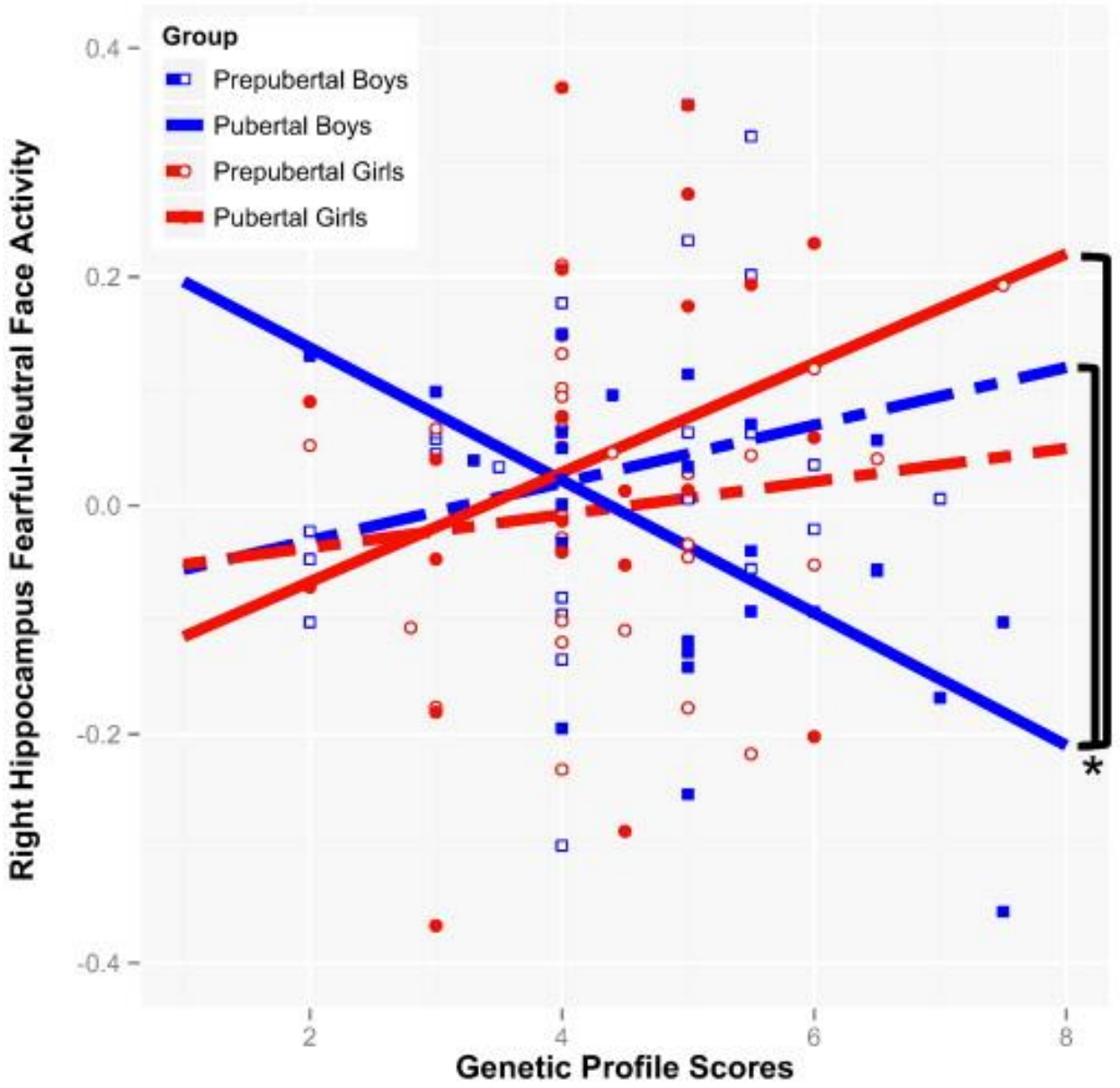



**Figure 3.2: Effects of genetic profile scores on right hippocampus activity.**
This figure shows the relationship between genetic profile scores and fearful–neutral face activity in the right hippocampus split by sex and pubertal status. Females are plotted with a circle and males with a square; pubertal children are denoted by filled symbols and prepubertal children by empty symbols. Brackets indicate relationships that are significantly different (i.e. there is a genetic profile score × sex interaction among pubertal children and a genetic profile score × pubertal status interaction among males). Pubertal males also show a significant simple slope effect * $p < 0.05$.

**Table 3.2: Follow-up regressions testing genetic profile scores (GPS) × puberty × sex interactions predicting fearful–neutral face activity.**

| | Left Amygdala | | | Right Amygdala | | | Left Hippocampus | | | Right Hippocampus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **p** | **b** | **β** | **p** | **b** | **β** | **p** | **b** | **β** | **p** |
| **Constant** | 0.044 | | 0.092 | 0.043 | | 0.157 | 0.031 | | 0.083 | 0.017 | | 0.352 |
| **Ethnicity** | -0.058 | -0.249 | 0.276 | 0.005 | 0.026 | 0.939 | 0.002 | 0.011 | 0.959 | -0.041 | 0.011 | 0.263 |
| **Sex** | 0.039 | 0.152 | 0.370 | 0.053 | 0.180 | 0.308 | 0.045 | 0.261 | 0.135 | 0.001 | 0.261 | 0.969 |
| **GPS** | -0.011 | -0.063 | 0.603 | 0.022 | 0.112 | 0.370 | 0.007 | 0.061 | 0.615 | 0.002 | 0.061 | 0.881 |
| **GPS x Ethnicity** | -0.016 | -0.091 | 0.702 | -0.023 | -0.117 | 0.630 | 0.002 | 0.019 | 0.934 | -0.002 | 0.019 | 0.945 |
| **GPS x Sex** | 0.058 | 0.337 | 0.090 | 0.070 | 0.353 | 0.083 | **0.052** | **0.437** | **0.028** | **0.050** | **0.437** | **0.037** |
| **Pubertal Status** | -0.002 | -0.029 | 0.966 | 0.003 | 0.000 | 0.950 | 0.027 | 0.173 | 0.381 | 0.008 | 0.173 | 0.787 |
| **GPS x Puberty** | 0.055 | 0.316 | 0.168 | 0.041 | 0.205 | 0.380 | -0.001 | -0.005 | 0.982 | -0.015 | -0.005 | 0.571 |
| **Puberty x Sex** | 0.166 | 0.661 | 0.063 | **0.228** | **0.813** | **0.030** | **0.148** | **0.888** | **0.017** | 0.093 | 0.888 | 0.129 |
| **Ethnicity x Puberty** | 0.143 | 0.630 | 0.169 | 0.130 | 0.499 | 0.286 | 0.060 | 0.382 | 0.400 | -0.001 | 0.382 | 0.991 |
| **GPS x Puberty x Sex** | **0.194** | **1.124** | **0.006** | **0.193** | **0.972** | **0.021** | **0.102** | **0.855** | **0.036** | **0.143** | **0.855** | **0.004** |
| | | | | | | | | | | | | |
| **Model $R^2$** | 0.161 | | | 0.123 | | | **0.168** | | | **0.172** | | |
| **Adjusted $R^2$** | 0.074 | | | 0.031 | | | **0.082** | | | **0.086** | | |
| **Model F** | 1.847 | | | 1.343 | | | **1.945** | | | **1.995** | | |
| **Model p** | 0.062 | | | 0.219 | | | **0.048** | | | **0.042** | | |
| **$R^2$ change** | **0.068** | | | **0.051** | | | **0.039** | | | **0.076** | | |

*Follow-up Regressions Testing Genetic Profile Scores (GPS) x Puberty x Sex Interactions Predicting Fearful-Neutral Face Activity: Unstandardized (b) and standardized (β) regression coefficients and their associated p-value are presented for the final step of each model. Model statistics are listed below each set of predictor statistics. $R^2$ change indicates the change in model $R^2$ adding the three-way genetic profile scores x puberty x sex interaction to the model after all other predictors listed. Effects significant at $p<0.05$ are in bold and effects reaching significance after False Discovery Rate (FDR) correction ($q<0.05$) are shaded gray (correcting for the three-way interaction tested in each of four regions). FDR corrected p-values for each regions were: Left Amygdala p=0.012, Right Amygdala p=0.028, Left Hippocampus p=0.036, Right Hippocampus p=0.012*

Given the strong relationship between pubertal status and age, we added age at scan and interactions with age (age × ethnicity, age × sex, GPS × age, and GPS × age × sex) as covariates to the model as a further statistical control (Supplementary Table 3.10). The three-way GPS × pubertal status × sex interaction remained significant in the left amygdala and left and right hippocampus ($p < 0.05$) and trend-level significant in the right amygdala ($p = 0.06$). Additionally, no significant main effects or interactions with age were found, suggesting that the above interaction is specific to pubertal effects rather than age in this sample. In a final statistical control, we found that the GPS × pubertal status × sex interaction held significant in all four regions (all $ps < 0.03$) when controlling for histories of major depressive disorder, anxiety disorders, and/or externalizing disorders (see Supplementary Table 3.11). Furthermore, we examined whether these effects were significant in subsets of children with histories of each of these types of disorders (Supplementary Table 3.12). The results also held significant when controlling for amygdala or hippocampal volume (data not shown).

To parse and understand this three-way GPS × pubertal status × sex interaction, we assessed two-way interactions with genetic profile scores in the sex and pubertal subgroups and isolated the simple slopes for GPS predicting fearful–neutral face activity for prepubertal and pubertal boys and girls (see Figure 3.2 and Table 3.3). A significant GPS × sex interaction was present among pubertal children in all 4 regions but was absent among prepubertal children. Further, there were significant GPS × pubertal status interactions among girls in the left and right amygdala and among boys in the right hippocampus ($p = 0.007$). Pubertal boys ($N = 28$) showed a negative relationship between genetic profile scores and activity in all four regions (e.g. right hippocampus: $b = -0.066$, $\beta = -0.495$, $t = -$

2.993, p = 0.004) whereas pubertal girls (N = 24) show the hypothesized positive relationship in all four regions (e.g. right amygdala: b = 0.126, β = 0.577, t = 2.375, p = 0.020).

**Table 3.3: Interactions with Pubertal Status or Sex and Simple Slope Effects of Genetic Profile Scores (GPS) Predicting Fearful-Neutral Face Activity**

| | Left Amygdala | | | Right Amygdala | | | Left Hippocampus | | | Right Hippocampus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **p** | **b** | **β** | **p** | **b** | **β** | **p** | **b** | **β** | **p** |
| **GPS x Puberty, Split by Sex** | | | | | | | | | | | | |
| Boys | -0.042 | -0.212 | 0.354 | -0.056 | -0.258 | 0.300 | -0.052 | -0.422 | 0.102 | **-0.087** | **-0.703** | **0.007** |
| Girls | **0.152** | **0.895** | **0.011** | **0.137** | **0.702** | **0.050** | 0.050 | 0.428 | 0.216 | 0.056 | 0.476 | 0.172 |
| **GPS x Sex Split, by Puberty** | | | | | | | | | | | | |
| Prepubertal | -0.039 | -0.383 | 0.426 | -0.026 | -0.240 | 0.644 | 0.001 | -0.041 | 0.973 | -0.022 | -0.278 | 0.517 |
| Pubertal | **0.155** | **0.724** | **0.002** | **0.166** | **0.721** | **0.005** | **0.103** | **0.808** | **0.003** | **0.121** | **0.901** | **0.001** |
| **Simple Slope Effects of GPS** | | | | | | | | | | | | |
| Prepubertal Boys | -0.019 | -0.053 | 0.576 | 0.015 | 0.114 | 0.704 | 0.007 | 0.077 | 0.762 | 0.021 | 0.208 | 0.373 |
| Pubertal Boys | -0.061 | -0.265 | 0.058 | -0.041 | -0.144 | 0.282 | **-0.045** | **-0.344** | **0.045** | **-0.066** | **-0.495** | **0.004** |
| Prepubertal Girls | -0.058 | -0.436 | 0.139 | -0.011 | -0.126 | 0.803 | 0.008 | 0.036 | 0.760 | -0.001 | -0.070 | 0.971 |
| Pubertal Girls | **0.094** | **0.459** | **0.040** | **0.126** | **0.577** | **0.020** | 0.059 | 0.464 | 0.062 | 0.055 | 0.406 | 0.081 |

*Interactions with Pubertal Status or Sex and Simple Slope Effects of Genetic Profile Scores (GPS) Predicting Fearful-Neutral Face Activity: For each of the four regions of interest, GPS x Pubertal Status interactions for each sex and GPS x Sex interactions within each pubertal status group are presented. Additionally, the simple slope effects of GPS predicting fearful-neutral face activity for each pubertal status x sex subgroup are presented. These effects were extracted using the PROCESS tool from the full models presented in Table 3.2, i.e. accounting for all covariates. Unstandardized (b) and standardized (β) regression coefficients and their associated p-values are presented for each effect in each model. Effects significant at $p<0.05$ are in bold.*

To examine whether the results represented differential effects on fearful and/or neutral faces, we examined whether the GPS × sex interaction predicted fearful-baseline and neutral-baseline activity among the pubertal children (given that all four regions showed a GPS × sex interaction only among pubertal children). We tested this with a GLM with emotion type as a 2-level within-subject factor (fearful face vs. baseline activity and neutral face vs. baseline activity), sex and ethnicity as binary between-subject factors, and GPS as a continuous predictor. The results indicated an emotion type × GPS × sex interaction in all four regions (see Supplementary Table 3.13). Figure 3.3 shows the relationships between GPS and fearful face and neutral face activity separately for pubertal boys and girls for the right hippocampus. For this, and the other regions, pubertal girls tended to show a positive relationship between GPS and fearful face activity while pubertal boys tend to show a positive relationship between GPS and neutral face activity. In other words, the positive relationship between GPS and fearful–neutral face activity among pubertal females was driven mainly by a positive effect on fearful face activity. Conversely, the negative relationship between GPS and fearful–neutral face activity observed among pubertal males was driven mainly by a positive effect on neutral face activity. Thus, more risk-conferring alleles among stress- and MDD-related genetic variants predicted greater responses to emotional faces among females, but greater responses to neutral faces among males.

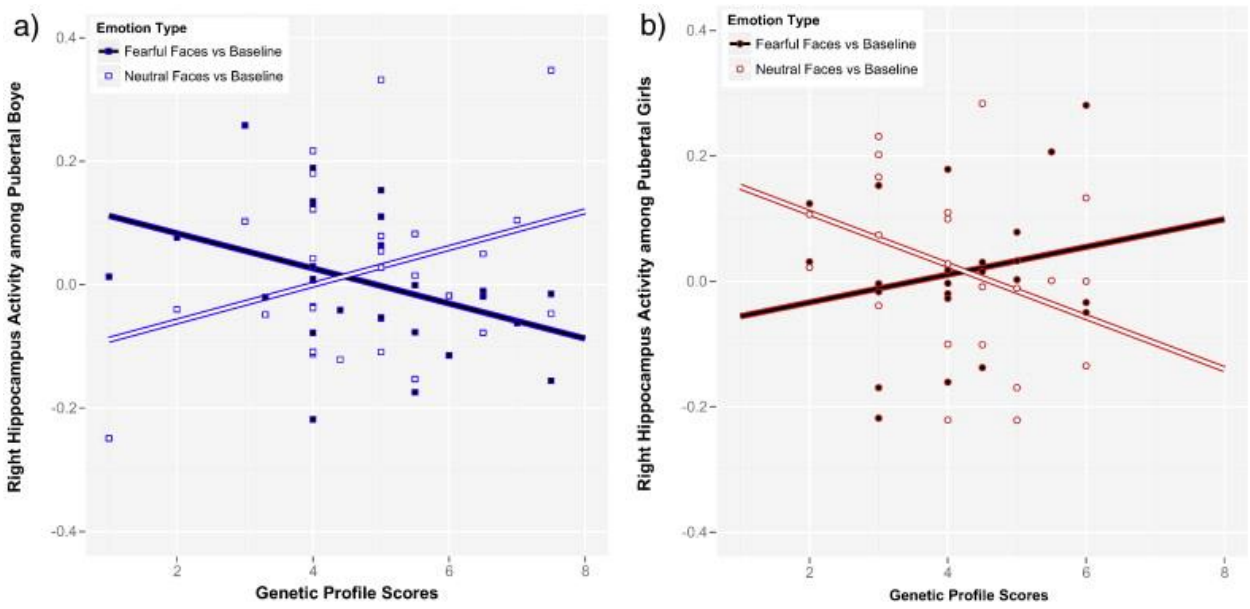



**Figure 3.3: Effects of genetic profile scores on fearful and neutral face activity among pubertal children.**

This figure shows the effects of genetic profiles scores on fearful face vs. baseline (filled black) and on neutral face vs. baseline activity (filled white) in the right hippocampus. These effects are shown only for pubertal children and are displayed separately for males (a) and females (b).

**3.4.4 Specificity of Results to Fearful Faces**: As a follow-up analysis, we tested whether these associations were specific to fearful faces or whether these stress-related factors predicted increased reactivity to negative emotional faces more generally. To do so, we performed the same sets of regressions as above but predicting sad–neutral face activity. Supplementary Table 3.14 shows a summary of the final step of each of the regression models including demographics, life events, GPS, and interactions predicting sad–neutral faces. Here, we found no significant main effects of or interactions with stressful life events. There was a significant GPS × sex interaction predicting left amygdala ($b = 0.091$, $\beta = 0.534$, $t = 2.669$, $p = 0.009$) and right amygdala activity ($b = 0.081$, $\beta = 0.432$, $t = 2.106$, $p = 0.038$); this was also trend level significant when predicting the left and right hippocampal activity ($ps < 0.1$). The pattern of simple slopes was similar to that predicting fearful–neutral faces, i.e. there was a positive relationship between GPS and activity among females but a negative relationship among males.

Next, we performed the same exploratory regression models predicting sad–neutral face activity to test the specificity of the three-way interactions to fearful–neutral faces (Supplementary Table 3.15). While each region showed a significant genetic profile score × sex effect (all $ps < 0.05$), we found that the GPS × pubertal status × sex interaction predicted sad–neutral face activity in the left hippocampus ($b = 0.098$, $\beta = 0.835$, $t = 2.109$, $p = 0.038$) and was a trend-level significant predictor of left amygdala and right hippocampus activity ($p < 0.1$). This suggests that the differential effects of stress-system genes by sex and pubertal status may generalize to negative emotional face stimuli, though pubertal effects were less strong.

**3.4.5 Power Calculations**: Consistent with current reporting standards for gene × environment studies, we calculated estimates of post-hoc power to detect our initial GPS × life events hypothesis (N = 107, 9 total predictors, assuming all other predictors account for 15% of variance). We would have over 80% power to detect a 6% or greater increase in variance explained by the addition of the GPS × life events interaction, but about 20% power to detect a 1% increase in variance explained. We estimated our power at 70% to detect the GPS × sex interaction among the pubertal children for a 10% increase in variance explained (N = 52, 5 total predictors, assuming all other predictors account for 10% of variance). Yet, our power was limited (20%) to detect the simple slope effects, expecting a 5% increase in variance explained (N = 26, 3 total predictors, assuming all other predictors account for 10% of variance).

# 3.5 Discussion

**3.5.1 Genetic Profile Scores, Sex, and Puberty**: HPA axis SNPs may moderate effects of life stress/adversity on amygdala reactivity (Bogdan et al., 2012 and White et al., 2012). While we did not observe a GPS × life events interaction here, it is possible that this type of effect is not detectable this early in childhood. Yet, we instead found a three-way interaction between genetic profile scores × pubertal status × sex. GPS predicted activity more strongly among pubertal than prepubertal children, even using a relatively coarse grouping based on Tanner Stages. Among pubertal children, higher GPS predicted higher fearful face activity among females but predicted higher neutral face activity among males. While this particular pattern of results was not anticipated, it may still be conceptually consistent, where both males and females with more genetic 'risk' variants show greater amygdala and hippocampus reactivity. Specifically, females

showed an expected positive association between GPS and negative emotional stimuli, while males showed greater responses to stimuli that we expect to be emotionally neutral, but that may be perceived as more negative in the context of other negative faces.

While this type of sex-moderated effect of genetic risk on amygdala and hippocampus reactivity is novel, there is potentially relevant precedent for sex differences in neural response to emotional stimuli. For example, boys but not girls may exhibit amygdala habituation to fearful faces (Thomas et al., 2001a) where this type of sex difference may relate to the apparently differential effects on fearful vs. neutral face activity. Further, there is much evidence for sex differences in stress-system and amygdala and hippocampus function. In previous work, we showed that GPS were a significantly stronger positive predictor of cortisol reactivity among females than males (Pagliaccio et al., 2013a). Sex also moderates cortisol reactivity to acute stressors (e.g. Kirschbaum et al., 1995 and Kirschbaum et al., 1992) and the effects of childhood trauma and CRHR1 variation on cortisol reactivity and depression (Heim et al., 2009). Sex is also a major moderator of the effects of environmental stress and stress-system genes on the HPA axis and the amygdala and hippocampus in a variety of animal studies (e.g. Bourke et al., 2013, Shors et al., 2001 and Zohar and Weinstock, 2011).

The relationship between puberty and neural/emotional development is also key in this age range, and likely interacts with factors like sex and stress. Structurally, the amygdala and hippocampus exhibit non-linear growth rates across pubertal development where females tend to show large increases in volume in early puberty which peak in mid puberty whereas males show increasing subcortical volumes throughout puberty (Goddings et al., 2014). It has been suggested that amygdala responses to emotional faces also show a U-shaped developmental curve where adolescents show greater responses to emotional faces than children or adults (Guyer et al., 2008 and Hare et al., 2008). Further, pubertal development specifically has been positively correlated with amygdala responses to emotional and neutral faces in early adolescence but not in late

childhood (Moore et al., 2012). In contrast, we observed interactions with pubertal status rather than main effects. Nonetheless, it will be important to test whether these main effects and/or interactions with puberty are observed longitudinally across development.

It should also be noted that interactions with genetic profile scores also predicted sad–neutral face activity. Thus, these genetic 'risk' factors may play a role in amygdala and hippocampus responses to emotionally salient stimuli more generally, rather than specifically relating to threat-related stimuli. These differences in the effect of HPA axis genetic risk factors and amygdala and hippocampus function based on sex and pubertal development may be particularly salient in understanding the increasing rates of internalizing psychopathology during puberty/adolescence and the increasingly high prevalence rates among females relative to males during this period (e.g. Angold et al., 1998 and Hankin et al., 1998).

**3.5.2 Stressful and Traumatic Life Events**: We found that the experience of more stressful and traumatic life events across childhood predicted higher fearful–neutral face activity in the left amygdala. This is consistent with previous work showing that the experience of severe adversity/trauma predicted greater amygdala responses to negative faces among children, adolescents, and adults (Ganzel et al., 2013, Grant et al., 2011 and Tottenham et al., 2011). Other work in this sample has explored the separate relationships between stressful vs. traumatic life events on responses to emotional faces in the amygdala, hippocampus, and other regions (Suzuki et al., 2014). These and our findings build upon prior results by suggesting that not only severe traumatic events relate to amygdala reactivity but particularly stressful life events predict amygdala reactivity even in school-age children. Additionally, we found that this effect was specific to fearful–neutral faces (i.e. life events did not predict sad–neutral face activity). While the amygdala generally responds to different facial emotion types (for meta-analysis, see Sergerie et al., 2008; for results in a healthy subsample of school-age children from the PDS, see Pagliaccio et al. (2013b); these results suggest that the effect of life stress may be particularly important for

amygdala response to threat-related stimuli rather than for negative emotional expressions more generally. Additionally, it is important to note that lack of a GPS × life events interaction may indicate more independent effects of stress-system genetics and childhood stressors, but may also be due to low power to detect interactions of small effect sizes.

**3.5.3 Limitations and Future Directions:** As previously discussed in greater detail (Pagliaccio et al., 2013a), there are several limitations to using single summary variables to encapsulate genetic variation or stressful/traumatic life events. Though this approach can increase power by combining multiple sources of variance and reducing the number of tests to be performed, it assumes that the effects of stressors or of SNPs sum additively with equal weights. Refining this approach is an important future direction that requires optimizing the relative weighting of life events or SNPs for testing in independent samples. To this end, Supplementary Table 3.16 presents the effects of each SNP independently predicting left amygdala fearful–neutral face activity split by sex and pubertal status. These results should be interpreted with caution as the counts of each genotype by subgroup are relatively small, but these regression coefficients may be useful in creating better-informed genetic profile scores in the future. Additionally, we have previously presented the relationship between these individual SNPs and cortisol reactivity and brain volumes (Pagliaccio et al., 2013a).

Additionally, the mood induction task used prior to the Emotional Face Processing Task and the presence of both half- and full-intensity emotional faces may introduce additional sources of variance into the effects of interest. Another limitation is that we examined pubertal status in a relatively coarse manner by collapsing across Tanner Stages 2–5. While we did not have sufficient sample sizes within each stage (as would be expected in this age range) to adequately test incremental changes across puberty, longitudinal data as prepubertal children transition into puberty would be key to truly confirm our results. Using measures of gonadal hormones may also be useful for exploring the underlying mechanisms of puberty's moderating effects on stress.

From a genetic perspective, both the likelihood of low power contributing to false negatives and also an increased false discovery rate contributing to false positives need to be considered. Our calculations indicate that we would have good power to detect medium to large effect sizes but that we lack power at smaller effect sizes. Thus, we may have false negatives in our results if we are unable to detect genetic influences of small effects. Second, while we were highly cautious in selecting polymorphisms for the GPS, one might argue that the priors associated with their inclusion may not be satisfactory (e.g. these SNPs have not emerged in genome-wide association studies of depression), which may increase the likelihood of false positives. All these issues highlight the importance of future replication regarding interactions with GPS. Furthermore, it is important to consider how effects may differ by ethnicity. While we did not find differences in brain activity by ethnicity, GPS and life events scores were higher among the African American children in the sample. As we did not have large enough sample sizes split by ethnicity, future studies will need to test the specificity and generalizability of these effects across ethnicity or genetic ancestry.

**3.5.4 Conclusions**: We found that having more 'risk' alleles in HPA axis genes predicted higher amygdala and hippocampus reactivity, especially among pubertal school-age children. This interacted with sex, such that higher genetic profile scores predicted higher fearful (and sad) face activity among girls but predicted higher neutral face activity among boys. The experience of more stressful/traumatic life events predicted higher left amygdala reactivity to fearful–neutral faces (but not sad–neutral faces). These findings help elucidate effects of normative genetic and environmental factors on individual differences in amygdala and hippocampus reactivity. Further, the results underscore that sex and puberty may be key factors to consider in studies of the neural measures of emotion reactivity in children. Overall, the current results suggest that how stress-related risk factors impact the neural underpinnings of emotion processing may be key to understanding the normative individual differences in neural responding to emotional

stimuli with potential salience for the developmental psychopathology of internalizing disorders, especially in the peripubertal period.

## 3.6 Supplementary Information

**Supplementary Table 3.1: Reported Instances of Each Type of Stressful and Traumatic Life Event**

| | | Count of Participants by Reported # of Instances | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Stressful Life Events** | **Missing** | **0** | **1** | **2** | **3** | **4** | **5** | **6+** |
| Broke Up with Best Friend | | 101 | 6 | | | | | |
| Broke Up with Boy/Girlfriend | | 106 | 1 | | | | | |
| Change Daycare/School | | 9 | 37 | 35 | 15 | 6 | 2 | 3 |
| Conflict Between Parents/Family | | 89 | 6 | 6 | 1 | | 2 | 3 |
| Death of Pet | | 55 | 34 | 15 | 1 | 1 | 1 | |
| Forced Separation from Home | | 102 | 3 | 1 | | | | 1 |
| Lived/Attended School in Unsafe Environment | | 96 | 10 | 1 | | | | |
| Loss of Home Without Family Separation | | 101 | 6 | | | | | |
| Lost Significant Person Through Moving | | 64 | 38 | 4 | 1 | | | |
| Moving House | | 18 | 33 | 23 | 15 | 12 | 1 | 5 |
| New Child in Home | | 35 | 42 | 17 | 7 | 4 | 2 | |
| New Parental Figure | | 84 | 12 | 9 | 2 | | | |
| Parental Arrest | | 85 | 16 | 4 | 2 | | | |
| Parental Divorce | | 96 | 10 | 1 | | | | |
| Parental Hospitalization | | 42 | 45 | 14 | 1 | 3 | 1 | 1 |
| Parental Separation | 4 | 66 | 26 | 7 | 4 | | | |
| Reduction in Standard of Living | | 65 | 25 | 15 | 2 | | | |
| Separation From Parent (1 week or more) | | 56 | 31 | 11 | 3 | 3 | | 3 |
| **Traumatic Life Events** | | | | | | | | |
| Accident or Crash with Automobile, Plane, or Boat | | 78 | 28 | 1 | | | | |
| Accidental Burning, Poisoning, or Drowning | | 90 | 17 | | | | | |
| Attacked by an Animal | | 102 | 4 | 1 | | | | |
| Death of Adult Loved One | 1 | 27 | 20 | 27 | 16 | 7 | 9 | |
| Death of Sibling or Peer | | 92 | 12 | 3 | | | | |
| Diagnosed with Physical Illness | | 84 | 21 | 2 | | | | |
| Domestic Violence | | 96 | 5 | 4 | 1 | | | 1 |
| Hospitalization, Emergency Room Visit, or Invasive Medical Procedure | | 37 | 34 | 14 | 10 | 2 | 4 | 6 |
| Learned about Traumatic Event | | 75 | 23 | 8 | 1 | | | |
| Man-made Disasters (fire, war, terrorism) | | 101 | 5 | 1 | | | | |
| Natural Disasters (flood, hurricane, tornado, earthquake) | | 70 | 25 | 7 | 3 | 2 | | |
| Physical Abuse | | 101 | 5 | | | | | 1 |
| Sexual Abuse, Sexual Assault, or Rape | | 98 | 5 | 3 | | | | 1 |
| Victim of Physical Violence | | 103 | 3 | 1 | | | | |
| Witnessed Another Person Being Threatened with Harm, Seriously Injured, or Killed | | 83 | 14 | 6 | 2 | 1 | | 1 |

*This table presents a list of stressful and traumatic life events assessed during the diagnostic interviews. The count of participants reporting different cumulative numbers of instances of each event through the time of scan are presented along with the number of missing/not reported values. The item for Hospitalization, Emergency Room Visit, or Invasive Medical Procedure did not assess psychiatric hospital visits.*

**Supplementary Table 3.2: Diagnoses Through and at Time of Scan**

| N = 107 | EVER | AT SCAN |
|---|---|---|
| **No Disorders** | 29 | 62 |
| **Any Anxiety Disorder** | 57 | 27 |
| **Generalized Anxiety Disorder** | 33 | 8 |
| **Post-Traumatic Stress Disorder** | 12 | 1 |
| **Separation Anxiety Disorder** | 33 | 9 |
| **Obsessive Compulsive Disorder** | 14 | 11 |
| **Panic Attack** | 3 | 1 |
| **Panic With Agoraphobia** | 1 | 0 |
| **Panic Without Agoraphobia** | 1 | 1 |
| **Agoraphobia Without Panic** | 2 | 0 |
| **Social Phobia** | 15 | 9 |
| **Any Externalizing Disorder** | 47 | 22 |
| **Attention Deficit Hyperactivity Disorder** | 35 | 16 |
| **Oppositional Defiant Disorder** | 38 | 12 |
| **Conduct Disorder** | 23 | 6 |
| **Major Depressive Disorder** | 55 | 22 |
| **PO-MDD #** | 42 | - |

*This table displays the specific diagnoses that children may have met criteria for at any assessment through the time of scan or at the assessment wave closest to the scan session. Diagnoses are not mutually exclusive, so the counts across diagnoses may add to more than the total 107 children. To clarify the observed co-morbidity through the time of scan, 29 children had no diagnoses by the time of scan, 23 children were diagnosed with either an anxiety disorder, an externalizing disorder, or MDD, 29 child had diagnoses from two disorder types, and 26 children had diagnoses of anxiety disorders, externalizing disorders, and MDD.*

*# PO-MDD is preschool-onset depression diagnosed before age 6.*

**Supplementary Table 3.3: Interrelationships Between Variables**

| Predictor | Ethn. | Puberty | Age | GPS | Life Events | Left Amyg. | Right Amyg. | Left Hipp. | Right Hipp. | MDD | Anx. | Ext. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sex** | 0.225 | 0.753 | 1.025 | 1.104 | -0.113 | -0.484 | -0.560 | -1.105 | 0.118 | 1.138 | 0.088 | 2.100 |
| **Ethnicity** | | 0.031 | -1.168 | **-5.673**** | **-2.611*** | -0.854 | -0.378 | -0.027 | -1.032 | 0.951 | 0.459 | 0.331 |
| **Pubertal Status** | | | **10.997***** | 0.139 | 1.872 | -0.302 | -0.157 | 0.544 | -0.264 | 0.448 | 2.778 | 1.226 |
| **Age at Scan (months)** | | | | -0.027 | **0.251**** | 0.000 | 0.050 | 0.081 | -0.047 | -0.870 | **2.506*** | -1.064 |
| **Genetic Profile Scores** | | | | | 0.006 | -0.108 | -0.004 | -0.056 | -0.018 | -0.675 | -1.680 | 0.027 |
| **Stressful/Traumatic Life Events** | | | | | | **0.378**** | 0.170 | 0.120 | 0.188 | **2.939**** | **3.195**** | **2.521*** |
| **Left Amygdala Activity** | | | | | | | **0.521**** | **0.599**** | **0.665**** | 0.545 | **3.872**** | 1.506 |
| **Right Amygdala Activity** | | | | | | | | **0.503**** | **0.562**** | -0.997 | **2.778**** | -0.216 |
| **Left Hippocampus Activity** | | | | | | | | | **0.711**** | -0.623 | **2.472*** | 0.089 |
| **Right Hippocampus Activity** | | | | | | | | | | 0.268 | **2.190*** | 0.659 |
| **Major Depressive History** | | | | | | | | | | | **6.749**** | **21.297**** |
| **Anxiety Disorder History** | | | | | | | | | | | | **15.129**** |
| **Externalizing Disorder History** | | | | | | | | | | | | |

*This chart displays the relationships between the variables of interest. Effects of sex, ethnicity, pubertal status, and diagnostic status are tested either by chi-squared test or independent samples t-test (females>males, White> African American, pubertal>prepubertal, disorder history>no history). Effects of continuous variables are tested by Pearson's correlation. Activity variables represent magnitude estimates for fearful-neutral faces.*

** p < 0.05, ** p < 0.01, * p < 0.001*

**Supplementary Table 3.4: Regression Predicting Left Amygdala Fearful-Neutral Face Activity**

| | Step 1 | | | | Step 2 | | | | Step 3 | | | | Step 4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **FDR p** |
| **Constant** | 0.034 | | 1.497 | 0.137 | **-0.111** | | **-2.519** | **0.013** | **-0.110** | | **-2.364** | **0.020** | **-0.113** | | **-2.423** | **0.017** | |
| **Ethnicity** | -0.040 | -0.176 | -0.874 | 0.384 | -0.024 | -0.105 | -0.471 | 0.639 | 0.005 | -0.037 | 0.050 | 0.961 | 0.053 | -0.017 | 0.482 | 0.631 | |
| **Sex** | 0.023 | 0.102 | 0.523 | 0.602 | 0.014 | 0.062 | 0.343 | 0.732 | -0.085 | 0.066 | -1.040 | 0.301 | -0.089 | 0.058 | -1.083 | 0.282 | |
| **Genetic Profile Scores** | | | | | -0.023 | -0.132 | -1.251 | 0.214 | -0.014 | -0.083 | -0.728 | 0.468 | 0.011 | -0.071 | 0.304 | 0.762 | 0.831 |
| **Life Events** | | | | | **0.008** | **0.365** | **3.828** | **0.000** | **0.008** | **0.372** | **3.918** | **0.000** | **0.008** | **0.368** | **3.800** | **0.000** | **0.003** |
| **GPS x Ethnicity** | | | | | | | | | 0.007 | 0.039 | 0.177 | 0.860 | -0.002 | -0.010 | -0.046 | 0.964 | |
| **GPS x Sex** | | | | | | | | | 0.059 | 0.342 | 1.824 | 0.071 | 0.053 | 0.310 | 1.616 | 0.109 | |
| **Life Events x Ethnicity** | | | | | | | | | -0.001 | -0.032 | -0.169 | 0.866 | -0.003 | -0.147 | -0.626 | 0.533 | |
| **Life Events x Sex** | | | | | | | | | 0.006 | 0.271 | 1.480 | 0.142 | 0.006 | 0.275 | 1.499 | 0.137 | |
| **GPS x Life Events** | | | | | | | | | | | | | -0.001 | -0.079 | -0.839 | 0.404 | 0.539 |
| **Model $R^2$** | 0.010 | | | | 0.158 | | | | 0.205 | | | | 0.210 | | | | |
| **Adjusted $R^2$** | -0.010 | | | | 0.125 | | | | 0.140 | | | | 0.137 | | | | |
| **Model F** | 0.499 | | | | 4.781 | | | | 3.152 | | | | 2.871 | | | | |
| **Model p** | 0.609 | | | | **0.001** | | | | **0.003** | | | | **0.005** | | | | |
| **Model Change p** | 0.609 | | | | **0.000** | | | | 0.227 | | | | 0.404 | | | | |

*GPS=Genetic Profile Scores. Unstandardized (b) and standardized (β) regression coefficients and their associated t- and p-value are presented for the each step of the model. Model statistics are listed below each set of predictor statistics. Effects significant at p<0.05 are in bold and effects reaching significance after False Discovery Rate (FDR) correction in the fourth step (q=0.05) are shaded gray (correcting for 12 hypothesized test, i.e. the three hypothesized effects [genetic profile scores, life events, and their interaction] predicting activity in four regions).*

**Supplementary Table 3.5: Regression Predicting Right Amygdala Fearful-Neutral Face Activity**

| | Step 1 | | | | Step 2 | | | | Step 3 | | | | Step 4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **FDR p** |
| **Constant** | 0.036 | | 1.386 | 0.169 | -0.043 | | -0.799 | 0.426 | -0.048 | | -0.834 | 0.406 | -0.048 | | -0.838 | 0.404 | |
| **Ethnicity** | -0.021 | -0.081 | -0.403 | 0.688 | 0.005 | 0.018 | 0.075 | 0.941 | 0.130 | 0.094 | 1.128 | 0.262 | 0.139 | 0.097 | 1.021 | 0.310 | |
| **Sex** | 0.029 | 0.112 | 0.576 | 0.566 | 0.027 | 0.105 | 0.538 | 0.592 | -0.044 | 0.089 | -0.440 | 0.661 | -0.045 | 0.088 | -0.444 | 0.658 | |
| **Genetic Profile Scores** | | | | | 0.001 | 0.004 | 0.039 | 0.969 | 0.011 | 0.056 | 0.459 | 0.647 | 0.016 | 0.058 | 0.354 | 0.724 | 0.831 |
| **Life Events** | | | | | 0.004 | 0.172 | 1.685 | 0.095 | 0.004 | 0.171 | 1.677 | 0.097 | 0.004 | 0.171 | 1.646 | 0.103 | 0.207 |
| **GPS x Ethnicity** | | | | | | | | | -0.001 | -0.003 | -0.013 | 0.989 | -0.002 | -0.011 | -0.045 | 0.964 | |
| **GPS x Sex** | | | | | | | | | 0.071 | 0.358 | 1.777 | 0.079 | 0.070 | 0.353 | 1.707 | 0.091 | |
| **Life Events x Ethnicity** | | | | | | | | | -0.006 | -0.238 | -1.162 | 0.248 | -0.006 | -0.257 | -1.012 | 0.314 | |
| **Life Events x Sex** | | | | | | | | | 0.004 | 0.166 | 0.842 | 0.402 | 0.004 | 0.166 | 0.840 | 0.403 | |
| **GPS x Life Events** | | | | | | | | | | | | | 0.000 | -0.013 | -0.124 | 0.901 | 0.901 |
| **Model $R^2$** | 0.005 | | | | 0.032 | | | | 0.081 | | | | 0.081 | | | | |
| **Adjusted $R^2$** | -0.015 | | | | -0.006 | | | | 0.006 | | | | -0.004 | | | | |
| **Model F** | 0.237 | | | | 0.840 | | | | 1.079 | | | | 0.951 | | | | |
| **Model p** | 0.790 | | | | 0.503 | | | | 0.384 | | | | 0.485 | | | | |
| **Model Change p** | 0.790 | | | | 0.242 | | | | 0.272 | | | | 0.901 | | | | |

*GPS=Genetic Profile Scores. Unstandardized (b) and standardized (β) regression coefficients and their associated t- and p-value are presented for the each step of the model. Model statistics are listed below each set of predictor statistics. Effects significant at p<0.05 are in bold and effects reaching significance after False Discovery Rate (FDR) correction in the fourth step (q=0.05) are shaded gray (correcting for 12 hypothesized test, i.e. the three hypothesized effects [genetic profile scores, life events, and their interaction] predicting activity in four regions).*

**Supplementary Table 3.6: Regression Predicting Left Hippocampus Fearful-Neutral Face Activity**

| | Step 1 | | | | Step 2 | | | | Step 3 | | | | Step 4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **FDR p** |
| **Constant** | 0.020 | | 1.256 | 0.212 | -0.013 | | -0.405 | 0.686 | -0.008 | | -0.249 | 0.804 | -0.014 | | -0.433 | 0.666 | |
| **Ethnicity** | -0.002 | -0.015 | -0.077 | 0.939 | 0.001 | 0.007 | 0.029 | 0.977 | 0.003 | 0.073 | 0.049 | 0.961 | 0.091 | 0.126 | 1.151 | 0.252 | |
| **Sex** | 0.033 | 0.214 | 1.102 | 0.273 | 0.031 | 0.201 | 1.028 | 0.306 | -0.062 | 0.211 | -1.047 | 0.298 | -0.069 | 0.191 | -1.179 | 0.241 | |
| **Genetic Profile Scores** | | | | | -0.005 | -0.044 | -0.390 | 0.697 | 0.001 | 0.007 | 0.057 | 0.955 | 0.047 | 0.039 | 1.803 | 0.075 | 0.207 |
| **Life Events** | | | | | 0.002 | 0.120 | 1.173 | 0.244 | 0.002 | 0.131 | 1.301 | 0.196 | 0.002 | 0.121 | 1.091 | 0.278 | 0.417 |
| **GPS x Ethnicity** | | | | | | | | | 0.015 | 0.122 | 0.519 | 0.605 | -0.001 | -0.008 | -0.034 | 0.973 | |
| **GPS x Sex** | | | | | | | | | **0.051** | **0.426** | **2.150** | **0.034** | 0.041 | 0.342 | 1.719 | 0.089 | |
| **Life Events x Ethnicity** | | | | | | | | | 0.001 | 0.034 | 0.171 | 0.865 | -0.004 | -0.266 | -1.090 | 0.278 | |
| **Life Events x Sex** | | | | | | | | | 0.006 | 0.373 | 1.928 | 0.057 | **0.006** | **0.384** | **2.016** | **0.047** | |
| **GPS x Life Events** | | | | | | | | | | | | | **-0.002** | **-0.206** | **-2.116** | **0.037** | 0.207 |
| **Model $R^2$** | 0.012 | | | | 0.028 | | | | 0.110 | | | | 0.150 | | | | |
| **Adjusted $R^2$** | -0.007 | | | | -0.010 | | | | 0.038 | | | | 0.071 | | | | |
| **Model F** | 0.608 | | | | 0.727 | | | | 1.519 | | | | 1.896 | | | | |
| **Model p** | 0.547 | | | | 0.576 | | | | 0.160 | | | | 0.061 | | | | |
| **Model Change p** | 0.547 | | | | 0.432 | | | | 0.067 | | | | **0.037** | | | | |

*GPS=Genetic Profile Scores. Unstandardized (b) and standardized (β) regression coefficients and their associated t- and p-value are presented for the each step of the model. Model statistics are listed below each set of predictor statistics. Effects significant at p<0.05 are in bold and effects reaching significance after False Discovery Rate (FDR) correction in the fourth step (q=0.05) are shaded gray (correcting for 12 hypothesized test, i.e. the three hypothesized effects [genetic profile scores, life events, and their interaction] predicting activity in four regions).*

**Supplementary Table 3.7: Regression Predicting Right Hippocampus Fearful-Neutral Face Activity**

| | Step 1 | | | | Step 2 | | | | Step 3 | | | | Step 4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **FDR p** |
| **Constant** | 0.005 | | 0.336 | 0.737 | -0.040 | | -1.235 | 0.220 | -0.033 | | -0.968 | 0.336 | -0.039 | | -1.143 | 0.256 | |
| **Ethnicity** | -0.032 | -0.205 | -1.023 | 0.309 | -0.028 | -0.176 | -0.735 | 0.464 | -0.055 | -0.131 | -0.807 | 0.422 | 0.026 | -0.082 | 0.322 | 0.748 | |
| **Sex** | -0.002 | -0.014 | -0.071 | 0.943 | -0.005 | -0.032 | -0.165 | 0.869 | -0.071 | -0.012 | -1.192 | 0.236 | -0.078 | -0.031 | -1.313 | 0.192 | |
| **Genetic Profile Scores** | | | | | -0.007 | -0.062 | -0.551 | 0.583 | -0.002 | -0.014 | -0.116 | 0.908 | 0.041 | 0.016 | 1.564 | 0.121 | 0.207 |
| **Life Events** | | | | | 0.002 | 0.165 | 1.626 | 0.107 | 0.003 | 0.177 | 1.762 | 0.081 | 0.002 | 0.168 | 1.569 | 0.120 | 0.207 |
| **GPS x Ethnicity** | | | | | | | | | 0.015 | 0.129 | 0.549 | 0.584 | 0.001 | 0.009 | 0.037 | 0.970 | |
| **GPS x Sex** | | | | | | | | | **0.051** | **0.425** | **2.145** | **0.034** | 0.042 | 0.347 | 1.741 | 0.085 | |
| **Life Events x Ethnicity** | | | | | | | | | 0.002 | 0.134 | 0.664 | 0.508 | -0.002 | -0.143 | -0.585 | 0.560 | |
| **Life Events x Sex** | | | | | | | | | 0.004 | 0.275 | 1.420 | 0.159 | 0.004 | 0.285 | 1.490 | 0.140 | |
| **GPS x Life Events** | | | | | | | | | | | | | -0.002 | -0.190 | -1.946 | 0.055 | 0.207 |
| **Model $R^2$** | 0.010 | | | | 0.041 | | | | 0.111 | | | | 0.144 | | | | |
| **Adjusted $R^2$** | -0.009 | | | | 0.003 | | | | 0.038 | | | | 0.065 | | | | |
| **Model F** | 0.530 | | | | 1.087 | | | | 1.524 | | | | 1.813 | | | | |
| **Model p** | 0.590 | | | | 0.367 | | | | 0.159 | | | | 0.075 | | | | |
| **Model Change p** | 0.590 | | | | 0.200 | | | | 0.113 | | | | 0.055 | | | | |

*GPS=Genetic Profile Scores. Unstandardized (b) and standardized (β) regression coefficients and their associated t- and p-value are presented for the each step of the model. Model statistics are listed below each set of predictor statistics. Effects significant at p<0.05 are in bold and effects reaching significance after False Discovery Rate (FDR) correction in the fourth step (q=0.05) are shaded gray (correcting for 12 hypothesized test, i.e. the three hypothesized effects [genetic profile scores, life events, and their interaction] predicting activity in four regions).*

**Supplementary Table 3.8: Regression Separating Effects of Stressful and Traumatic Life Events on Left Amygdala Fearful-Neutral Face Activity**

| | b | β | t | p | First-order correlation |
|---|---|---|---|---|---|
| **Constant** | -0.138 | | -3.079 | 0.003 | |
| **Ethnicity** | -0.008 | -0.017 | -0.18 | 0.857 | |
| **Sex** | 0.018 | 0.039 | 0.431 | 0.668 | |
| **Stressful Life Events** | **0.013** | **0.355** | **3.494** | **0.001** | **r(105)=0.395, p<0.001** |
| **Traumatic Life Events** | 0.004 | 0.098 | 0.966 | 0.337 | **r(105)=0.249, p=0.01** |
| **Model $R^2$** | 0.165 | | | | |
| **Adjusted $R^2$** | 0.132 | | | | |
| **Model F** | 5.040 | | | | |
| **Model p** | 0.001 | | | | |

*Unstandardized (b) and standardized (β) regression coefficients and their associated t- and p-value are presented with model statistics listed below. Effects significant at p<0.05 are in bold.*

**Supplementary Table 3.9: Effects of Family Income**

| | Sex | Ethnicity | Pubertal Status | Life Events | Genetic Profile Scores | Left Amygdala Activity | Right Amygdala Activity | Left Hippocampus Activity | Right Hippocampus Activity | Major Depression History | Anxiety Disorder History | Externalizing Disorder History |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Spearman's Rho** | -0.038 | **0.560** | 0.183 | **-0.415** | -0.107 | -0.170 | -0.062 | 0.025 | -0.084 | **-0.283** | **-0.210** | **-0.226** |
| **p-value** | 0.698 | **<0.001** | 0.060 | **<0.001** | 0.273 | 0.081 | 0.525 | 0.801 | 0.388 | **0.003** | **0.030** | **0.019** |

Correlations between total family income at the time of scan and variables of interest are presented. Income is an ordinal variable: 1=<$20,000/year (N=24), 2=$20,001-$40,000 (N=20), 3=$40,001-$60,000 (N=17), 4=>$60,000 (N=46). Effects significant at p<0.05 are in bold and shaded gray. African American families reported lower income. Children with lower family income experienced more stressful/traumatic life events. Children with lower family income were more likely to have a history of all types of psychopathology.

| | Step 1 | | | Step 2 | | | Step 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | **b** | **t** | **p** | **b** | **t** | **p** | **b** | **t** | **p** |
| **Intercept** | 0.123 | 1.979 | 0.050 | 0.137 | 1.967 | 0.052 | -0.076 | -0.845 | 0.400 |
| **Sex** | 0.011 | 0.258 | 0.797 | -0.235 | -1.118 | 0.266 | -0.267 | -1.338 | 0.184 |
| **Ethnicity** | -0.028 | -0.438 | 0.662 | -0.170 | -0.708 | 0.480 | -0.076 | -0.334 | 0.739 |
| **Genetic Profile Scores** | -0.027 | -1.404 | 0.163 | -0.023 | -0.360 | 0.720 | -0.042 | -0.709 | 0.480 |
| ***Family Income*** | -0.033 | -1.488 | 0.140 | -0.043 | -1.828 | 0.071 | -0.019 | -0.807 | 0.422 |
| **GPS x Sex** | | | | 0.064 | 1.804 | 0.074 | 0.063 | 1.869 | 0.065 |
| **GPS x Ethnicity** | | | | 0.007 | 0.114 | 0.909 | -0.015 | -0.266 | 0.791 |
| **Income x Sex** | | | | -0.010 | -0.256 | 0.798 | 0.004 | 0.101 | 0.920 |
| **Income x Ethnicity** | | | | 0.050 | 0.841 | 0.403 | 0.060 | 1.063 | 0.291 |
| **GPS x Income** | | | | 0.001 | 0.062 | 0.951 | 0.012 | 0.526 | 0.600 |
| **Life Events** | | | | | | | **0.008** | **3.452** | **0.001** |

Hierarchical regression results examining potential effects of family income predicting left amygdala fearful-neutral face activity are presented. Income did not significant predict activity nor did its interaction with genetic profile scores (GPS), sex, and ethnicity. Life events was a highly significant predictor of activity (as in the main text regression), even controlling for income. This would imply a relatively specific effect of stressful life events rather than a more general effect of socio-economic status, given the strong relationship between life events and income (see above correlation table).

**Supplementary Table 3.10: Regressions Predicting Fearful-Neutral Face Activity, Controlling for Age Effects**

| | Left Amygdala | | | Right Amygdala | | | Left Hippocampus | | | Right Hippocampus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **t** | **p** | **b** | **t** | **p** | **b** | **t** | **p** | **b** | **t** | **p** |
| **Constant** | 0.148 | 0.646 | 0.519 | -0.166 | -0.661 | 0.510 | -0.003 | -0.020 | 0.984 | 0.117 | 0.699 | 0.486 |
| **Ethnicity** | 0.028 | 0.054 | 0.957 | -0.453 | -0.800 | 0.425 | 0.292 | 0.814 | 0.417 | 0.026 | 0.069 | 0.945 |
| **Sex** | 0.226 | 0.509 | 0.611 | -0.434 | -0.886 | 0.377 | -0.003 | -0.008 | 0.993 | 0.150 | 0.458 | 0.648 |
| **Genetic Profile Scores** | 0.258 | 1.267 | 0.207 | 0.247 | 1.103 | 0.272 | 0.243 | 1.711 | 0.089 | 0.175 | 1.171 | 0.243 |
| **GPS x Ethnicity** | -0.024 | -0.691 | 0.490 | -0.020 | -0.539 | 0.590 | -0.011 | -0.454 | 0.650 | -0.019 | -0.752 | 0.453 |
| **GPS x Sex** | 0.211 | 0.620 | 0.536 | 0.225 | 0.599 | 0.550 | 0.168 | 0.705 | 0.482 | 0.061 | 0.245 | 0.807 |
| **Pubertal Status** | 0.016 | 0.303 | 0.762 | -0.046 | -0.771 | 0.442 | 0.027 | 0.713 | 0.477 | 0.022 | 0.553 | 0.581 |
| **GPS x Puberty** | 0.065 | 1.387 | 0.167 | 0.037 | 0.717 | 0.474 | 0.025 | 0.760 | 0.448 | 0.004 | 0.123 | 0.902 |
| **Puberty x Sex** | 0.167 | 1.534 | 0.127 | 0.074 | 0.622 | 0.535 | 0.107 | 1.411 | 0.160 | 0.132 | 1.646 | 0.102 |
| **Ethnicity x Puberty** | 0.049 | 0.438 | 0.662 | -0.039 | -0.311 | 0.756 | 0.079 | 1.002 | 0.318 | -0.028 | -0.341 | 0.734 |
| **GPS x Puberty x Sex** | **0.201** | **2.351** | **0.020** | 0.178 | 1.892 | 0.060 | **0.122** | **2.039** | **0.043** | **0.174** | **2.757** | **0.007** |
| **Age at Scan** | -0.001 | -0.522 | 0.603 | 0.002 | 0.890 | 0.375 | 0.000 | 0.119 | 0.905 | -0.001 | -0.654 | 0.514 |
| **GPS x Age** | -0.002 | -1.275 | 0.204 | -0.002 | -1.007 | 0.315 | -0.002 | -1.704 | 0.090 | -0.001 | -1.166 | 0.245 |
| **Age x Sex** | -0.002 | -0.422 | 0.674 | 0.004 | 0.992 | 0.323 | 0.000 | 0.099 | 0.921 | -0.001 | -0.459 | 0.647 |
| **Age x Ethnicity** | 0.000 | -0.111 | 0.912 | 0.004 | 0.834 | 0.405 | -0.002 | -0.826 | 0.410 | 0.000 | -0.128 | 0.898 |
| **GPS x Age x Sex** | -0.002 | -0.564 | 0.574 | -0.001 | -0.501 | 0.617 | -0.001 | -0.619 | 0.537 | 0.000 | -0.164 | 0.87 |

*GPS=Genetic Profile Scores. Unstandardized (b) regression coefficients and their associated t- and p-value are presented for the each step of the model. Effects significant at p<0.05 are in bold.*

**Supplementary Table 3.11: Regressions Predicting Fearful-Neutral Face Activity, Controlling for Diagnostic Status**

| | Left Amygdala | | | Right Amygdala | | | Left Hippocampus | | | Right Hippocampus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **t** | **p** | **b** | **t** | **p** | **b** | **t** | **p** | **b** | **t** | **p** |
| **Constant** | 0.038 | 1.495 | 0.138 | 0.027 | 0.900 | 0.370 | 0.027 | 1.466 | 0.146 | 0.013 | 0.704 | 0.483 |
| **Ethnicity** | -0.036 | -0.701 | 0.485 | 0.022 | 0.368 | 0.713 | 0.010 | 0.266 | 0.791 | -0.032 | -0.868 | 0.388 |
| **Sex** | 0.042 | 0.993 | 0.323 | 0.039 | 0.786 | 0.434 | 0.042 | 1.410 | 0.162 | 0.000 | 0.012 | 0.990 |
| **Genetic Profile Scores** | 0.001 | 0.041 | 0.967 | 0.035 | 1.441 | 0.153 | 0.012 | 0.855 | 0.395 | 0.007 | 0.509 | 0.612 |
| **GPS x Ethnicity** | -0.010 | -0.240 | 0.811 | -0.037 | -0.777 | 0.439 | 0.001 | 0.027 | 0.979 | -0.002 | -0.055 | 0.957 |
| **GPS x Sex** | 0.050 | 1.528 | 0.130 | 0.062 | 1.589 | 0.115 | **0.049** | **2.106** | **0.038** | 0.046 | 1.949 | 0.054 |
| **Pubertal Status** | -0.029 | -0.655 | 0.514 | -0.040 | -0.772 | 0.442 | 0.011 | 0.349 | 0.728 | -0.006 | -0.173 | 0.863 |
| **GPS x Puberty** | 0.048 | 1.269 | 0.208 | 0.030 | 0.663 | 0.509 | -0.006 | -0.215 | 0.830 | -0.019 | -0.697 | 0.488 |
| **Puberty x Sex** | 0.115 | 1.340 | 0.184 | 0.158 | 1.549 | 0.125 | 0.117 | 1.919 | 0.058 | 0.069 | 1.110 | 0.270 |
| **Ethnicity x Puberty** | 0.153 | 1.556 | 0.123 | 0.133 | 1.132 | 0.261 | 0.060 | 0.855 | 0.395 | 0.003 | 0.044 | 0.965 |
| **GPS x Puberty x Sex** | **0.211** | **3.150** | **0.002** | **0.215** | **2.699** | **0.008** | **0.109** | **2.291** | **0.024** | **0.151** | **3.123** | **0.002** |
| **Depressive Disorder** | -0.013 | -0.269 | 0.789 | -0.060 | -1.083 | 0.282 | -0.026 | -0.780 | 0.438 | -0.011 | -0.327 | 0.744 |
| **Anxiety Disorder** | **0.158** | **3.338** | **0.001** | **0.184** | **3.268** | **0.002** | **0.079** | **2.341** | **0.021** | **0.073** | **2.129** | **0.036** |
| **Externalizing Disorder** | 0.009 | 0.177 | 0.860 | -0.060 | -1.002 | 0.319 | -0.006 | -0.161 | 0.872 | -0.004 | -0.116 | 0.908 |

*GPS=Genetic Profile Scores. Unstandardized (b) regression coefficients and their associated t- and p-value are presented for the each step of the model. Effects significant at p<0.05 are in bold.*

**Supplementary Table 3.12: Regression Effects within Diagnostic History Groups**

| Regression Effects | | Major Depression History | | Anxiety Disorder History | | Externalizing Disorder History | |
|---|---|---|---|---|---|---|---|
| | | Absent | Present | Absent | Present | Absent | Present |
| | **N** | 52 | 55 | 50 | 57 | 60 | 47 |
| **Effect of Life Events:** | **b** | **0.102** | **0.087** | 0.077 | **0.064** | **0.164** | 0.031 |
| | **p** | **0.025** | **0.003** | 0.109 | **0.030** | **0.000** | 0.280 |
| **Life Events x Diagnostic History Interaction:** | **b** | | -0.015 | | -0.014 | | **-0.132** |
| | **p** | | 0.771 | | 0.816 | | **0.006** |
| **Effect of GPS x Puberty x Sex Interaction:** | **b** | **0.188** | **0.186** | **0.204** | **0.203** | **0.213** | **0.157** |
| | **p** | **0.009** | **0.010** | **0.002** | **0.004** | **0.003** | **0.023** |
| **GPS x Pub. X Sex x Diagnostic History Interaction:** | **b** | | -0.002 | | -0.001 | | -0.055 |
| | **p** | | 0.958 | | 0.983 | | 0.138 |

**Supplementary Table 3.13: GLM Results Among Pubertal Children**

| | Left Amygdala | | Right Amygdala | | Left Hippocampus | | Right Hippocampus | |
|---|---|---|---|---|---|---|---|---|
| | **F** | **p** | **F** | **p** | **F** | **p** | **F** | **p** |
| **Emotion Type** | 1.404 | 0.242 | 0.888 | 0.351 | 3.463 | 0.069 | 3.261 | 0.078 |
| **Emotion Type x Sex** | 4.849 | 0.033 | 4.813 | 0.033 | 6.393 | 0.015 | 2.353 | 0.132 |
| **Emotion Type x Ethnicity** | 0.016 | 0.9 | 0.141 | 0.709 | 0 | 0.99 | 2.353 | 0.132 |
| **Emotion Type x GPS** | 0.318 | 0.576 | 0.619 | 0.435 | 0.067 | 0.797 | 0.29 | 0.593 |
| **Emotion Type x GPS x Ethnicity** | 0.143 | 0.707 | 0.37 | 0.546 | 0.051 | 0.822 | 0.541 | 0.466 |
| **Emotion Type x GPS x Sex** | **8.093** | **0.007** | **8.766** | **0.005** | **6.628** | **0.013** | **7.189** | **0.01** |

*General Linear Model analysis with Emotion Type as a 2-level within-subject factor (Fearful Face vs. Baseline Activity; Neutral Face vs. Baseline Activity), Sex and Ethnicity as between-subject factors, and Genetic Profile Scores (GPS) as a continuous predictor. This analysis only includes pubertal children (N=52). Effects significant at $p<0.05$ are in bold. Degrees of freedom for F-test = (1,46).*

**Supplementary Table 3.14: Regression Predicting Sad-Neutral Face Activity**

| | Left Amygdala | | | | Right Amygdala | | | | Left Hippocampus | | | | Right Hippocampus | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** |
| **Constant** | -0.047 | | -0.970 | 0.334 | 0.068 | | 1.249 | 0.215 | 0.000 | | -0.005 | 0.996 | 0.000 | | -0.002 | 0.998 |
| **Ethnicity** | 0.091 | 0.036 | 0.800 | 0.426 | 0.110 | -0.152 | 0.862 | 0.391 | 0.083 | -0.086 | 1.034 | 0.304 | 0.049 | -0.203 | 0.599 | 0.550 |
| **Sex** | 0.031 | 0.256 | 0.366 | 0.715 | 0.057 | 0.162 | 0.605 | 0.547 | -0.019 | 0.135 | -0.308 | 0.758 | -0.028 | 0.092 | -0.461 | 0.646 |
| **Genetic Profile Scores** | 0.022 | 0.053 | 0.594 | 0.554 | -0.006 | 0.029 | -0.137 | 0.891 | 0.007 | -0.042 | 0.257 | 0.798 | 0.016 | 0.012 | 0.594 | 0.554 |
| **Life Events** | 0.004 | 0.195 | 1.929 | 0.057 | -0.002 | -0.068 | -0.640 | 0.524 | 0.001 | 0.047 | 0.418 | 0.677 | 0.000 | 0.011 | 0.061 | 0.951 |
| **GPS x Ethnicity** | -0.008 | -0.048 | -0.201 | 0.841 | -0.013 | -0.071 | -0.287 | 0.775 | -0.006 | -0.049 | -0.198 | 0.843 | -0.013 | -0.107 | -0.433 | 0.666 |
| **GPS x Sex** | **0.091** | **0.534** | **2.669** | **0.009** | **0.081** | **0.432** | **2.106** | **0.038** | 0.043 | 0.368 | 1.787 | 0.077 | 0.046 | 0.386 | 1.868 | 0.065 |
| **Life Events x Ethnicity** | -0.005 | -0.218 | -0.888 | 0.377 | -0.008 | -0.354 | -1.407 | 0.163 | -0.005 | -0.369 | -1.458 | 0.148 | -0.005 | -0.307 | -1.211 | 0.229 |
| **Life Events x Sex** | 0.002 | 0.089 | 0.464 | 0.644 | -0.001 | -0.026 | -0.134 | 0.894 | 0.002 | 0.163 | 0.827 | 0.410 | 0.003 | 0.173 | 0.876 | 0.383 |
| **GPS x Life Events** | -0.001 | -0.045 | -0.456 | 0.650 | 0.001 | 0.035 | 0.347 | 0.729 | -0.001 | -0.059 | -0.580 | 0.563 | -0.001 | -0.072 | -0.706 | 0.482 |

*GPS=Genetic Profile Scores. Unstandardized (b) and standardized (β) regression coefficients and their associated t- and p-value are presented for the each step of the model. Effects significant at p<0.05 are in bold.*

**Supplementary Table 3.15: Follow-Up Regressions Predicting Sad-Neutral Face Activity**

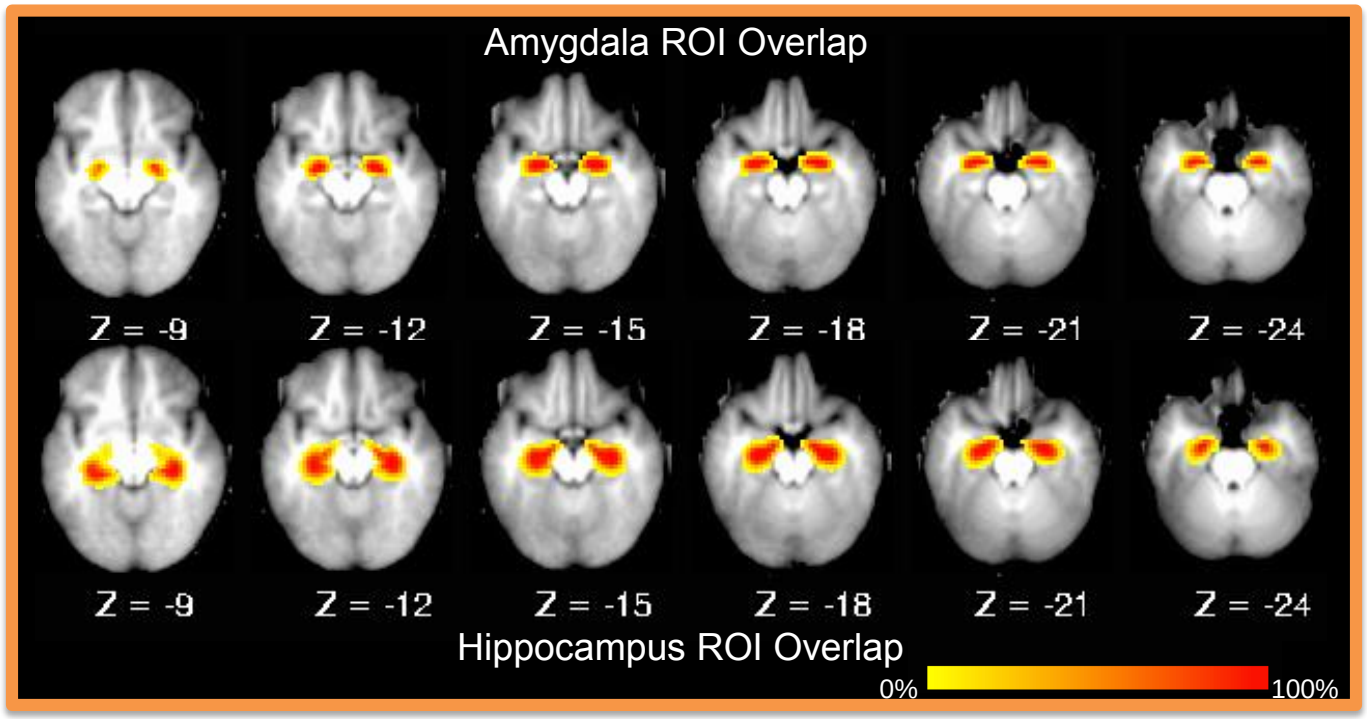
| | Left Amygdala | | | | Right Amygdala | | | | Left Hippocampus | | | | Right Hippocampus | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** | **b** | **β** | **t** | **p** |
| **Constant** | 0.039 | | 1.554 | 0.123 | 0.046 | | 1.621 | 0.108 | 0.023 | | 1.347 | 0.181 | 0.012 | | 0.671 | 0.504 |
| **Ethnicity** | -0.009 | -0.030 | -0.165 | 0.870 | 0.002 | 0.020 | 0.036 | 0.972 | -0.009 | -0.050 | -0.253 | 0.801 | -0.037 | -0.227 | -0.984 | 0.327 |
| **Sex** | **0.089** | **0.361** | **2.066** | **0.041** | 0.071 | 0.264 | 1.471 | 0.145 | 0.047 | 0.276 | 1.600 | 0.113 | 0.029 | 0.159 | 0.930 | 0.355 |
| **Genetic Profile Scores** | 0.017 | 0.099 | 0.816 | 0.417 | 0.024 | 0.131 | 1.058 | 0.293 | 0.005 | 0.043 | 0.361 | 0.719 | 0.006 | 0.049 | 0.389 | 0.698 |
| **GPS x Ethnicity** | -0.022 | -0.130 | -0.555 | 0.580 | -0.034 | -0.185 | -0.769 | 0.444 | -0.014 | -0.119 | -0.516 | 0.607 | -0.017 | -0.138 | -0.568 | 0.571 |
| **GPS x Sex** | **0.096** | **0.561** | **2.865** | **0.005** | **0.075** | **0.405** | **2.014** | **0.047** | **0.048** | **0.412** | **2.131** | **0.036** | **0.049** | **0.407** | **2.004** | **0.048** |
| **Pubertal Status** | 0.012 | 0.036 | 0.276 | 0.783 | -0.044 | -0.197 | -0.896 | 0.373 | 0.033 | 0.199 | 1.108 | 0.271 | 0.007 | 0.040 | 0.227 | 0.821 |
| **GPS x Puberty** | 0.049 | 0.284 | 1.255 | 0.213 | 0.050 | 0.266 | 1.146 | 0.255 | 0.028 | 0.239 | 1.071 | 0.287 | 0.012 | 0.097 | 0.412 | 0.681 |
| **Puberty x Sex** | **0.188** | **0.789** | **2.162** | **0.033** | **0.233** | **0.932** | **2.397** | **0.018** | **0.167** | **1.028** | **2.831** | **0.006** | 0.070 | 0.392 | 1.104 | 0.272 |
| **Ethnicity x Puberty** | 0.145 | 0.645 | 1.426 | 0.157 | 0.172 | 0.704 | 1.518 | 0.132 | 0.136 | 0.880 | 1.975 | 0.051 | 0.060 | 0.377 | 0.805 | 0.423 |
| **GPS x Puberty x Sex** | 0.131 | 0.767 | 1.909 | 0.059 | 0.060 | 0.321 | 0.779 | 0.438 | **0.098** | **0.835** | **2.109** | **0.038** | 0.096 | 0.801 | 1.922 | 0.058 |

*GPS=Genetic Profile Scores. Unstandardized (b) and standardized (β) regression coefficients and their associated t- and p-value are presented for the each step of the model. Effects significant at p<0.05 are in bold.*

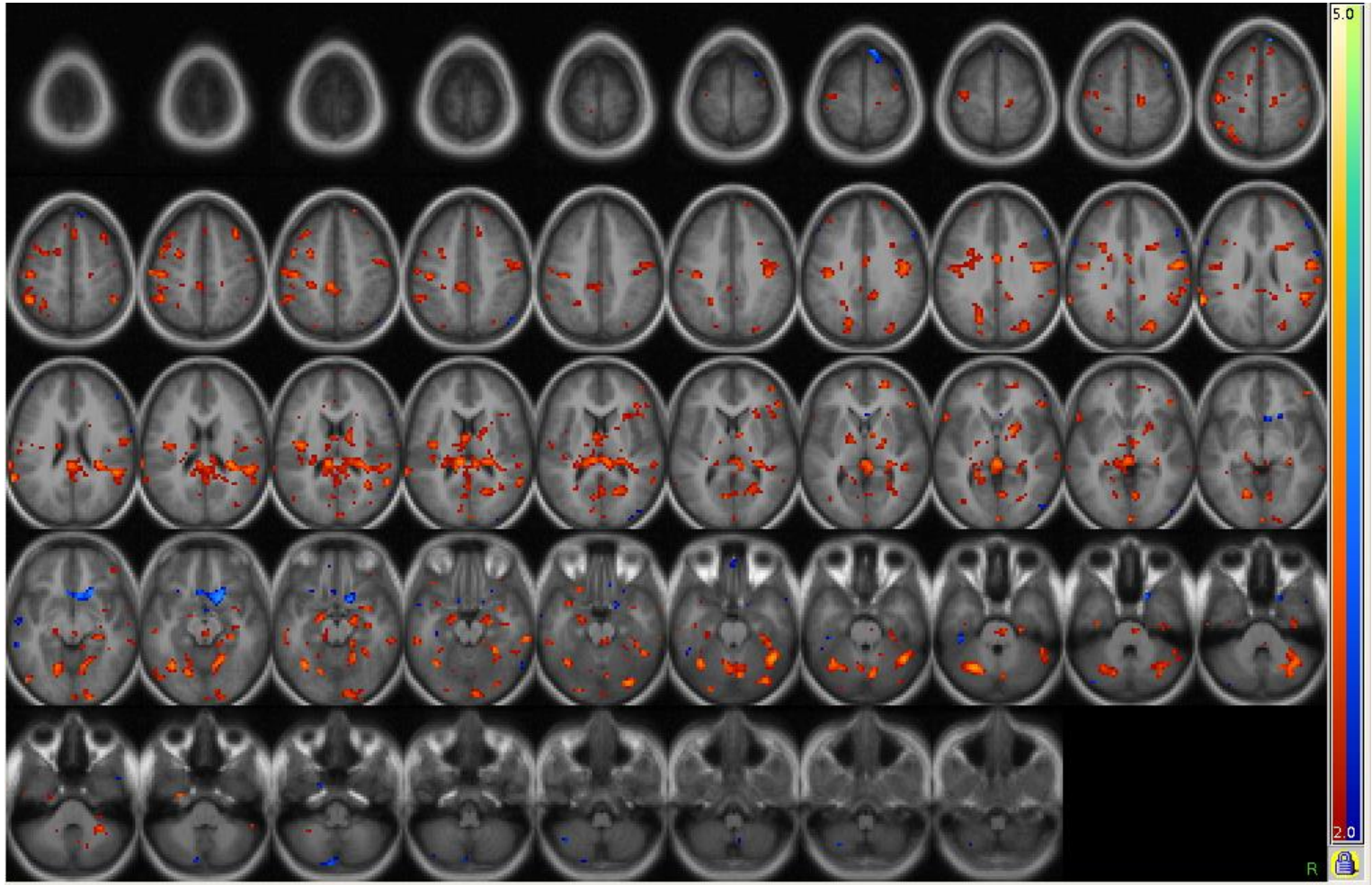
**Supplementary Table 3.16: Effect of Each SNP on Left Amygdala Fearful-Neutral Face Activity, Split by Sex and Pubertal Status**

| | | Prepubertal Boys | | Pubertal Boys | | Prepubertal Girls | | Pubertal Girls | |
|---|---|---|---|---|---|---|---|---|---|
| **SNP** | **Gene** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** |
| **rs4792887** | **CRHR1** | -0.377 | -0.590 | -0.220 | -0.272 | -0.322 | -0.391 | 0.416 | 0.411 |
| **rs110402** | **CRHR1** | -0.634 | -1.294 | 0.152 | 0.294 | 1.024 | 1.814 | 0.503 | 0.801 |
| **rs242941** | **CRHR1** | -0.147 | -0.406 | -0.764 | -1.734 | -0.778 | -2.347 | -0.099 | -0.190 |
| **rs242939** | **CRHR1** | -0.291 | -0.776 | 0.285 | 0.604 | -0.020 | -0.032 | -0.430 | -0.467 |
| **rs1876828** | **CRHR1** | -0.119 | -0.212 | -1.067 | -1.513 | - | - | 0.928 | 0.787 |
| **rs5522** | **NR3C2** | 0.120 | 0.207 | -0.013 | -0.028 | -0.175 | -0.377 | 1.021 | 1.865 |
| **rs41423247** | **NR3C1** | 0.403 | 0.771 | 0.177 | 0.285 | -1.758 | -1.886 | -0.860 | -1.422 |
| **rs10482605** | **NR3C1** | -0.533 | -1.600 | -0.574 | -1.138 | -0.258 | -0.675 | 0.051 | 0.094 |
| **rs10052957** | **NR3C1** | 1.004 | 1.943 | -0.297 | -0.404 | -0.184 | -0.258 | 2.665 | 2.549 |
| **rs1360780** | **FKBP5** | 0.329 | 0.816 | -0.222 | -0.525 | -0.171 | -0.472 | 0.162 | 0.323 |

*These results are provided to aid in the creation of better informed genetic profile scores in future research. These values represent the standardized regression coefficients and t-statistics for each single nucleotide polymorphism in the genetic profile scores predicting left amygdala fearful-neutral face activity, controlling for ethnicity, split by sex and pubertal status. These results were obtained from ten separate regression models rather than one model with all SNPs entered as predictors. Note that the sample sizes for each subgroup were relatively small and, for example, all pubertal girls in this sample had the major rs1876828 allele.*




**Supplementary Figure 3.1: Heat Map of Overlap in Individually Defined Anatomical ROIs**
Color bar represents the percent of participants with anatomically defined amygdala or hippocampal segmentations overlapping at any voxel. Note that while the group level maps show some overlap in the boundaries between the amygdala and hippocampus, there is no overlap between amygdala and hippocampal ROIs for any individual participant, i.e. all overlap is across participants.




**Supplementary Figure 3.2: Whole-Brain Extent of Genetic Profile Scores x Puberty x Sex Interaction Effect**
Voxel-wise z-statistics for the interaction of GPS, puberty, and sex from the main regression analyses performed whole-brain are presented here to show the extent and specificity of the amygdala and hippocampus ROI results. The results are displayed at a minimum z value of 2. Even at this relatively low threshold there are distinct, separable clusters of activity over the amygdala and hippocampus. As we had specific a priori hypotheses, these results are displayed only to show the specific of the effects to our ROIs (Supplementary Figure 3.1); as such, we have not corrected this image for multiple comparisons as it is not meant to be interpreted statistically. Thus, there are a variety of other regions showing activity, at z>2, that may or may not pass correction for multiple comparisons, but could be investigated in future studies.

# Chapter 4: Amygdala Functional Connectivity, HPA Axis Genetic Variation, and Life Stress in Children and Relations to Anxiety and Emotion Regulation

Reference: Pagliaccio, D., Luby, J. L., Bogdan, R., Agrawal, A., Gaffrey, M. S., Belden, A. C., Botteron, K. N., Harms, M. P., and Barch, D. M. (*under review*). Amygdala functional connectivity, HPA axis genetic variation, and life stress in children and relations to anxiety and emotion regulation. Journal of Abnormal Psychiatry.

# 4.1 Abstract

Internalizing pathology has been related to alterations in amygdala resting state functional connectivity, potentially implicating altered emotional reactivity and/or emotion regulation in the etiological pathway. Importantly, there is accumulating evidence that stress exposure and genetic vulnerability impact amygdala structure/function and risk for internalizing pathology. The present study examined whether early life stress and genetic profile scores (10 single nucleotide polymorphisms within four hypothalamic-pituitary-adrenal axis genes: *CRHR1*, *NR3C2*, *NR3C1*, and *FKBP5*) predicted individual differences in amygdala functional connectivity in school-age children (9-14 year olds; N=120). Whole-brain regression analyses indicated that increasing genetic 'risk' predicted alterations in amygdala connectivity to the caudate and postcentral gyrus. Experience of more stressful and traumatic life events predicted weakened amygdala-anterior cingulate cortex connectivity. Genetic 'risk' and stress exposure interacted to predict weakened connectivity between the amygdala and the inferior and middle frontal gyri, caudate, and parahippocampal gyrus in those children with the greatest genetic and environmental risk load. Furthermore, amygdala connectivity longitudinally predicted anxiety symptomology and emotion regulation skills at a later follow-up. Amygdala connectivity mediated effects of life stress on anxiety and of genetic variants on emotion regulation. The current results suggest that considering the unique and interacting effects of biological vulnerability and environmental risk factors may be key to understanding the development of altered amygdala functional connectivity, a potential factor in the risk trajectory for internalizing pathology.

## 4.2 Introduction

Major depressive disorder (MDD) and anxiety disorders are among the most prevalent and disabling psychiatric conditions (Kessler et al., 2005) and are characterized by deficits in emotional and overall adaptive functioning (American Psychiatric Association, 2013). Neuroimaging studies have implicated the amygdala as a key region involved in emotional reactivity (e.g. Sergerie, Chochol, & Armony, 2008) and successful emotion regulation has been suggested to modulate amygdala reactivity (Lapate et al., 2012; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). Importantly, amygdala structure and function are altered in patients with depression and anxiety (for meta-analyses, see Etkin & Wager, 2007; Hamilton et al., 2012; Hamilton, Siemer, & Gotlib, 2008) and have been linked to individual differences in emotional experience and regulation (Abler et al., 2010; Drabant, McRae, Manuck, Hariri, & Gross, 2009). Recent work has examined the functional connectivity of the amygdala with a variety of regions, particularly prefrontal cortex, cingulate cortex, the striatum, and the hippocampus, with the aim of understanding communication between regions that likely subserves effective emotion reactivity and regulation. Disruptions in amygdala connectivity have gained increasing focus as potential intermediate phenotypes between risk factors and psychological outcomes. This type of work aims to identify the mechanisms linking risk factors to pathology by understanding the relations between risk factors and more proximal outcomes, like brain function. Particularly, genetic predispositions and individual environmental factors, such as stressful life events, are among the most potent predictors of depression and anxiety onset (e.g. Kendler, Gardner, & Lichtenstein, 2008; Kendler, Hettema, Butera, Gardner, & Prescott, 2003; Kendler, Karkowski, & Prescott, 1999; Kendler,

Neale, Kessler, Heath, & Eaves, 1992; Kessler, Davis, & Kendler, 1997). As such, the goal of the current study was to investigate whether stressful life events and/or genetic risk factors that influence stress responses also influence amygdala functional connectivity, which in turn may be a potentially mediating factor in the pathway to emotion regulation impairments and pathology, such as anxiety or depression.

**4.2.1 Normative Amygdala Connectivity**: Resting state functional connectivity (rsFC) MRI measures correlations in intrinsic, low frequency fluctuations across brain areas (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995). Levels of rsFC MRI have been suggested to represent the accumulated history of co-activation of brain areas (e.g. Dosenbach et al., 2007; Fair et al., 2007; Kelly et al., 2009). Examination of functional brain connectivity has the potential to provide much useful information about intrinsic functional brain networks and thus many recent studies have examined normative patterns of global rsFC across the brain (e.g. Greicius, Krasnow, Reiss, & Menon, 2003; Power et al., 2011), as well as some work characterizing the development of rsFC (e.g. Power, Barnes, Snyder, Schlaggar, & Petersen, 2012).

Several studies have begun to characterize normative rsFC with the amygdala and identify key networks of brain regions associated with the amygdala, which is of particular importance for studies of emotion and psychopathology. For example, work in adults has suggested normative patterns of positive connectivity (i.e. correlations) between the amygdala and a number of regions, including the hippocampus, insula, thalamus, striatum, and medial frontal gyrus. This work has also identified typical patterns of negative connectivity (anti-correlation) between the amygdala and superior and middle frontal gyrus (MFG), posterior cingulate, parietal regions, and occipital regions, as well as

providing potential evidence for differences in connectivity among amygdala subregions (Roy et al., 2009). These patterns are similar to regions often co-activated with the amygdala in fMRI studies of emotion processing, congruous with the idea that rsFC represents a history of co-activation. Specifically, meta-analyses have identified many of the regions that typically show positive rsFC with the amygdala to also be commonly activated in response to emotional faces (Fusar-Poli et al., 2009) or during emotional memory tasks (Murty, Ritchey, Adcock, & Labar, 2010).

Furthermore, many of the regions typically showing negative connectivity with the amygdala have been suggested to be involved in cognitive reappraisal of emotion and related down-regulation amygdala activity (Buhle et al., 2013; Frank et al., 2014; Kohn et al., 2013), i.e. activity in regions down-regulating amygdala reactivity would tend to be negatively correlated with amygdala activity. While little is known about the normative development of amygdala connectivity during childhood and adolescence, there is evidence that amygdala connectivity with medial PFC (mPFC) may become more positive with age, while connectivity with the insula and posterior cingulate may become more negative with age (Gabard-Durnam et al., 2014). Additionally, related work has suggested that shifts in amygdala-mPFC connectivity from weakly positive to negative may mediate the normative age-related decline in separation anxiety over childhood development (Gee, Humphreys, et al., 2013b).

### 4.2.2 Alterations in Amygdala Connectivity Associated with Internalizing Pathology

: Work in adults has suggested that generalized anxiety disorder may be associated with disruptions in amygdala connectivity to the frontoparietal executive control network and insula- and cingulate-based salience network regions (Etkin,

Prater, Schatzberg, Menon, & Greicius, 2009). Further, anxiety disorder patients may have weaker positive connectivity between the amygdala and medial orbital frontal cortex (OFC), where reduced positive connectivity correlated with increased state anxiety levels (Hahn et al., 2011). Amygdala-mPFC connectivity has also been suggested to be key to emotional function and anxiety disorders (Kim, Loucks, Palmer, Brown, Solomon, Marchante, et al., 2011b) and specifically, elevated state anxiety levels also predict a weakening of positive amygdala connectivity with the vmPFC and of negative connectivity with dmPFC (Kim, Gee, Loucks, Davis, & Whalen, 2011a). Relatedly, weaker amygdala positive rsFC with the insula has been cited in adult depression (Veer, 2010) and with the pregenual region of the anterior cingulate cortex (ACC) in depressed and bipolar adults (Anand, Li, Wang, & Lowe, 2009). Additionally, amygdala functional connectivity (during an emotion task rather than during rest) with regions of the cingulate, inferior frontal gyrus (IFG), and MFG have been suggested to be reduced in MDD patients in several studies (Dannlowski et al., 2009; Lui et al., 2011; Matthews, Strigo, & Simmons, 2008). Further, functional connectivity (during an emotion task) of the amygdala with these regions and the insula, thalamus, caudate, and putamen may strengthen in MDD patients over the course of antidepressant treatment (Chen et al., 2007).

Overall, internalizing pathology has been related to a weakening of typical patterns of amygdala connectivity and there is some suggestion that treatment may strengthen such connectivity. Disruptions in amygdala communication with key regions involved in effective emotional function thus likely contribute to some of the core dysfunctions in mood pathology. These alterations in amygdala connectivity could result from several different factors. For example, alterations in the anatomical connectivity of the brain (e.g.,

white matter pathways) could contribute, though functional connectivity is not isomorphic with structural connectivity (Damoiseaux & Greicius, 2009). Additionally, altered functional co-activation of different brain regions (e.g., hyper-reactivity of the amygdala and/or impaired activation of prefrontal and or cingulate regions) could lead to altered functional connectivity over time and across development.

### 4.2.3 Alterations in Amygdala Connectivity Associated with Risk Factors

**Factors**: Risk factors that are predictive of mood and anxiety disorders have also been associated with altered amygdala functional connectivity. For example, work by (Luking et al., 2011) has suggested that children with a personal and/or maternal history of depression (who are at increased risk for future depression) show reductions in amygdala connectivity with regions typically showing both positive (e.g. parahippocampal gyrus, putamen) or negative connectivity (e.g. MFG, postcentral gyrus). Alterations in amygdala connectivity have also been related to other risk key factors for internalizing pathology, particularly early life stress. For example, childhood emotional maltreatment was shown to be associated with decreased negative connectivity between the right amygdala and the bilateral precuneus and decreased positive connectivity with the left insula, hippocampus, and putamen (van der Werff et al., 2012). Another study found that childhood maltreatment predicted decreased connectivity between the subgenual cingulate and amygdala among female adolescents and hippocampus among males and females (Herringa, Birn, & Ruttle, 2013). Importantly, this connectivity also mediated associations between maltreatment and internalizing symptomology (Herringa et al., 2013). In contrast, other work has suggested that childhood cortisol levels may predict stronger negative amygdala-ventromedial PFC connectivity among females, mediating effects of early life

stress on connectivity (Burghy et al., 2012). Further, elevated baseline cortisol among adults has been related to stronger negative amygdala-mPFC connectivity (Veer et al., 2012). Other work has suggested that deprivation from early life institutionalization predicts more negative amygdala-mPFC connectivity, mediated by cortisol, in a region that showed typical development from positive to negative connectivity across childhood-adolescence (Gee, Gabard-Durnam, et al., 2013a).

Finally, it has been suggested that commonly occurring genetic variants, like the short allele of serotonin transporter promoter polymorphism (Pezawas et al., 2005) and the higher active alleles (3.5 or 4 repeats) of the monoamine oxidase A variable number tandem repeat polymorphism (Dannlowski et al., 2009), may exert effects on amygdala connectivity. Specifically, those carrying these 'risk' variants (these alleles have shown main effects or interactions with environmental factors in predicting increased risk for psychopathology) tend to have weaker amygdala connectivity with regions of the ACC and PFC. While the limited literature relating genetic variants to rsFC has focused mainly on the monoamine/serotonin system, prior work has also implicated hypothalamic-pituitary-adrenal (HPA) axis genetic variants in influencing amygdala structure/function (e.g. Pagliaccio et al., 2013; 2015). Particularly, variants in key genes regulating the reactivity/regulation of the HPA axis have been related to decreases in volume and increases in reactivity to emotional stimuli in the amygdala and hippocampus. These findings might also suggest an impact on amygdala connectivity with other regions as well, however these putative relationships have not yet been tested, but again also build on prior work relating cortisol to amygdala connectivity (e.g. Burghy et al., 2012; Gee, Gabard-

Durnam, et al., 2013a; Veer et al., 2012) and showing that corticosteroid can induce weakening of amygdala connectivity (Henckens, van Wingen, Joëls, & Fernández, 2012).

Overall, this literature suggests that both the presence of internalizing disorders as well as the presence of environmental or genetic risk factors are associated with weakening of amygdala connectivity with key regions implicated in emotion reactivity or regulation. While a variety of studies have examined how environmental or genetic factors predict alterations in amygdala connectivity, as noted above, there have been no studies to our knowledge that examine the interaction of such factors predicting amygdala connectivity or testing whether such connectivity predicts future internalizing disorder symptomology or emotional functioning. Importantly, the role of gene x environment or stress-diathesis interactions is being increasingly considered in studies of risk for psychopathology (e.g. Belsky & Pluess, 2009; Caspi & Moffitt, 2006; Caspi et al., 2003; Moffitt, Caspi, & Rutter, 2006; Rutter, Moffitt, & Caspi, 2006) as these interactions can account for significant variance over and above main effects of genotype and environment. In parallel, intermediate phenotypes, like brain structure and function, have gained attention in the literature as they may provide a powerful means of elucidating the mechanistic pathway from biological and/or environmental risk factors to psychiatric outcomes (for examples of studies examining interactions of stress and HPA axis genes on brain structure/function, see Bogdan, Williamson, & Hariri, 2012; Pagliaccio et al., 2013; 2015; White et al., 2012).

Given this, the goal of the current study was to test whether stress-related environmental and/or genetic risk factors predicted amygdala connectivity in school-age children (N=120 9-14-year-olds) and whether connectivity patterns related to psychiatric

outcomes. Particularly, we present the normative resting state functional connectivity patterns observed in this age range and then tested whether main effects and/or interactions of early life stress exposure and HPA axis genetic variants predicted amygdala connectivity patterns. Based on prior literature linking weakened amygdala connectivity and stress-related risk factors to internalizing pathology, we hypothesized that stress-related factors would predict weaker connectivity between the amygdala and regions typically showing negative connectivity, such as the dorsomedial and lateral PFC and the cingulate, often implicated in the regulation of emotion as noted above, and weaker connectivity with regions typically showing positive connectivity, such as the hippocampus and striatum. We then tested whether these patterns predicted psychopathology or emotion regulation concurrent to scan or at a 1-year follow-up. This built on prior work in this sample linking these stress-related factors to amygdala and hippocampus structure and function (Pagliaccio et al., 2013; 2015).

# 4.3 Materials and Methods

**4.3.1 Participants**: A subsample of participants enrolled in the prospective longitudinal Preschool Depression Study (PDS; total N=306) were included in the current analyses. The PDS is being conducted by the Washington University in St. Louis School of Medicine Early Emotional Development Program (WUSM EEDP); its broad goals are to explore clinical and neural outcomes related to preschool-onset depression. The details of the study methods have been published previously (see, Luby, Si, Belden, Tandon, & Spitznagel, 2009). Briefly, 3- to 5-year old children and their primary caregivers were

recruited from the St. Louis metropolitan area to complete in-depth clinical interviews annually and three neuroimaging sessions with the children. The first imaging wave occurred when children were 7-12 years of age; the current study examines data from the second wave of imaging when children were 9-14 years old (current subsample: mean=11.21 ± 1.24 years). Parental written consent and child assent were obtained prior to study participation and the Institutional Review Board at Washington University approved all experimental procedures.

Of the 182 children who completed the second scan wave, 6 were excluded for poor quality structural scans or missing functional connectivity scans. 32 children were excluded during due to excessive head motion (see fMRI pre-processing section below). Nine children were excluded for missing key measures of interest. Finally, an additional 15 participants who identified as ethnicities other than White or African American were removed from the current analysis to reduce population stratification leaving a final sample size of 120 participants (65 White, 55 African American).

**4.3.2 Diagnostic Assessments**: Trained WUSM EEDP staff conducted up to seven in-person assessments (median=6 assessments) with participants and their parents/guardians from study enrollment through the time of scan and most children had completed a follow-up assessment ~1 year after the scan session (13.73±4.65 months). A reliable and age-appropriate semi-structured parent-report diagnostic interview was used to assess psychiatric symptoms in children younger than 8 years of age, the Preschool-Age Psychiatric Assessment (PAPA; Egger, Ascher, & Angold, 2003). The Childhood and Adolescent Psychiatric Assessment (CAPA; Angold & Costello, 2000) was used when children were 8 years or older, which also includes child-report. Interviews were

audiotaped, reviewed for reliability, and calibrated for accuracy (Luby et al., 2009). This data was used to assess whether children met criteria for relevant psychiatric disorders through the time of scan (Supplementary Table 4.1) and to create continuous measures of depressive disorder, anxiety disorder (generalized anxiety disorder, social anxiety disorder, post-traumatic stress disorder), and externalizing disorder (attention-deficit hyperactivity disorder, conduct disorder, oppositional defiant disorder) symptomology. Data from the PAPA/CAPA were also used to assess the child's experience of stressful and traumatic life events from birth through the scan session (a full list of events and their frequencies is reported in Supplementary Table 4.2). We examined the sum count of instances of these life events in the current analyses. As we had no *a priori* method for weighting individual events and as counts of stressful versus traumatic events were highly correlated ($r(118) = 0.443$, $p<0.001$), all events were summed equally. Parents also reported on their child's emotion regulation abilities using the Emotion Regulation Checklist (ERC; Shields & Cicchetti, 1997) at the assessment wave closest to scan and at the follow-up assessment. We focused on the emotion regulation subscale of the ERC where higher scores indicated better emotion regulation skills in the children. Demographic and clinical information is presented in Table 4.1.

**Table 4.1: Demographic and Clinical Variables**

| | Mean | Std | Minimum | Maximum |
|---|---|---|---|---|
| **Genetic Profile Scores** | 5.508 | 1.193 | 3 | 8 |
| **Stressful and Traumatic Life Events** | 15.917 | 10.398 | 1 | 54 |
| **Age at Scan (months)** | 140.200 | 14.941 | 109 | 179 |
| **Depression Symptomology** | 2.284 | 2.841 | 0 | 15 |
| **Anxiety Symptomology** | 1.530 | 1.749 | 0 | 13 |
| **Externalizing Symptomology** | 2.930 | 4.136 | 0 | 24 |
| **ERC Emotion Regulation Scores** | 28.040 | 3.785 | 17 | 32 |
| | **Counts for Each Subgroup** | | | |
| **Sex** | Female = 58, Male =62 | | | |
| **Ethnicity** | White = 65, African American = 55 | | | |

*Mean, standard deviation (std), minimum, and maximum values are presented for predictors of interest, age at scan, and symptomology and emotion regulation scores. Counts of participants by sex and ethnicity are also presented.*

**4.3.3 Genetic Profile Scores (GPS)**: Extensive details on the rationale, methods, and limitations of our HPA axis genetic profile score (GPS) creation have been published previously (Pagliaccio et al., 2013). In short, prior work documented the utility of additively combining genetic variants to study their polygenic effects on brain structure and function, whereas a single polymorphism alone may not be a significant predictor (Nikolova, Ferrell, Manuck, & Hariri, 2011). The current additive genetic profile scores sum across 10 SNPs within 4 integral HPA axis genes where higher scores indicate more alleles previously associated with increased cortisol, depression prevalence/severity, and/or related phenotypes (e.g. antidepressant treatment response, suicidality, etc.). These SNPs were narrowed down from a larger selection to reduce linkage disequilibrium (all pairwise r2<0.49). In prior work, higher GPS predicted elevated cortisol reactivity to a stressor, indicative of their construct validity (Pagliaccio et al., 2013). The variants of interest included SNPs from *CRHR1* (rs4792887, rs110402, rs242941, rs242939, rs1876828), *NR3C2* (rs5522), *NR3C1* (rs41423247, rs10482605, rs10052957), and *FKBP5* (rs1360780). For more background on each SNP and linkage disequilibrium plots, see (Pagliaccio et al., 2013).

**4.3.4 MRI Scanning**: Participants completed a neuroimaging battery including high-resolution structural, functional task, and resting state scans collected using a 3.0 Tesla TIM TRIO Siemens whole body scanner at Washington University in St. Louis. The resting state data were the focus of the current analysis. T1-weighted structural images were acquired in the sagittal plane using an MPRAGE 3D sequence (TR=2400ms, TE=3.16ms, flip angle=8°, slab=176mm, 176 slices, matrix size=256x256, field of view (FOV)=256 mm, voxel size=1x1x1 mm; interslice skip=0). T2-weighted images were

collected for registration purposes using a 3D SPACE acquisition (TR=3200ms, TE=497ms, 160 slices, FOV=256, voxel size=1x1x1mm).

Up to two resting state fMRI scans were acquired, each including 164 frames (each lasting ~6.8 minutes). Participants were instructed to rest with their eyes closed and to remain awake during the resting state scan. Data were acquired using an asymmetric spin-echo, echo-planar sequence, which was maximally sensitive to blood oxygenation level–dependent (BOLD) contrast (T2*) (TR=2500ms, TE=27ms, FOV=256mm, flip=90°, voxel size=4x4x4mm, slices=36).

**4.3.5 fMRI Pre-Processing**: Imaging data were preprocessed using the following steps: (1) correction for slice-dependent time shifts; (2) removal of first 4 images of each run to allow BOLD signal to reach steady state; (3) elimination of odd/even slice intensity differences due to interpolated acquisition; (4) realignment of data acquired from each participant within and across runs to compensate for rigid body motion (Ojemann et al., 1997); (5) image intensity normalization to a whole-brain mode value of 1000; (6) registration of the 3D structural volume (T1) to an atlas template (WU "711-2B") in the Talairach coordinate system (Talairach & Tournoux, 1988) using a 12-parameter affine transform and re-sampling to 1mm cubic representation (Buckner et al., 2004; Ojemann et al., 1997); (7) co-registration of the 3D fMRI volume to the T2, and the T2 to the participant's structural image; and (8) transformation of the fMRI data to 3x3x3mm voxel atlas space using a single affine 12-parameter transform.

**4.3.6 Functional Connectivity Data Processing**: Resting state functional connectivity processing occurred in three stages using in-house software. First, nuisance variables were regressed from the BOLD data (average signal from the ventricles, white

matter, and whole brain as defined by FreeSurfer segmentation as well as 6 head realignment parameters and their derivates [24 parameters from Volterra series expansion]), a temporal band-pass filter was applied (0.009 Hz < f < 0.08 Hz), and spatial smoothing was applied (6 mm full width at half maximum). Further, average global signal and its derivate were regressed out of the BOLD data, which has been shown to reduce motion and signal artifacts (Power et al., 2012; Power, Barnes, Snyder, Schlaggar, & Petersen, 2013; Power et al., 2014; Satterthwaite et al., 2013).

Next, frames with excess head motion artifact were censored based on frame-wise displacement (FD) as previously described Power et al., (2012). FD is a sum of the absolute values of the 6 linear and rotational head displacement values from the realignment parameters estimated in Step 4 of the above preprocessing (the 3 rotational values are converted to millimeters as displacement on the surface of a sphere of radius 50mm). Volumes with FD greater than 0.2 were censored from all subsequent analyses. Furthermore, runs with less than 40 frames remaining after censoring and participants with less than 110 total frames remaining were excluded from further analyses. Finally, the initial rs-fcMRI processing (nuisance regressors, band-pass filtering, smoothing) was reapplied to the raw data (output of the initial preprocessing) interpolating over the frames censored in the previous stage Power et al., (2013).

**4.3.7 fMRI Analysis**: We used FreeSurfer v5.1 (Fischl et al., 2004; 2002) to create anatomical region of interest (ROI) masks. The amygdala was segmented bilaterally from each participant's T1 anatomical image, down-sampled to match the functional resolution of the atlas space (3x3x3mm), and registered to the common atlas space. These images were summed and a group-level anatomical mask was created by thresholding the

region where at least half of participants had overlap in their amygdala segmentations, allowing a more anatomically precise ROI than relying on atlas ROIs. See Figure 4.1 and Supplementary Figure 4.4.

The time-series from these two ROIs were correlated with the time-series at every other voxel in the brain to create two whole brain voxel-wise correlation maps for each participant. Values in these maps were converted to z-statistics using Fisher's r-to-z transform.

**4.3.8 Statistical Analysis**: First, to establish the overall normative patterns of amygdala connectivity in our sample, two whole-brain one-sample t-tests (null hypothesis = zero) were run using in-house software (FIDL analysis package, http://www.nil.wustl.edu/labs/fidl/index.html; Ollinger, Corbetta, & Shulman, 2001) to characterize significant voxel-wise resting state functional connectivity (r-to-z transformed) with the left or right amygdala. Whole-brain t-test results were thresholded based on Monte Carlo simulations (3dClustSim, afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html) at z≥3 and ≥ 17 contiguous voxels. A summary of peak locations was created using a peak finding program to isolate local maxima/minima in these whole brain thresholded maps and to consolidate nearby peaks less than 20mm from each other.

Next, to explore our main hypotheses of interest, we examined two whole-brain regression analyses predicting voxel-wise rsFC with the left or right amygdala. GPS, life events, and their interaction (GPS x LE) were the predictors of interest, controlling for ethnicity (White vs. African American), sex (females vs. males), and interactions between these covariates and GPS and life events (for discussion of controlling for interactions with

covariates, see (Keller, 2013). The GPS and life events variables were z-scored to center and normalize both variables. Whole-brain z-maps for the effects of GPS, life events, and the GPS x life events interaction were thresholded as above based on Monte Carlo simulations at z≥3 and ≥ 21 contiguous voxels to control for multiple comparisons. Average connectivity values within each significant cluster were extracted for each participant to parse interaction effects and to perform control analyses.

We used linear regressions in IBM SPSS Statistics v20 (Armonk, NY: IBM Corp.) to extract statistics for the above regressions predicting average cluster activity. In post-hoc regression, we then tested whether the effects observed at the whole-brain level remained significant when controlling for a variety of covariates. Particularly, we examined how dimensional scores of depression, anxiety, or externalizing disorder severity at scan and ERC emotion regulation skills related to connectivity. In a final step, we tested whether effects remained significant when controlling for age at scan and interactions between age and GPS or life events. We used the moderation model from the PROCESS tool for SPSS (Hayes, 2013) to parse significant GPS x life events interaction effects by isolating simple slopes.

In cases where there was an association between diagnostic severity or emotion regulation and connectivity, we tested whether that region's connectivity with the amygdala predicted symptom severity (MDD N=98, externalizing N=90, anxiety N=91) or regulation (N=98) by the time of the follow-up wave. This follow-up wave was ~1 year (13.73±4.65 months) after the scan when connectivity was examined. To do this, we ran a linear regression with connectivity predicting the follow-up outcome. In a subsequent step, we tested whether connectivity predicted change in scores by controlling for concurrent

severity or regulation skills and the number of months between the scan and the follow-up. In a final step, we controlled for all other factors in the main regressions, i.e. sex, ethnicity, GPS, life events, and their interactions. In cases where connectivity predicted future outcomes, we tested whether connectivity mediated the association between GPS or life events (whichever predictor identified the ROI) and outcome scores using the PROCESS tool.

# 4.4 Results

**4.4.1 Characterizing Amygdala Connectivity Patterns**: Figure 4.1 and Supplementary Figure 4.1 present the results of whole-brain one-sample t-tests exploring left amygdala connectivity in this sample. Peak coordinates are presented in Supplementary Table 4.3. Right amygdala connectivity is presented in Supplementary Figure 4.2 and Supplementary Table 4.4. Consistent with the prior literature, both the left and right amygdala show strong positive connectivity with much of the subcortex, including the bilateral hippocampus, striatum, and contralateral amygdala as well as the brain stem, posterior insula, and vmPFC. Additionally, the amygdala shows strong negative connectivity with much of the dmPFC, lateral PFC, anterior insula, cingulate cortex, and parietal lobe. The patterns of connectivity for the left and right amygdala were very similar/overlapping.

The whole-brain regression results predicting left amygdala connectivity revealed two significant clusters showing a main effect of GPS (putamen and postcentral gyrus), one cluster in the ACC/mPFC showing a main effect of life events, and four clusters showing a

significant GPS x life events interaction (parahippocampal gyrus, caudate tail, MFG, IFG). Figure 4.1 and Supplementary Figure 4.3 display these regions and Table 4.2 presents coordinates, voxel extents, and t-statistics from one-sample t-tests examining whether rsFC significantly differed from zero on average. No significant clusters were found predicting right amygdala connectivity. Table 4.3 presents regression results predicting connectivity between the left amygdala and each of these regions (averaged across the region) controlling for diagnostic severity and emotion regulation skills at scan (Supplementary Table 4.5 presents unstandardized regression coefficient and confidence intervals for these result). Supplementary Table 4.6 also controls for age effects. We discuss the effects of interest here in the main text and provide more discussion of effects/interactions of covariates in the Supplementary Materials. Additionally, we present regressions separately by ethnicity in Supplementary Table 4.7.

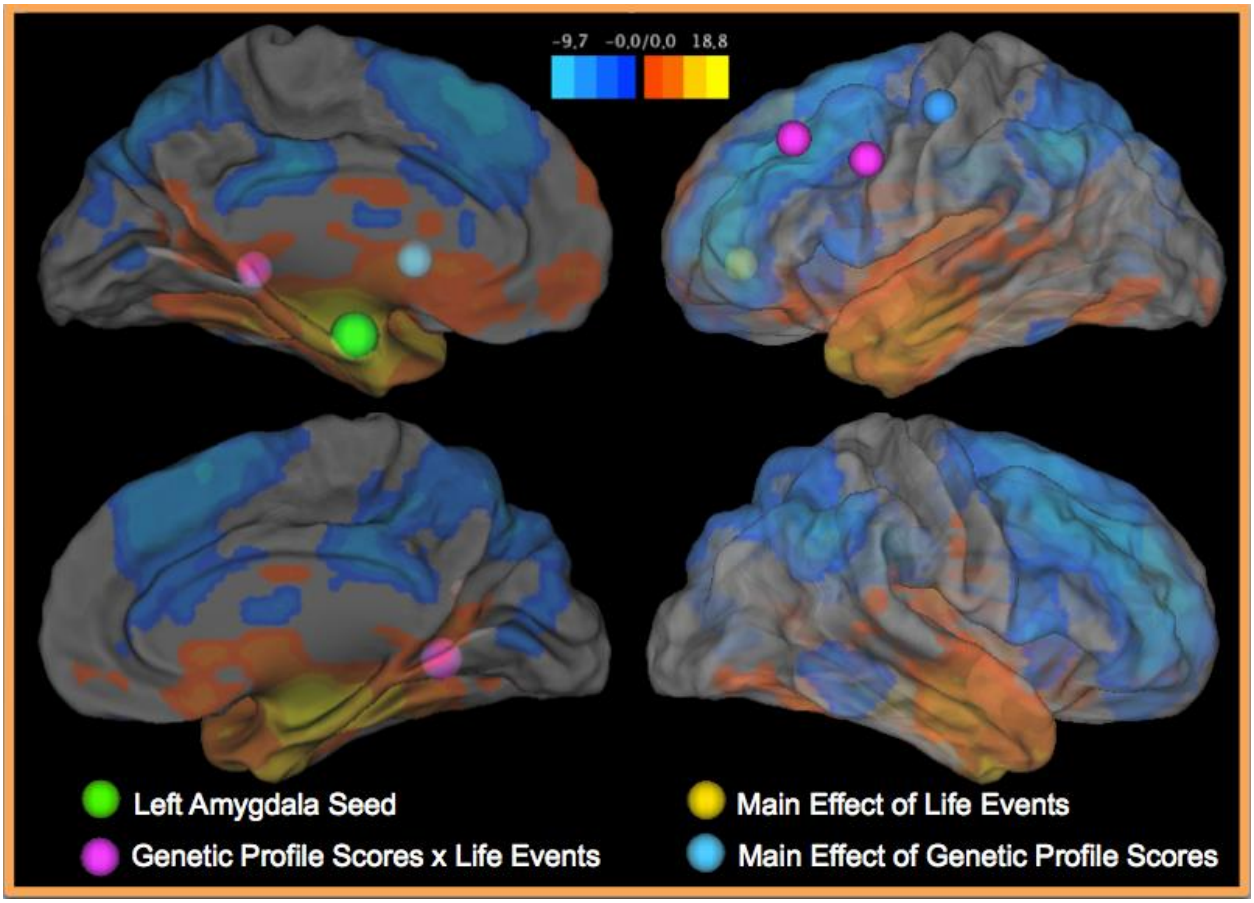



**Figure 4.1: Normative Left Amygdala Connectivity and Regions Showing Significant Regression Effects**

This figure presents a surface rendering of the normative resting state connectivity patterns found with the left amygdala. Specifically, colors on the surface indicate z-statistics for the whole-brain one-sample t-test indicating areas that show significant connectivity with the left amygdala. These results are also presented in axial slices in Supplementary Figure 4.1. The center of left amygdala seed is indicated by a green sphere. Other spheres indicate the peaks of regression effects: blue = main effects of genetic profile scores; yellow = main effects of life events; purple = genetic profile score x life events interactions. Axial slices through these regions are presented in Supplementary Table 4.3.

**Table 4.2: Summary of Clusters Showing Effects in Whole-Brain Regressions**

| Cluster | X | Y | Z | Voxels | BA | Mean r-to-z | t | Effect | Concurrent | Follow-Up |
|---|---|---|---|---|---|---|---|---|---|---|
| *Main Effects of GPS* | | | | | | | | ↑ GPS | | |
| Left Putamen | -16 | 9 | 3 | 41 | - | 0.043 | 3.338** | ↓/weaker connectivity | - | - |
| Left Post-Central Gyrus | -50 | -15 | 48 | 47 | 3 | -0.011 | -0.891 | ↑/weaker connectivity | Ext, Reg | Reg |
| *Main Effect of Life Events* | | | | | | | | ↑ LE | | |
| Left Anterior Cingulate | -20 | 42 | 3 | 23 | 32 | -0.101 | -7.346*** | ↑/weaker connectivity | Anx, Ext | Anx |
| *GPS x LE Interactions* | | | | | | | | ↑ LE with high GPS, ↓LE with low GPS | | |
| Right Parahippocampal Gyrus | 28 | -48 | 0 | 129 | 19 | 0.053 | 4.756*** | ↓/weaker connectivity | Reg | - |
| Left Caudate Tail | -34 | -33 | 0 | 22 | - | 0.093 | 7.683*** | ↓/weaker connectivity | - | - |
| Left Middle Frontal Gyrus | -40 | 27 | 39 | 66 | 8 | -0.139 | -11.563*** | ↑/weaker connectivity | - | - |
| Left Inferior Frontal Gyrus | -50 | 6 | 33 | 22 | 9 | -0.045 | -3.048** | ↑/weaker connectivity | Dep, Anx | - |

*Clusters showing significant effects of genetic profile scores (GPS), life events (LE), or their interaction in the whole brain regressions are listed here. Their peak Talairach co-ordinates (X,Y,Z) for each cluster, voxel extent, and Brodmann area (BA) are presented. The mean r-to-z connectivity values for each cluster with the left amygdala and associated one sample t-statistic testing a null hypothesis of mean zero connectivity are also presented. The direction of effects are summarized along with relationships with concurrent and follow-up scores (Dep=depressive, Anx=anxiety, Ext=externalizing disorder symptomology, Reg=ERC emotion regulation scores)* ↑/↓ = higher or lower values of a variable. ** p<0.05, ** p<0.01, ***p<0.001*

First, GPS negatively predicted connectivity between the left amygdala and the cluster in the putamen. As this region tended to show positive connectivity with the left amygdala on average (Table 4.2), higher GPS predicted weakened connectivity. As shown in Tables 4.3 and Supplementary Table 4.6, this effect remained significant when controlling for all other factors. The post-hoc regression also notes a GPS x life events interaction predicting amygdala-putamen connectivity (simple slopes presented in Supplementary Table 4.8), though this effect was not significant at the level of the whole-brain multiple comparisons cluster correction. In addition, GPS positively predicted connectivity between the left amygdala and the postcentral gyrus. Though this postcentral gyrus cluster showed near zero connectivity at the group level with the left amygdala (Table 4.2), children with high GPS tended to have weak positive connectivity whereas those with low GPS tended to have weak negative connectivity. This effect of GPS held when controlling for all other factors (Tables 4.3 and Supplementary Table 4.6). Additionally, there was a negative association between amygdala-postcentral gyrus connectivity and emotion regulation skills (significant when controlling for effects of age; Supplementary Table 4.6), i.e. more negative connectivity predicted better emotion regulation skills. Finally, we noted a negative association between amygdala-postcentral gyrus connectivity and concurrent externalizing disorders symptomology, i.e. more negative connectivity correlated with higher symptomology.

**Table 4.3: Results of Main Linear Regression Models**

| | Left Putamen | | Left Post-Central Gyrus | | Left Anterior Cingulate | | Right Parahippocampal Gyrus | | Left Caudate Tail | | Left Middle Frontal Gyrus | | Left Inferior Frontal Gyrus | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Predictor** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** |
| **Sex** | 0.258 | 1.436 | 0.038 | 0.206 | -0.203 | -1.184 | 0.043 | 0.255 | 0.209 | 1.156 | -0.140 | -0.742 | -0.245 | -1.308 |
| **Ethnicity** | **-0.808** | **-3.986*****| **0.755** | **3.63***** | -0.229 | -1.181 | -0.039 | -0.203 | -0.043 | -0.210 | 0.142 | 0.663 | 0.034 | 0.161 |
| **GPS** | **-0.421** | **-4.345*****| **0.438** | **4.411***** | -0.074 | -0.798 | -0.110 | -1.194 | -0.124 | -1.269 | 0.051 | 0.504 | 0.075 | 0.738 |
| **Life Events** | 0.141 | 1.435 | 0.168 | 1.676# | **0.501** | **5.347***** | 0.095 | 1.029 | **0.199** | **2.024*** | 0.040 | 0.384 | -0.078 | -0.760 |
| **GPS x Sex** | 0.197 | 1.177 | -0.129 | -0.749 | 0.202 | 1.263 | -0.237 | -1.494 | -0.221 | -1.310 | 0.136 | 0.772 | 0.047 | 0.267 |
| **LE x Sex** | 0.298 | 1.635 | -0.099 | -0.529 | 0.203 | 1.167 | 0.075 | 0.433 | 0.183 | 1.000 | -0.104 | -0.540 | **-0.321** | **-1.689#** |
| **GPS x Ethnicity** | 0.107 | 0.562 | 0.272 | 1.391 | 0.235 | 1.285 | -0.170 | -0.940 | -0.062 | -0.325 | **0.455** | **2.259*** | **0.426** | **2.137*** |
| **LE x Ethnicity** | -0.179 | -0.876 | **0.447** | **2.134*** | **0.417** | **2.134*** | **-1.048** | **-5.417***** | **-0.730** | **-3.550**** | **0.891** | **4.136***** | 0.326 | 1.526 |
| **GPS x LE** | **-0.348** | **-2.693***** | **0.245** | **1.844#** | 0.167 | 1.348 | **-0.755** | **-6.161***** | **-0.631** | **-4.853***** | **0.665** | **4.876***** | **0.500** | **3.703***** |
| **Depressive Symptoms** | 0.041 | 0.444 | 0.020 | 0.207 | -0.031 | -0.352 | -0.101 | -1.155 | -0.150 | -1.621 | 0.029 | 0.298 | **0.166** | **1.721#** |
| **Anxiety Symptoms** | 0.095 | 0.934 | -0.016 | -0.154 | **0.366** | **3.759***** | 0.040 | 0.413 | 0.093 | 0.903 | 0.031 | 0.292 | **-0.192** | **-1.799#** |
| **Externalizing Symptoms** | -0.141 | -1.290 | **-0.294** | **-2.612*** | **-0.298** | **-2.843**** | 0.069 | 0.669 | -0.094 | -0.852 | -0.030 | -0.260 | -0.042 | -0.365 |
| **Emotion Regulation** | 0.105 | 1.117 | **-0.175** | **-1.809#** | -0.106 | -1.171 | **0.167** | **1.865#** | 0.036 | 0.384 | -0.051 | -0.511 | -0.056 | -0.570 |
| | | | | | | | | | | | | | | |
| **$R^2$** | | 0.313 | | 0.276 | | 0.372 | | 0.383 | | 0.304 | | 0.236 | | 0.251 |
| **Adjusted $R^2$** | | 0.229 | | 0.189 | | 0.296 | | 0.308 | | 0.220 | | 0.143 | | 0.160 |
| **Model F** | | 3.746*** | | 3.145** | | 4.882*** | | 5.114*** | | 3.600*** | | 2.544** | | 2.752** |

*Standardized regression coefficients (β) and their associated t-values are presented for all predictors in the main regression results predicting all seven regions of interest. Model R2, adjusted R2, and model F values are presented for each model. Effects that identified each region are shaded gray. Effects with p<0.10 are in bold. GPS= genetic profile scores, LE= life events. ^ GxE effect not significant at whole-brain threshold level. # p<0.10, * p<0.05, **p<0.01, ***p<0.001*

Negative life events showed a strong positive association with left amygdala-ACC connectivity, which remained significant when controlling for all other factors (Tables 4.3 and Supplementary Table 4.6). This region showed strong negative connectivity with the amygdala at the group-level (Table 4.2), and thus the experience of more negative life events predicted weaker/less negative connectivity. Importantly, we also found a significant negative association between connectivity and concurrent externalizing symptomology and a significant positive association with anxiety, i.e. weaker negative connectivity related to greater anxiety symptoms but fewer externalizing symptoms. This effect also interacted with ethnicity where the relationship between connectivity and life events experience was slightly stronger among the White children (Supplementary Table 4.7).

Four clusters showed significant GPS x life events interaction, all of which remained significant when controlling for diagnostic, emotion regulation, and age effects (Tables 4.3 and Supplementary Table 4.6). These interactions all took a similar form where greater life events experience predicted weaker connectivity among children with higher GPS and predicted stronger connectivity among those with low GPS (Figure 4.2, Supplementary Table 4.8). This interaction predicted left amygdala connectivity with the parahippocampal gyrus, which was positive at the group level (Table 4.2). Additionally, there was a trend-level positive association between amygdala-parahippocampal gyrus connectivity and emotion regulation skills (Table 4.3) that reached significance when controlling for age effects (Supplementary Table 4.6), i.e. stronger connectivity predicted better emotion regulation. Connectivity with the caudate tail was also strongly positive at the group level (Table 4.2) and showed a similar GPS x life events interaction. Further, life events

experience positively predicted amygdala-caudate connectivity at mean levels of GPS. Finally, this GPS x life events interaction predicted connectivity between the left amygdala and two left PFC regions, MFG and IFG, which both show strong negative connectivity at the group level (Table 4.2). These interaction effects mirror effects on the parahippocampal gyrus and caudate but with signs reversed (Figure 4.2), i.e. children with the highest GPS and life events experience showed the weakest connectivity (least negative/closest to zero).

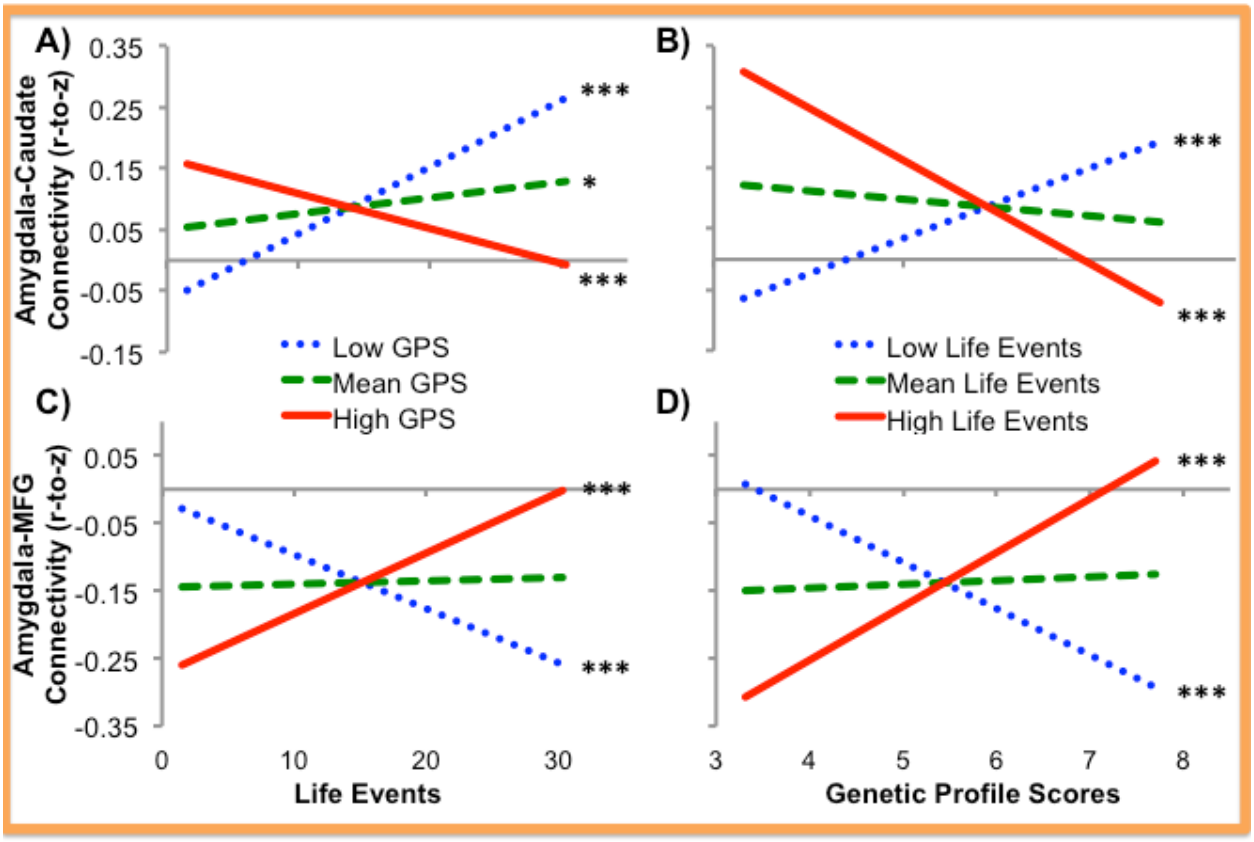



**Figure 4.2: Simple Slope Plots for Interaction Effects**

Simple slope effects of life events (top row) and genetic profile scores (bottom row) predicting two regions showing a genetic profile score x life events interaction on left amygdala connectivity are shown here, specifically a cluster in the caudate (left column) and the middle frontal gyrus (right column). These regions were chosen to exemplify the interaction patterns predicting regions showing typically positive connectivity, e.g. the caudate, or negative connectivity, e.g. the middle frontal gyrus. Simple slopes for each variable were presented at high (mean + 1 SD), mean, and low (mean – 1 SD) levels of the interacting variable and effects controlled for all other variables in the regressions (Table 4.3). * $p<0.05$, ** $p<0.01$, *** $p<0.001$

### 4.4.2 Predicting Symptomology/Emotion Regulation at Follow-Up:

Next, we tested whether any regions that showed an association with concurrent symptomology or emotion regulation skills also predicted future outcomes (Supplementary Table 4.9). Particularly, given the association between left amygdala-postcentral gyrus connectivity and externalizing symptoms and emotion regulation skills, we tested associations with these variables at a follow-up assessment. We found that amygdala-postcentral gyrus connectivity did not predict externalizing symptomology at follow-up, but did negatively predict emotion regulation skills at follow-up. Importantly, amygdala-postcentral gyrus connectivity continued to predict follow-up emotion regulation when controlling for concurrent emotion regulation, i.e. stronger negative connectivity predicted better emotion regulation skills at follow-up. Furthermore, postcentral gyrus connectivity significantly mediated the association between GPS and improvements in emotion regulation skills (Figure 4.3A).

Similarly, we examined associations between left amygdala-ACC connectivity and externalizing and anxiety symptomology at follow-up (Supplementary Figure 4.9). Left amygdala-ACC connectivity did not significant predict externalizing symptomology at follow-up, but positively predicted anxiety symptomology at follow-up, i.e. weaker/less negative connectivity predicted greater anxiety symptomology. This did not remain significant when controlling for concurrent symptomology, i.e. amygdala-ACC connectivity predicted future anxiety symptomology but not change in symptomology (though current symptomology was highly predictive of symptomology at follow-up). Given this relationship and that amygdala-ACC connectivity was predicted by both life event exposure and anxiety symptoms, we tested whether connectivity mediated the relationship between

life events and concurrent symptoms and whether this shared variance predicted follow-up symptoms. Particularly, we found evidence for a significant indirect effect in a serial mediation model where greater life events exposure predicted weaker amygdala-ACC connectivity which predicted higher concurrent anxiety which in turn predicted higher future anxiety (Figure 4.3B). Thus, amygdala-ACC connectivity partially mediated the effects of life events on anxiety symptomology and this likely accounts for connectivity predicting future but not change in symptomology, i.e. connectivity and current symptoms shared variance in predicting future symptomology.

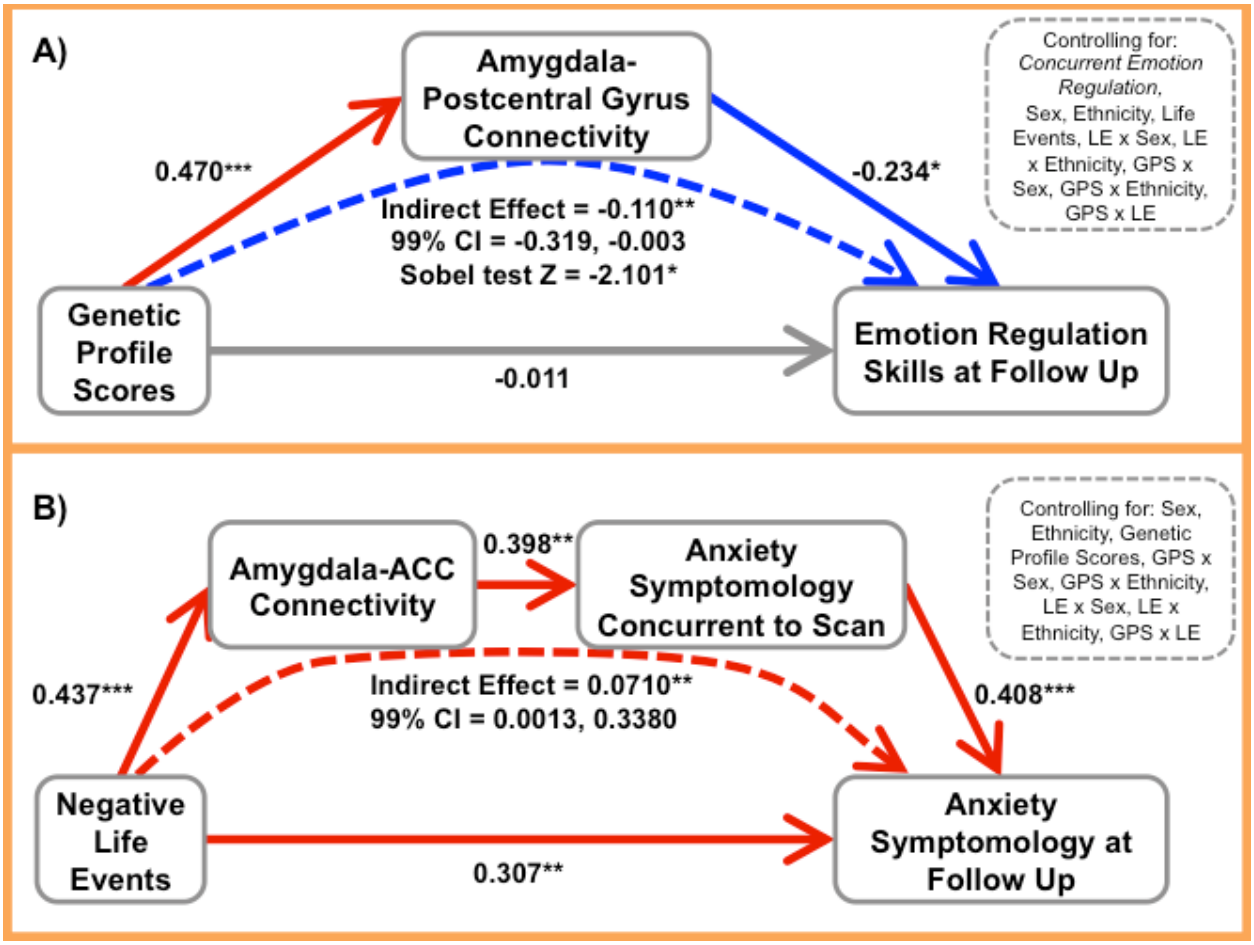



**Figure 4.3: Mediation Models Predicting Outcomes at Follow-Up**

This figure presents a schematic of the mediation results testing two model: (A) left amygdala-postcentral gyrus connectivity mediates the relations between genetic profile scores (GPS) and emotion regulation skills at follow-up and (B) left amygdala-anterior cingulate cortex (ACC) connectivity and concurrent anxiety symptomology act as serial mediators of the effects of life events (LE) on follow-up anxiety symptomology. Standardized regression coefficients (β) are presented for all effects. The path from the independent to dependent variable represents the total effect. * p<0.05, ** p<0.01, *** p<0.001

## 4.5 Discussion

**4.5.1 Summary**: The goal of the current study was test whether normal variation in HPA axis genes and childhood stress exposure predicted or interacted to predict resting state functional connectivity with the amygdala in school-age children. Further, we examined how this connectivity related to concurrent depressive, externalizing, and anxious symptoms and emotion regulation skills and whether connectivity predicted these outcomes ~1 year later. We found that (1) greater HPA axis genetic profile scores predicted weaker/less positive connectivity with the putamen and predicted more positive connectivity with the postcentral gyrus, that (2) greater negative life events experience predicted weaker/less negative connectivity with ACC, and that (3) genetic profile scores and life events experience interacted to predict connectivity with the parahippocampal gyrus, caudate tail, MFG, and IFG where children with the highest GPS and life events showed the weakest connectivity. Finally, (4) connectivity with the postcentral gyrus related to concurrent externalizing symptoms and concurrent and future emotion regulation skills while connectivity with the ACC related to concurrent externalizing symptoms and concurrent and future anxiety symptoms.

**4.5.2 Stress-Related Risk Factors Predicting Connectivity**: The current results indicate that HPA axis genetic variation and early life stress exert main and interacting effects on amygdala resting state connectivity in children. Particularly, increasing risk from these stress-related factors related to weakened connectivity across several frontal and subcortical regions, some of which have shown depression- and anxiety-related alterations in function and connectivity in prior work (e.g. Dannlowski et

al., 2009; Kim, Gee, Loucks, Davis, & Whalen, 2011a; Lui et al., 2011; Matthews et al., 2008). Importantly, while research has related commonly occurring genetic variants modulating the serotonin system to weakened amygdala rsFC (Dannlowski et al., 2009; Pezawas et al., 2005), the current results suggest a key role for HPA axis genetic variation as well. We found main effects of GPS similar to these prior studies, such that increasing genetic 'risk' (more variants previously associated with increased depression and/or cortisol) predicted a weakening of typically positive amygdala-putamen connectivity and predicted more positive amygdala-postcentral gyrus connectivity, which is typically negative in adults (Roy et al., 2009). Furthermore, we found that genetic profile scores interacted with childhood negative life events experience to predict weakened amygdala connectivity, i.e. less positive connectivity with regions typically showing positive connectivity (parahippocampal gyrus and caudate) and less negative connectivity with regions typically showing negative connectivity (MFG and IFG). Specifically, this interaction indicated that increasing life events exposure predicted weaker connectivity particularly among children with high genetic profile scores and vice versa.

Further, we noted a crossover interaction such that in the presence of elevated genetic risk, high life events exposure predicted weak connectivity with the amygdala while in the presence of low genetic risk, high life events exposure predicted stronger connectivity. This type of cross-over interaction has been observed previously in the literature, particularly between environmental stress and several of the genes in our profile scores in prior work (e.g. Bogdan et al., 2012; Klengel et al., 2012). These type of results have pushed the field to re-conceptualize many genetic 'risk' factors as 'for-better-or-for-worse' plasticity factors, which may be detrimental in poor environmental conditions, but

adaptive in healthy/beneficial environments (Belsky et al., 2009). Additionally, it is important to point out that only one of these four regions showed a significant main effect of life events or GPS in the regressions (life events predicted amygdala-caudate connectivity at means levels of GPS). Thus, examining gene x environment interactions can be critical, as these stress-related alterations may not have been identified in a study examining only environmental or genetic risk factors independently.

**4.5.3 Negative Connectivity Regions**: The current results are in line with many prior studies linking depression/anxiety to weakening of both typical positive and negative amygdala connectivity. For example, prior work found that children with a personal and/or maternal history of depression showed reductions in amygdala connectivity with similar regions, including the parahippocampal gyrus, MFG, putamen and postcentral gyrus (Luking et al., 2011). Based on the idea that rsFC represents a cumulative history of co-activation, weakened negative amygdala-PFC connectivity potentially can be understood in the context of poor emotion regulation skills, e.g. less PFC down-regulation of amygdala reactivity relates to less successful emotion regulation (Wager et al., 2008) potentially leading to weaker negative rsFC over time. Particularly, the regions identified in the current study that showed negative connectivity with the amygdala, i.e. the ACC, MFG, and IFG, have been implicated in the regulation of emotion (and of amygdala activity) (e.g. Ochsner, Bunge, Gross, & Gabrieli, 2002; Ochsner et al., 2004, for meta-analysis see Frank et al., 2014). Further, regulation-related activity in postcentral gyrus, MFG, and other regions tends to show normative change across development (McRae et al., 2012). Our results regarding the postcentral gyrus also support this explanation as stronger negative amygdala-postcentral gyrus connectivity predicted better emotion regulation skills at scan

and improvements in emotion regulation at the follow-up assessment. While we did not observe strong negative connectivity at the group level between the amygdala and postcentral gyrus, negative connectivity is typical of healthy adults (Roy et al., 2009), and development of this negative connectivity is thus potentially adaptive, relating to improve emotion regulation skills.

**4.5.4 Positive Connectivity Regions**: As noted above, prior work has implicated weakened positive connectivity in internalizing disorders, though the functional meaning of this need to be explored further in the future. Particularly, typical limbic hyper-reactivity to emotional stimuli in depression/anxiety (for meta-analyses, see Etkin & Wager, 2007; Groenewold, Opmeer, de Jonge, Aleman, & Costafreda, 2012) might suggest the hypothesis that greater co-activation of the amygdala and other subcortical regions over time in patients with internalizing disorders would predict stronger positive connectivity between the amygdala and these regions. However, given the evidence for weakened positive connectivity observed here with regions often implicated in processing of emotional face stimuli (Fusar-Poli et al., 2009) and similar findings in previous literature focused on internalizing psychopathology (e.g. Chen et al., 2007; Hahn et al., 2011), alternative explanations are needed. For example, one possibility is that specific disruptions in PFC regulation of amygdala activity could lead to uncoupling of amygdala activity from other subcortical responses to emotional stimuli. Further, it will be important to explore whether this is an alteration in intrinsic amygdala connectivity or whether it develops with age and experience.

**4.5.5 Associations with Symptomology and Emotion Regulation**: The current results also suggest associations between amygdala resting state connectivity and

concurrent or future psychiatric outcomes. Particularly, weaker/less positive connectivity with the parahippocampal gyrus was related to worse emotion regulation skills at the time of the scan. As the parahippocampal gyrus typically shows positive connectivity with the amygdala in adults (Roy et al., 2009) and in this sample, the current results suggest that weakened connectivity or decoupling of these regions is associated with poor emotional outcomes. As noted above, the functional implications of this weakened connectivity need to be explored further, particularly to understand its role in the development of internalizing symptomology. Relatedly, less negative connectivity with the postcentral gyrus was related to less externalizing symptomology at scan but worse emotion regulation skills at scan and worsening of emotion regulation over time. Furthermore, amygdala-postcentral gyrus connectivity served as a mediator of the effect of GPS on worsening of emotion regulation skills. This result presents a potential mechanism by which HPA axis genetic variation may influence one's emotional functioning via alterations in amygdala connectivity, likely by moderating one's intrinsic HPA axis reactivity/regulation in the face of environmental stressors.

Finally, weaker/less negative connectivity with the ACC was related to greater anxiety symptomology but less externalizing symptomology. While amygdala-ACC connectivity did not significantly predict future externalizing symptomology, it did predict future anxiety symptoms. Further, connectivity and concurrent anxiety acted as serial mediators of the effect of life events on later anxiety symptomology, i.e. greater negative life events exposure predicted weaker amygdala-ACC connectivity, which in turn predicted worse anxiety symptomology at scan and at the subsequent follow-up. Thus, amygdala-ACC connectivity shared variance with concurrent symptomology in predicting later anxiety.

Nonetheless, amygdala connectivity likely plays a role in the effects of childhood stress experience on the development of anxiety. Prior work has, for example, suggested that changes in amygdala-mPFC may mediate normative age-related changes in anxiety (Gee, Humphreys, et al., 2013b). Thus, early stress may act on this circuit to perturb normative developmental trajectories. Overall, we find that weaker amygdala connectivity, be that less positive connectivity with the parahippocampal gyrus or less negative connectivity with the ACC or postcentral gyrus, related to poor emotional outcomes, i.e. worse emotion regulation scores or greater anxiety. Interestingly, we find the opposite effect with externalizing symptomology, though connectivity did not predict externalizing symptomology at follow-up. This should be examined further to determine the specificity and generalizability of these associations. Additionally, it is important to note symptomology and emotion regulation likely relate to amygdala connectivity with other regions not identified here, as our focus was on connectivity patterns relating to stress-related risk factors. Thus, other normative relations to symptoms or emotional regulation in children should be explored further in future studies.

**4.5.6 Normative Connectivity**: While the goal of the current study was not specifically to characterize the normative resting state connectivity patterns of the amygdala in school-age children, we presented this data for reference to aid future work. Consistent with prior work (Gabard-Durnam et al., 2014; Roy et al., 2009), we found that the left and right amygdala showed significant positive connectivity with the much of the subcortex (e.g. hippocampus and striatum), the brainstem, the posterior insula, the anterior temporal lobe, and part of the vmPFC whereas the left and right amygdala show negative connectivity with much of the dmPFC, lateral PFC, anterior insula, cingulate

cortex, and parietal lobe. The left and right amygdala show very similar patterns of connectivity only differing slightly in the strength of association with contra/ipsilateral regions, i.e. the left amygdala tended to show slightly stronger connectivity with left hemisphere regions than the right amygdala and vice versa. Despite this very similar connectivity, we only noted associations between stress-related risk factors and left amygdala connectivity. Though this left-lateralization of effect has also been observed in some prior work, e.g. examining effects of antidepressant treatment on amygdala functional coupling (Chen et al., 2007).

These normative connectivity patterns may be useful for future research given that the literature characterizing normative resting state amygdala connectivity has focused primarily on adults (Roy et al., 2009) or consistencies/differences across development (Gabard-Durnam et al., 2014). The current patterns suggest that amygdala connectivity in childhood is quite similar to that shown in adulthood (Roy et al., 2009). While normative connectivity in this specific age range has not been established previously, the current patterns are also consistent with patterns of connectivity previously observed across development controlling for age (Gabard-Durnam et al., 2014). Gabard-Durnam et al., (2014) also noted age-related differences in amygdala connectivity, specifically more positive connectivity with regions of MFG and ACC with increasing age and more negative connectivity with posterior cingulate, insula, superior temporal gyrus, inferior parietal lobe, and parahippocampal gyrus. We did not observe any significant main effects of age on connectivity with any of the regions identified in the current study. This is consistent with prior work as the regions identified here generally fell within the connectivity patterns observed by Gabard-Durnam et al., controlling for age (rather than changing with age).

Nonetheless, there are likely age-related differences in amygdala connectivity in the current sample/age range but with regions other than those identified based on relations with life events and/or GPS.

**4.5.6 Limitations and Future Directions**: First, there are several limitations to using single summary variables for genetic variation or stressful/traumatic life events, as has been discussed previously in greater detail (Pagliaccio et al., 2013). While combining across multiple sources of variance and reducing the number of tests performed can increase power, it assumes that the effects of stressors/SNPs sum additively with equal weights. Optimizing the relative weighting of events or SNPs can be very useful for future studies; to this end, we have previously presented SNP-wise relations with cortisol reactivity, amygdala and hippocampus volumes (Pagliaccio et al., 2013), and amygdala reactivity to fearful-neutral faces (Pagliaccio et al., 2015). Additionally, as we did not have an a priori method for weighting different life events or differential hypotheses about stressor severity/trauma, we combined across all events assessed. This could be explored further in the future to assess the specificity or magnitude of effects of certain types of stressors/traumas or to assess the effect of stressor timing during development on connectivity alterations.

We were also limited in our ability to examine change in diagnostic status across development. While examining change in more continuous variables can be more powerful, studying the onset of or presence/absence of a diagnosis has been a focus in the field to date. While we were limited in our ability to examine this in the current study (e.g. only four children with no prior history of MDD through the time of scan that had developed

MDD by the time of the follow-up assessment), this could be examined in the future when further diagnostic longitudinal data is available.

**4.5.7 Conclusions**: The current study finds that increasing negative life events exposure and HPA axis genetic 'risk' factors predict and interact to predict weakened amygdala resting state functional connectivity in school-age children. Particularly, these factors predicted weaker negative connectivity between the amygdala and regions of prefrontal cortex and postcentral gyrus and weakened positive connectivity with the parahippocampal gyrus and striatum. Further, these connectivity patterns were associated with anxiety disorder symptomology and emotion regulation skills. Overall, these results suggest that amygdala connectivity may place a key role in the mechanism between stress-related risk factors and the development of internalizing psychopathology.

# 4.6 Supplementary Materials

### 4.6.1 Further Explanation of Main Effects and Interactions with Covariates:

**GPS Regions:** Amygdala-putamen connectivity showed a main effect of ethnicity (Table 4.3 and Supplementary Table 4.6), such that amygdala-putamen connectivity was weaker on average among the White children (Supplementary Table 4.7).

Amygdala-postcentral gyrus connectivity showed a significant main effect of ethnicity (Table 4.3 and Supplementary Table 4.6), such that connectivity was more positive among White children (Supplementary Table 4.7). There was also a weak life events x ethnicity interaction (Table 4.3) where the main effect of life events trended

towards positively predicting connectivity among White but not African American children (Supplementary Table 4.7).

**LE Region:** Amygdala-ACC connectivity showed an age x life events interaction (Supplementary Table 4.6). This effect was only significant when controlling for all factors in the main regression and diagnostic/ERC scores, but tended to indicate a stronger effect of life events on connectivity among older vs. younger children. Additionally, a life events x sex interaction also became significant when controlling for age effects (Supplementary Table 4.6), where life events positively predicted connectivity stronger among females than males (simple slopes: females: $\beta=0.689$, $t=5.198$, $p<0.001$; males: $\beta=0.148$, $t=1.086$, $p=0.280$).

**GPS x LE Regions:** Amygdala-parahippocampal gyrus connectivity showed a life events x ethnicity interaction (Table 4.3 and Supplementary Table 4.6) where life events were a significant negative predictor among African American children (Supplementary Table 4.7).

Amygdala-caudate connectivity showed a life events x ethnicity interaction (Table 4.3 and Supplementary Table 4.6) where life events experience positively predicted connectivity at mean levels of GPS more strongly for African American children (Supplementary Table 4.7).

Amygdala connectivity with the IFG and MFG both showed GPS x ethnicity interactions (Table 4.3 and Supplementary Table 4.6) where GPS showed a trend-level positive relationship with connectivity among White children but a non-significant (Supplementary Table 4.7), negative relationship among African American children. There was also a life events x ethnicity interaction (Table 4.3 and Supplementary Table 4.6)

predicting amygdala-MFG activity, similarly where life events positively predicted connectivity among White children but negatively predicted among African American children (Supplementary Table 4.7).

**Supplementary Table 4.1: Diagnoses through Time of Scan**

| N = 120 | # |
|---|---|
| **No Disorders** | 48 |
| **Any Anxiety Disorder** | 54 |
| **Generalized Anxiety Disorder** | 25 |
| **Post-Traumatic Stress Disorder** | 6 |
| **Separation Anxiety Disorder** | 25 |
| **Obsessive Compulsive Disorder** | 14 |
| **Panic Attack** | 4 |
| **Panic Disorder With Agoraphobia** | 1 |
| **Panic Disorder Without Agoraphobia** | 0 |
| **Agoraphobia Without Panic** | 2 |
| **Social Phobia** | 26 |
| **Any Externalizing Disorder** | 41 |
| **Attention Deficit Hyperactivity Disorder** | 27 |
| **Oppositional Defiant Disorder** | 31 |
| **Conduct Disorder** | 19 |
| **Major Depressive Disorder** | 48 |
| **PO-MDD #** | 34 |

*This table displays the specific diagnoses that children may have met criteria for at any assessment through the time of scan. Diagnoses are not mutually exclusive, so the counts across diagnoses add to more than the total 120 children. To clarify the observed co-morbidity through the time of scan, 48 children had no diagnoses by the time of scan, 23 children were diagnosed with either an anxiety disorder, an externalizing disorder, or MDD, 29 child had diagnoses from two disorder types, and 26 children had diagnoses of anxiety disorders, externalizing disorders, and MDD.*

*# PO-MDD is preschool-onset depression diagnosed before age 6.*

**Supplementary Table 4.2: Reported Instances of Stressful and Traumatic Life Events**

| | Count of Participants by # of Instances | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Stressful Life Events** | **Missing** | **0** | **1** | **2** | **3** | **4** | **5** | **6+** |
| Broke Up with Best Friend | 26 | 86 | 8 | | | | | |
| Broke Up with Boy/Girlfriend | 1 | 114 | 4 | 1 | | | | |
| Change Daycare/School | 0 | 18 | 40 | 34 | 14 | 7 | 4 | 3 |
| Conflict Between Parents/Family | 28 | 82 | 2 | 4 | 1 | | 1 | 2 |
| Death of Pet | 0 | 26 | 17 | | | | | 1 |
| Forced Separation from Home | 0 | 112 | 6 | 1 | | 1 | | |
| Lived/Attended School in Unsafe Environment | 0 | 108 | 10 | 1 | 1 | | | |
| Loss of Home Without Family Separation | 28 | 86 | 6 | | | | | |
| Lost Significant Person Through Moving | 0 | 78 | 34 | 6 | 1 | 1 | | |
| Moving House | 0 | 28 | 32 | 21 | 18 | 12 | 4 | 5 |
| New Child in Home | 0 | 38 | 38 | 31 | 4 | 6 | 2 | |
| New Parental Figure | 0 | 92 | 21 | 4 | 3 | | | |
| Parental Arrest | 0 | 92 | 19 | 7 | 1 | 1 | | |
| Parental Divorce | 0 | 102 | 16 | 2 | | | | |
| Parental Hospitalization | 28 | 37 | 40 | 9 | 2 | 3 | | 1 |
| Parental Separation | 0 | 77 | 33 | 8 | 2 | | | |
| Reduction in Standard of Living | 0 | 81 | 21 | 12 | 6 | | | |
| Separation From Parent (1 week or more) | 28 | 55 | 26 | 6 | 2 | 3 | | |
| **Traumatic Life Events** | | | | | | | | |
| Accident or Crash with Automobile, Plane, or Boat | 0 | 92 | 26 | 2 | | | | |
| Accidental Burning, Poisoning, or Drowning | 9 | 80 | 9 | 2 | | | | |
| Attacked by an Animal | 29 | 84 | 7 | | | | | |
| Death of Adult Loved One | 1 | 29 | 21 | 23 | 21 | 11 | 7 | 7 |
| Death of Sibling or Peer | 0 | 97 | 17 | 4 | 2 | | | |
| Diagnosed with Physical Illness | 0 | 92 | 23 | 4 | 1 | | | |
| Domestic Violence | 114 | 3 | 1 | 1 | | | | 1 |
| Hospitalization, Emergency Room Visit, or Invasive Medical Procedure | 0 | 62 | 32 | 11 | 4 | 1 | 2 | 4 |
| Learned about Traumatic Event | 0 | 86 | 22 | 10 | 2 | | | |
| Man-made Disasters (fire,war,terrorism) | 0 | 115 | 2 | 2 | 1 | | | |
| Natural Disasters (flood,hurricane,tornado,earthquake) | 0 | 86 | 25 | 7 | 1 | 1 | | |
| Physical Abuse | 0 | 116 | 4 | | | | | |
| Sexual Abuse, Sexual Assault, or Rape | 0 | 116 | 2 | 1 | | | | 1 |
| Victim of Physical Violence | 0 | 117 | 3 | | | | | |
| Witnessed Another Person Being Threatened with Harm, Seriously Injured, or Killed | 0 | 90 | 22 | 8 | | | | |

*This table presents a list of stressful and traumatic life events assessed during the diagnostic interviews. The count of participants reporting different cumulative numbers of instances of each event through the time of scan are presented along with the number of missing/not reported values.*

**Supplementary Table 4.3: Peaks of Normative Connectivity with the Left Amygdala**

| X | Y | Z | Voxels | Z | Side | Lobe | Region | BA |
|---|---|---|---|---|---|---|---|---|
| *Peaks showing positive connectivity with the left amygdala* | | | | | | | | |
| -27 | 28 | -8 | 232 | 7.2 | L | Frontal | Inferior Frontal Gyrus | 47 |
| 30 | 28 | -5 | 200 | 5.6 | R | Frontal | Inferior Frontal Gyrus | 47 |
| -2 | 50 | -4 | 213 | 6.1 | L | Frontal | Medial Frontal Gyrus | 10 |
| 0 | 50 | 46 | 194 | 5.7 | L | Frontal | Medial Frontal Gyrus | 8 |
| -9 | 61 | 28 | 129 | 6.3 | L | Frontal | Superior Frontal Gyrus | 9 |
| 0 | 23 | -6 | 225 | 5.6 | L | Limbic | Anterior Cingulate | 24 |
| 4 | 1 | -2 | 314 | 8.1 | R | Limbic | Anterior Cingulate | 25 |
| -21 | -10 | -15 | 803 | 20.2 | L | Limbic | Parahippocampal Gyrus | 34 |
| 24 | -11 | -14 | 600 | 16.6 | R | Limbic | Amygdala | - |
| -15 | -45 | 10 | 456 | 6.1 | L | Limbic | Posterior Cingulate | 29 |
| 15 | -44 | 7 | 358 | 7.3 | R | Limbic | Posterior Cingulate | 29 |
| -41 | -70 | -7 | 145 | 6.8 | L | Occipital | Inferior Occipital Gyrus | 19 |
| -21 | -95 | -10 | 39 | 3.3 | L | Occipital | Inferior Occipital Gyrus | 17 |
| 27 | -95 | -3 | 56 | 4.0 | R | Occipital | Lingual Gyrus | 18 |
| 49 | -80 | 8 | 201 | 4.9 | R | Occipital | Middle Occipital Gyrus | 19 |
| 55 | -10 | 47 | 142 | 5.7 | R | Parietal | Postcentral Gyrus | 3 |
| -14 | 13 | 16 | 147 | 6.6 | L | Sub-lobar | Caudate Body | - |
| 16 | 13 | 17 | 95 | 3.6 | R | Sub-lobar | Caudate Body | - |
| -34 | -22 | 16 | 492 | 9.0 | L | Sub-lobar | Insula | 13 |
| 32 | -23 | 25 | 230 | 8.6 | R | Sub-lobar | Insula | 13 |
| 33 | 2 | 17 | 164 | 6.2 | R | Sub-lobar | Insula | 13 |
| -1 | -7 | 19 | 106 | 6.9 | L | Sub-lobar | Thalamus | - |
| -35 | 6 | -22 | 931 | 11.9 | L | Temporal | Superior Temporal Gyrus | 38 |
| -51 | -12 | -1 | 727 | 9.4 | L | Temporal | Superior Temporal Gyrus | 22 |
| -51 | -33 | 12 | 255 | 5.6 | L | Temporal | Superior Temporal Gyrus | 41 |
| 36 | 6 | -26 | 760 | 12.2 | R | Temporal | Superior Temporal Gyrus | 38 |
| 48 | -4 | -9 | 549 | 8.3 | R | Temporal | Superior Temporal Gyrus | 22 |
| 53 | -28 | 12 | 169 | 6.4 | R | Temporal | Superior Temporal Gyrus | 41 |
| 63 | -8 | 8 | 72 | 3.8 | R | Temporal | Superior Temporal Gyrus | 22 |
| -9 | -33 | -26 | 580 | 10.0 | L | Cerebellum | Culmen | - |
| 28 | -34 | -18 | 599 | 11.2 | R | Cerebellum | Culmen | - |
| 10 | -38 | -37 | 369 | 5.9 | R | Cerebellum | Cerebellar Tonsil | - |
| 8 | -59 | -36 | 151 | 4.3 | R | Cerebellum | Cerebellar Tonsil | - |
| -13 | -61 | -16 | 192 | 3.5 | L | Cerebellum | Declive | - |
| 35 | -64 | -8 | 189 | 5.2 | R | Cerebellum | Declive | - |
| -13 | -61 | -42 | 137 | 5.2 | L | Cerebellum | Inferior Semi-Lunar Lobule | - |

*The co-ordinates (X,Y,Z), voxel extent, and Z-value from the whole-brain t-test, and Brodmann Area (BA) are presented for peaks of clusters showing significant normative connectivity with the left amygdala.*

## Supplementary Table 4.3, Continued

| X | Y | Z | Voxels | Z | Side | Lobe | Region | BA |
|---|---|---|---|---|---|---|---|---|
| *Peaks showing negative connectivity with the left amygdala* | | | | | | | | |
| -45 | 17 | 4 | 365 | -6.7 | L | Frontal | Inferior Frontal Gyrus | 45 |
| 44 | 20 | 0 | 285 | -6.6 | R | Frontal | Inferior Frontal Gyrus | 47 |
| 46 | 12 | 21 | 285 | -5.9 | R | Frontal | Inferior Frontal Gyrus | 9 |
| 6 | 26 | 46 | 628 | -10.0 | R | Frontal | Medial Frontal Gyrus | 8 |
| 16 | 9 | 56 | 540 | -9.0 | R | Frontal | Medial Frontal Gyrus | 6 |
| -33 | 47 | 7 | 706 | -9.1 | L | Frontal | Middle Frontal Gyrus | 10 |
| 37 | 20 | 41 | 490 | -11.4 | R | Frontal | Middle Frontal Gyrus | 8 |
| 29 | 0 | 43 | 442 | -8.3 | R | Frontal | Middle Frontal Gyrus | 6 |
| 29 | 41 | -12 | 433 | -6.4 | R | Frontal | Middle Frontal Gyrus | 11 |
| -30 | -6 | 32 | 328 | -4.2 | L | Frontal | Precentral Gyrus | 6 |
| -24 | 4 | 57 | 668 | -7.2 | L | Frontal | Sub-Gyral | 6 |
| -32 | 34 | 33 | 803 | -10.1 | L | Frontal | Superior Frontal Gyrus | 9 |
| -12 | 50 | -15 | 123 | -4.9 | L | Frontal | Superior Frontal Gyrus | 11 |
| 27 | 42 | 22 | 937 | -11.2 | R | Frontal | Superior Frontal Gyrus | 10 |
| -10 | 23 | 22 | 373 | -7.2 | L | Limbic | Anterior Cingulate | 32 |
| 11 | 21 | 25 | 330 | -7.1 | R | Limbic | Anterior Cingulate | 24 |
| -2 | -33 | 31 | 497 | -7.9 | L | Limbic | Cingulate Gyrus | 31 |
| -20 | -72 | 32 | 259 | -5.2 | L | Occipital | Cuneus | 7 |
| 4 | -78 | 13 | 217 | -5.3 | R | Occipital | Cuneus | 17 |
| 12 | -95 | -16 | 38 | -4.6 | R | Occipital | Lingual Gyrus | 17 |
| -47 | -51 | 40 | 678 | -9.1 | L | Parietal | Inferior Parietal Lobule | 40 |
| 43 | -53 | 43 | 893 | -10.2 | R | Parietal | Inferior Parietal Lobule | 40 |
| 34 | -24 | 36 | 63 | -3.6 | R | Parietal | Postcentral Gyrus | 2 |
| -23 | -54 | 53 | 435 | -4.6 | L | Parietal | Precuneus | 7 |
| 3 | -70 | 44 | 781 | -7.9 | R | Parietal | Precuneus | 7 |
| 1 | -48 | 64 | 345 | -6.3 | R | Parietal | Precuneus | 7 |
| 22 | -47 | 56 | 393 | -5.1 | R | Parietal | Sub-Gyral | 7 |
| 5 | 2 | 14 | 56 | -6.5 | R | Sub-lobar | Caudate Body | - |
| -17 | -3 | 10 | 185 | -3.3 | L | Sub-lobar | Lateral Globus Pallidus | - |
| 24 | -11 | 7 | 189 | -5.0 | R | Sub-lobar | Putamen | - |
| 3 | -22 | 1 | 243 | -4.6 | R | Sub-lobar | Thalamus | - |
| -52 | -46 | -18 | 152 | -3.8 | L | Temporal | Fusiform Gyrus | 37 |
| 49 | -41 | -1 | 229 | -5.9 | R | Temporal | Middle Temporal Gyrus | 22 |
| -29 | -31 | -35 | 328 | -6.1 | L | Cerebellum | Cerebellar Tonsil | - |
| -46 | -53 | -40 | 151 | -4.7 | L | Cerebellum | Cerebellar Tonsil | - |
| 29 | -34 | -39 | 195 | -5.4 | R | Cerebellum | Cerebellar Tonsil | - |
| 51 | -45 | -36 | 71 | -5.0 | R | Cerebellum | Cerebellar Tonsil | - |
| 31 | -89 | -21 | 26 | -3.5 | R | Cerebellum | Declive | - |
| -32 | -71 | -38 | 203 | -5.1 | L | Cerebellum | Inferior Semi-Lunar Lobule | - |
| 34 | -72 | -47 | 73 | -4.1 | R | Cerebellum | Inferior Semi-Lunar Lobule | - |

**Supplementary Table 4.4: Peaks of Normative Connectivity with the Right Amygdala**

| X | Y | Z | Z | Voxels | Side | Lobe | Region | BA |
|---|---|---|---|---|---|---|---|---|
| *Peaks showing positive connectivity with the left amygdala* | | | | | | | | |
| -27 | 28 | -8 | 71 | 4.0 | L | Frontal | Inferior Frontal Gyrus | 47 |
| 25 | 26 | -6 | 208 | 5.4 | R | Frontal | Inferior Frontal Gyrus | 47 |
| -60 | -5 | 36 | 75 | 4.3 | L | Frontal | Precentral Gyrus | 6 |
| 54 | -8 | 48 | 89 | 4.6 | R | Frontal | Precentral Gyrus | 4 |
| 8 | 53 | 42 | 168 | 4.2 | R | Frontal | Superior Frontal Gyrus | 8 |
| 0 | 42 | -7 | 111 | 4.5 | L | Limbic | Anterior Cingulate | 32 |
| 2 | 19 | -5 | 214 | 5.8 | R | Limbic | Anterior Cingulate | 25 |
| -22 | -9 | -15 | 777 | 15.8 | L | Limbic | Amygdala | - |
| -18 | -42 | 10 | 266 | 5.7 | L | Limbic | Parahippocampal Gyrus | 30 |
| 23 | -14 | -14 | 780 | 19.4 | R | Limbic | Parahippocampal Gyrus | 28 |
| -25 | -90 | -6 | 58 | 3.7 | L | Occipital | Inferior Occipital Gyrus | 18 |
| -33 | -71 | -7 | 143 | 4.4 | L | Occipital | Lingual Gyrus | 18 |
| 37 | -86 | 0 | 342 | 5.4 | R | Occipital | Middle Occipital Gyrus | 18 |
| 54 | -73 | 6 | 119 | 4.1 | R | Occipital | Middle Occipital Gyrus | 19 |
| -59 | -20 | 51 | 26 | 3.3 | L | Parietal | Postcentral Gyrus | 2 |
| 31 | -1 | 16 | 286 | 6.4 | R | Sub-lobar | Claustrum | - |
| -35 | -9 | 20 | 279 | 7.4 | L | Sub-lobar | Insula | 13 |
| 36 | -23 | 16 | 402 | 8.5 | R | Sub-lobar | Insula | 13 |
| 59 | -36 | 16 | 110 | 4.7 | R | Sub-lobar | Insula | 13 |
| -2 | -3 | 0 | 325 | 9.6 | L | Sub-lobar | Thalamus | - |
| 18 | -37 | 11 | 339 | 6.0 | R | Sub-lobar | Pulvinar | - |
| -31 | -45 | -14 | 554 | 9.0 | L | Temporal | Fusiform Gyrus | 37 |
| 40 | -51 | -11 | 534 | 8.8 | R | Temporal | Fusiform Gyrus | 37 |
| -46 | -2 | -11 | 606 | 7.8 | L | Temporal | Middle Temporal Gyrus | 21 |
| 61 | -8 | 10 | 134 | 3.6 | R | Temporal | Precentral Gyrus | 42 |
| -32 | 12 | -29 | 537 | 10.1 | L | Temporal | Superior Temporal Gyrus | 38 |
| -40 | -29 | 14 | 304 | 7.1 | L | Temporal | Superior Temporal Gyrus | 41 |
| -55 | -11 | 8 | 222 | 5.3 | L | Temporal | Superior Temporal Gyrus | 22 |
| -60 | -31 | 11 | 74 | 4.7 | L | Temporal | Superior Temporal Gyrus | 42 |
| 33 | 5 | -30 | 623 | 10.9 | R | Temporal | Superior Temporal Gyrus | 38 |
| 47 | 1 | -8 | 578 | 8.3 | R | Temporal | Superior Temporal Gyrus | 38 |
| 48 | 20 | -18 | 168 | 5.8 | R | Temporal | Superior Temporal Gyrus | 38 |
| 4 | -38 | -7 | 451 | 6.6 | R | Cerebellum | Culmen | - |
| -10 | -50 | -35 | 232 | 6.0 | L | Cerebellum | Cerebellar Tonsil | - |
| 15 | -48 | -35 | 340 | 6.6 | R | Cerebellum | Cerebellar Tonsil | - |
| -8 | -63 | -15 | 153 | 5.4 | L | Cerebellum | Declive | - |
| -1 | -23 | -22 | 503 | 8.6 | L | Brainstem | Pons | |

*The co-ordinates (X,Y,Z), voxel extent, and Z-value from the whole-brain t-test, and Brodmann Area (BA) are presented for peaks of clusters showing significant normative connectivity with the left amygdala.*

**Supplementary Table 4.4, continued**

| X | Y | Z | Voxels | Z | Side | Lobe | Region | BA |
|---|---|---|---|---|---|---|---|---|
| *Peaks showing negative connectivity with the right amygdala* | | | | | | | | |
| 42 | 8 | 23 | 219 | -5.5 | R | Frontal | Inferior Frontal Gyrus | 9 |
| 48 | 21 | -1 | 135 | -5.4 | R | Frontal | Inferior Frontal Gyrus | 47 |
| -1 | 26 | 45 | 662 | -9.8 | L | Frontal | Medial Frontal Gyrus | 8 |
| 9 | -7 | 61 | 149 | -3.3 | R | Frontal | Medial Frontal Gyrus | 6 |
| -27 | 38 | 27 | 699 | -10.5 | L | Frontal | Middle Frontal Gyrus | 9 |
| -36 | 25 | 39 | 477 | -10.3 | L | Frontal | Middle Frontal Gyrus | 8 |
| -30 | 51 | 3 | 633 | -9.1 | L | Frontal | Middle Frontal Gyrus | 10 |
| -41 | 38 | -14 | 148 | -3.9 | L | Frontal | Middle Frontal Gyrus | 11 |
| 33 | 27 | 32 | 624 | -9.9 | R | Frontal | Middle Frontal Gyrus | 9 |
| 31 | 42 | 3 | 626 | -8.1 | R | Frontal | Middle Frontal Gyrus | 10 |
| 28 | -3 | 39 | 271 | -5.6 | R | Frontal | Middle Frontal Gyrus | 6 |
| -35 | 4 | 38 | 542 | -8.7 | L | Frontal | Precentral Gyrus | 9 |
| -50 | 16 | 7 | 331 | -6.8 | L | Frontal | Precentral Gyrus | 44 |
| -18 | 13 | 56 | 532 | -7.1 | L | Frontal | Superior Frontal Gyrus | 6 |
| 26 | 45 | 25 | 557 | -9.7 | R | Frontal | Superior Frontal Gyrus | 10 |
| 22 | 8 | 56 | 384 | -7.5 | R | Frontal | Superior Frontal Gyrus | 6 |
| 0 | 29 | 21 | 508 | -7.6 | L | Limbic | Anterior Cingulate | 24 |
| 0 | -36 | 25 | 570 | -8.2 | L | Limbic | Cingulate Gyrus | 31 |
| -11 | 10 | 30 | 203 | -6.0 | L | Limbic | Cingulate Gyrus | 24 |
| 14 | -14 | -35 | 188 | -3.7 | R | Limbic | Uncus | 36 |
| 6 | -73 | 8 | 99 | -6.1 | R | Occipital | Cuneus | 23 |
| -46 | -49 | 38 | 716 | -9.6 | L | Parietal | Inferior Parietal Lobule | 40 |
| 45 | -51 | 42 | 629 | -8.5 | R | Parietal | Inferior Parietal Lobule | 40 |
| -30 | -26 | 40 | 171 | -4.5 | L | Parietal | Postcentral Gyrus | 3 |
| 31 | -29 | 39 | 104 | -4.1 | R | Parietal | Postcentral Gyrus | 2 |
| -16 | -69 | 39 | 686 | -8.3 | L | Parietal | Precuneus | 7 |
| -2 | -48 | 49 | 645 | -7.6 | L | Parietal | Precuneus | 7 |
| -27 | -53 | 50 | 504 | -4.9 | L | Parietal | Precuneus | 7 |
| 22 | -70 | 44 | 574 | -6.4 | R | Parietal | Precuneus | 7 |
| 24 | -50 | 60 | 135 | -3.7 | R | Parietal | Superior Parietal Lobule | 7 |
| 7 | -3 | 16 | 130 | -5.6 | R | Sub-lobar | Caudate Body | - |
| -27 | 19 | 5 | 237 | -7.5 | L | Sub-lobar | Claustrum | - |
| -12 | -6 | 14 | 153 | -5.5 | L | Sub-lobar | Ventral Anterior Nucleus | - |
| -53 | -41 | -8 | 172 | -5.3 | L | Temporal | Middle Temporal Gyrus | 20 |
| 55 | -39 | -5 | 130 | -5.8 | R | Temporal | Middle Temporal Gyrus | 21 |
| -31 | -33 | -38 | 156 | -5.5 | L | Cerebellum | Cerebellar Tonsil | - |
| 27 | -33 | -40 | 222 | -4.9 | R | Cerebellum | Cerebellar Tonsil | - |
| -37 | -66 | -41 | 177 | -4.6 | L | Cerebellum | Inferior Semi-Lunar Lobule | - |
| 38 | -74 | -44 | 18 | -3.5 | R | Cerebellum | Inferior Semi-Lunar Lobule | - |
| -8 | -80 | -25 | 26 | -3.7 | L | Cerebellum | Pyramis | - |
| 14 | -85 | -24 | 127 | -4.7 | R | Cerebellum | Uvula | - |
| 0 | -23 | 0 | 129 | -4.9 | L | Brainstem | Red Nucleus | - |

**Supplementary Table 4.5: Additional Information for Main Regressions (Supp. to Table 4.3)**

| | Left Putamen | | | | Left Post-Central Gyrus | | | | Left Anterior Cingulate | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **p** | **CI-L** | **CI-U** | **b** | **p** | **CI-L** | **CI-U** | **b** | **p** | **CI-L** | **CI-U** |
| **Intercept** | -0.06 | 0.55 | -0.28 | 0.15 | 0.21 | 0.05 | 0.00 | 0.42 | 0.02 | 0.84 | -0.19 | 0.23 |
| **Sex** | 0.04 | 0.15 | -0.01 | 0.09 | 0.01 | 0.83 | -0.05 | 0.06 | -0.03 | 0.27 | -0.08 | 0.02 |
| **Ethnicity** | **-0.12** | **0.00** | **-0.17** | **-0.06** | **0.10** | **0.00** | **0.05** | **0.16** | -0.04 | 0.19 | -0.10 | 0.02 |
| **GPS** | **-0.05** | **0.00** | **-0.07** | **-0.03** | **0.05** | **0.00** | **0.03** | **0.07** | -0.01 | 0.42 | -0.03 | 0.01 |
| **Life Events** | 0.00 | 0.15 | 0.00 | 0.01 | 0.00 | 0.10 | 0.00 | 0.01 | **0.01** | **0.00** | **0.01** | **0.01** |
| **GPS x Sex** | 0.02 | 0.24 | -0.02 | 0.06 | -0.02 | 0.46 | -0.06 | 0.03 | 0.03 | 0.19 | -0.01 | 0.07 |
| **LE x Sex** | 0.00 | 0.11 | 0.00 | 0.01 | 0.00 | 0.60 | -0.01 | 0.00 | 0.00 | 0.25 | 0.00 | 0.01 |
| **GPS x Ethnicity** | 0.01 | 0.57 | -0.03 | 0.06 | 0.03 | 0.17 | -0.01 | 0.08 | 0.03 | 0.19 | -0.02 | 0.08 |
| **LE x Ethnicity** | 0.00 | 0.39 | -0.01 | 0.00 | **0.01** | **0.04** | **0.00** | **0.01** | **0.01** | **0.03** | **0.00** | **0.01** |
| **GPS x LE** | **0.00** | **0.01** | **-0.01** | **0.00** | 0.00 | 0.07 | 0.00 | 0.01 | 0.00 | 0.17 | 0.00 | 0.01 |
| **Depressive Symptoms** | 0.08 | 0.66 | -0.29 | 0.46 | 0.04 | 0.84 | -0.34 | 0.42 | -0.07 | 0.72 | -0.45 | 0.31 |
| **Anxiety Symptoms** | 0.01 | 0.33 | -0.01 | 0.03 | 0.00 | 0.89 | -0.02 | 0.02 | **0.03** | **0.00** | **0.02** | **0.05** |
| **Ext. Symptoms** | -0.01 | 0.20 | -0.01 | 0.00 | **-0.01** | **0.01** | **-0.02** | **0.00** | **-0.01** | **0.00** | **-0.02** | **0.00** |
| **Emotion Regulation** | 0.00 | 0.27 | 0.00 | 0.01 | -0.01 | 0.07 | -0.01 | 0.00 | 0.00 | 0.24 | -0.01 | 0.00 |

| | Right Parahippocampal Gyrus | | | | Left Caudate Tail | | | | Left Middle Frontal Gyrus | | | | Left Inferior Frontal Gyrus | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **b** | **p** | **CI-L** | **CI-U** | **b** | **p** | **CI-L** | **CI-U** | **b** | **p** | **CI-L** | **CI-U** | **b** | **p** | **CI-L** | **CI-U** |
| **Intercept** | -0.12 | 0.18 | -0.29 | 0.05 | 0.06 | 0.58 | -0.14 | 0.25 | -0.07 | 0.53 | -0.27 | 0.14 | 0.06 | 0.66 | -0.19 | 0.30 |
| **Sex** | 0.01 | 0.73 | -0.03 | 0.05 | 0.03 | 0.30 | -0.02 | 0.07 | -0.02 | 0.49 | -0.07 | 0.03 | -0.04 | 0.19 | -0.10 | 0.02 |
| **Ethnicity** | -0.01 | 0.76 | -0.05 | 0.04 | 0.00 | 0.99 | -0.05 | 0.05 | 0.02 | 0.57 | -0.04 | 0.07 | 0.01 | 0.86 | -0.06 | 0.07 |
| **GPS** | -0.01 | 0.24 | -0.03 | 0.01 | -0.01 | 0.20 | -0.04 | 0.01 | 0.01 | 0.63 | -0.02 | 0.03 | 0.01 | 0.47 | -0.02 | 0.04 |
| **Life Events** | 0.00 | 0.29 | 0.00 | 0.00 | **0.00** | **0.04** | **0.00** | **0.01** | 0.00 | 0.71 | 0.00 | 0.00 | 0.00 | 0.44 | 0.00 | 0.00 |
| **GPS x Sex** | -0.02 | 0.15 | -0.06 | 0.01 | -0.03 | 0.16 | -0.06 | 0.01 | 0.02 | 0.42 | -0.02 | 0.05 | 0.01 | 0.80 | -0.04 | 0.05 |
| **LE x Sex** | 0.00 | 0.67 | 0.00 | 0.01 | 0.00 | 0.31 | 0.00 | 0.01 | 0.00 | 0.59 | -0.01 | 0.00 | -0.01 | 0.10 | -0.01 | 0.00 |
| **GPS x Ethnicity** | -0.02 | 0.37 | -0.05 | 0.02 | -0.01 | 0.69 | -0.05 | 0.03 | **0.05** | **0.03** | **0.01** | **0.10** | **0.06** | **0.04** | **0.00** | **0.11** |
| **LE x Ethnicity** | **-0.01** | **0.00** | **-0.02** | **-0.01** | **-0.01** | **0.00** | **-0.02** | **0.00** | **0.01** | **0.00** | **0.01** | **0.02** | 0.01 | 0.13 | 0.00 | 0.01 |
| **GPS x LE** | **-0.01** | **0.00** | **-0.01** | **-0.01** | **-0.01** | **0.00** | **-0.01** | **0.00** | **0.01** | **0.00** | **0.00** | **0.01** | **0.01** | **0.00** | **0.00** | **0.01** |
| **Depressive Symptoms** | -0.18 | 0.24 | -0.49 | 0.13 | -0.29 | 0.11 | -0.64 | 0.06 | 0.05 | 0.77 | -0.31 | 0.42 | 0.38 | 0.09 | -0.06 | 0.83 |
| **Anxiety Symptoms** | 0.00 | 0.60 | -0.01 | 0.02 | 0.01 | 0.47 | -0.01 | 0.02 | 0.00 | 0.72 | -0.01 | 0.02 | -0.02 | 0.07 | -0.04 | 0.00 |
| **Ext. Symptoms** | 0.00 | 0.53 | 0.00 | 0.01 | 0.00 | 0.43 | -0.01 | 0.00 | 0.00 | 0.77 | -0.01 | 0.01 | 0.00 | 0.72 | -0.01 | 0.01 |
| **Emotion Regulation** | 0.01 | 0.07 | 0.00 | 0.01 | 0.00 | 0.68 | -0.01 | 0.01 | 0.00 | 0.60 | -0.01 | 0.01 | 0.00 | 0.57 | -0.01 | 0.01 |

*Additional information is presented here to supplement the main regressions presented in Table 4.3. Here, we provide unstandardized regression coefficients (b) and their associated p-value and 95% confidence interval (CI) estimate (L=lower bound, U=upper bound).Effects significant at p<0.05 are shaded bold. Effects that defined each region are shaded gray. GPS= genetic profile scores, LE = life events, Ext.=externalizing disorder*

**Supplementary Table 4.6: Regression Models Controlling for Effects of Age**

| | Left Putamen | | Left Postcentral Gyrus | | Left Anterior Cingulate | | Right Parahippocampal Gyrus | | Left Caudate Tail | | Left Middle Frontal Gyrus | | Left Inferior Frontal Gyrus | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** |
| **Sex** | 0.217 | 1.153 | 0.051 | 0.268 | -0.205 | -1.159 | 0.002 | 0.009 | 0.207 | 1.082 | -0.137 | -0.689 | -0.264 | -1.334 |
| **Ethnicity** | **-0.780** | **-3.774*** ** | **0.770** | **3.693*** ** | -0.197 | -1.012 | -0.037 | -0.193 | -0.025 | -0.118 | 0.128 | 0.585 | 0.010 | 0.047 |
| **GPS** | **-0.407** | **-4.155*** ** | **0.458** | **4.635*** ** | -0.082 | -0.890 | -0.121 | -1.324 | -0.120 | -1.208 | 0.040 | 0.390 | 0.075 | 0.728 |
| **Life Events** | 0.147 | 1.435 | **0.176** | **1.709#** | **0.468** | **4.866*** ** | 0.093 | 0.973 | **0.191** | **1.841#** | 0.036 | 0.337 | -0.054 | -0.503 |
| **GPS x Sex** | 0.222 | 1.277 | -0.095 | -0.541 | 0.229 | 1.401 | -0.259 | -1.592 | -0.200 | -1.133 | 0.115 | 0.626 | 0.015 | 0.081 |
| **LE x Sex** | 0.264 | 1.297 | -0.234 | -1.137 | **0.385** | **2.01*** | 0.190 | 0.999 | 0.208 | 1.006 | -0.046 | -0.213 | **-0.402** | **-1.878#** |
| **GPS x Ethnicity** | 0.062 | 0.315 | 0.258 | 1.305 | 0.212 | 1.150 | -0.184 | -1.005 | -0.079 | -0.398 | **0.472** | **2.277*** | **0.437** | **2.123*** |
| **LE x Ethnicity** | -0.217 | -1.046 | **0.413** | **1.976#** | **0.426** | **2.183*** | **-1.037** | **-5.355*** ** | **-0.739** | **-3.518** ** | **0.913** | **4.165*** ** | 0.324 | 1.491 |
| **GPS x LE** | **-0.352** | **-2.686** ** | **0.226** | **1.708#** | 0.168 | 1.368 | **-0.736** | **-6.019*** ** | **-0.636** | **-4.793*** ** | **0.674** | **4.866*** ** | **0.508** | **3.698*** ** |
| **Depressive Symptoms** | 0.053 | 0.566 | 0.025 | 0.262 | -0.016 | -0.181 | -0.099 | -1.130 | -0.142 | -1.497 | 0.023 | 0.236 | 0.155 | 1.574 |
| **Anxiety Symptoms** | 0.078 | 0.746 | -0.068 | -0.640 | **0.410** | **4.151*** ** | 0.083 | 0.842 | 0.092 | 0.869 | 0.056 | 0.501 | **-0.203** | **-1.841#** |
| **Externalizing Symptoms** | -0.150 | -1.331 | **-0.275** | **-2.418*** | **-0.303** | **-2.855** ** | 0.043 | 0.411 | -0.092 | -0.803 | -0.036 | -0.302 | -0.051 | -0.433 |
| **Emotion Regulation** | 0.092 | 0.965 | **-0.198** | **-2.053*** | -0.089 | -0.991 | **0.182** | **2.035*** | 0.035 | 0.360 | -0.039 | -0.384 | -0.061 | -0.609 |
| **Age at Scan** | -0.084 | -0.957 | -0.020 | -0.226 | 0.014 | 0.170 | -0.037 | -0.444 | -0.009 | -0.106 | 0.026 | 0.280 | -0.029 | -0.311 |
| **Age x GPS** | 0.082 | 0.875 | 0.125 | 1.325 | 0.010 | 0.111 | -0.082 | -0.935 | 0.045 | 0.474 | -0.073 | -0.732 | -0.050 | -0.511 |
| **Age x LE** | -0.039 | -0.398 | **-0.187** | **-1.884#** | **0.212** | **2.298*** | **0.167** | **1.817#** | 0.021 | 0.212 | 0.080 | 0.772 | -0.077 | -0.748 |
| **$R^2$** | | 0.325 | | 0.313 | | 0.403 | | 0.411 | | 0.306 | | 0.245 | | 0.256 |
| **Adjusted $R^2$** | | 0.221 | | 0.207 | | 0.311 | | 0.320 | | 0.199 | | 0.129 | | 0.142 |
| **Model F** | | 3.124*** | | 2.961*** | | 4.384*** | | 4.527*** | | 2.869** | | 2.108* | | 2.242** |

*Standardized regression coefficients (β) and their associated t-values are presented for all predictors in the main regression results predicting all seven regions of interest. Model $R^2$, adjusted $R^2$, and model F values are presented for each model. Effects that identified each region are shaded gray. Effects with p<0.10 are in bold. GPS= genetic profile scores, LE= life events. ^ GxE effect not significant at whole-brain threshold level. # p<0.10, * p<0.05, **p<0.01, ***p<0.001*

**Supplementary Table 4.7: Regressions Results for White and African American Subsamples**

| | Left Putamen | | Left Post-Central Gyrus | | Left Anterior Cingulate | | Right Parahippocampal Gyrus | | Left Caudate Tail | | Left Middle Frontal Gyrus | | Left Inferior Frontal Gyrus | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **White** | **AA** | **White** | **AA** | **White** | **AA** | **White** | **AA** | **White** | **AA** | **White** | **AA** | **White** | **AA** |
| **Sex** | 0.192 | 0.361 | 0.277 | -0.189 | **-0.69*** | 0.173 | 0.239 | 0.117 | 0.190 | 0.410 | -0.314 | -0.036 | -0.494 | -0.135 |
| **GPS** | **-0.388*** | **-0.466**** | **0.59***** | **0.338*** | -0.021 | -0.196 | -0.089 | -0.031 | -0.141 | -0.073 | 0.304# | -0.176 | 0.267# | -0.133 |
| **Life Events** | **-0.028** | **0.321*** | 0.335 | -0.015 | **0.735***** | **0.286*** | -0.330 | **0.69***** | -0.050 | **0.593***** | **0.539*** | **-0.378*** | 0.191 | **-0.292*** |
| **GPS x Sex** | 0.108 | 0.166 | -0.067 | 0.074 | -0.259 | 0.229 | -0.077 | -0.367 | -0.072 | -0.348 | -0.048 | 0.224 | -0.289 | 0.184 |
| **LE x Sex** | 0.354 | 0.080 | 0.153 | -0.193 | 0.134 | 0.059 | 0.204 | 0.000 | 0.499# | -0.042 | -0.249 | -0.063 | -0.228 | -0.319 |
| **GPS x LE** | -0.346 | **-0.334*** | 0.140 | 0.29# | 0.259 | 0.127 | **-0.505*** | **-0.826***** | **-0.672**** | **-0.665***** | **0.860**** | **0.555**** | **0.629*** | **0.464**** |
| **Depressive Symptoms** | 0.117 | -0.168 | -0.063 | 0.124 | -0.090 | -0.034 | -0.116 | -0.153 | -0.032 | **-0.361*** | -0.030 | 0.046 | 0.019 | **0.393*** |
| **Anxiety Symptoms** | 0.076 | 0.168 | 0.070 | -0.361# | **0.385**** | **0.400*** | 0.051 | 0.033 | 0.097 | -0.049 | 0.115 | -0.145 | -0.149 | -0.282 |
| **Ext. Symptoms** | 0.007 | -0.249# | -0.230 | -0.265# | **-0.365*** | -0.241# | 0.213 | 0.013 | **-0.344*** | 0.117 | -0.060 | 0.065 | -0.055 | -0.010 |
| **Emotion Regulation** | 0.129 | 0.107 | -0.281# | -0.091 | **-0.279*** | 0.015 | 0.132 | 0.190 | -0.007 | 0.134 | -0.235 | 0.071 | -0.088 | -0.053 |

*Standardized regression coefficients (β) for the White and African American (AA) subsamples are presented for all predictors in the main regression results predicting all seven regions of interest. Effects that identified each region are shaded gray. Effects with p<0.05 are in bold. GPS= genetic profile scores, LE= life events. Ext.=externalizing disorder. # p<0.10, * p<0.05, **p<0.01, ***p<0.001*

**Supplementary Table 4.8: Simple Slope Effects for Regions Showing GPS x LE Interactions**

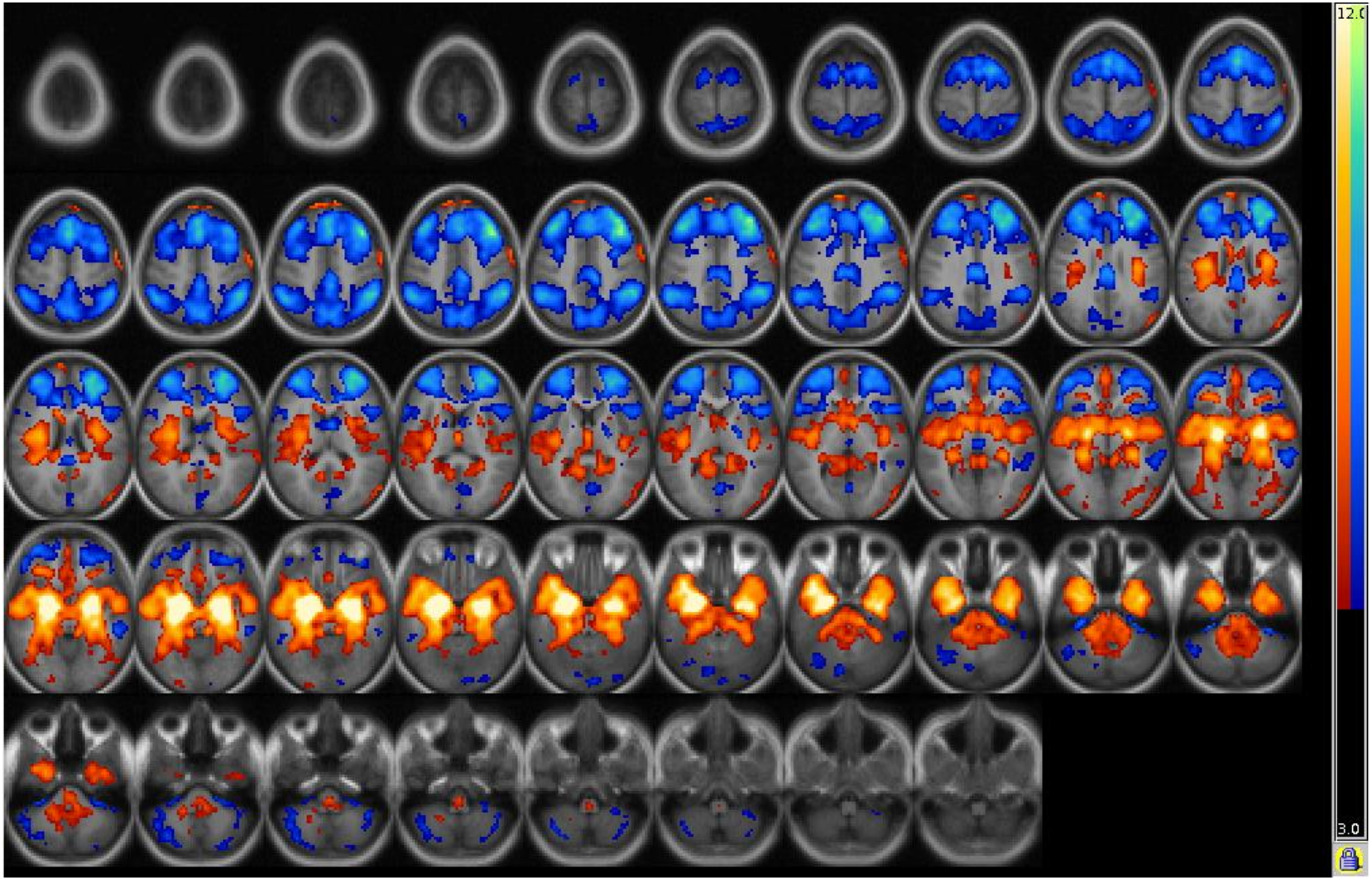
| | Right Parahippocampal Gyrus | | Left Caudate Tail | | Left Middle Frontal Gyrus | | Left Inferior Frontal Gyrus | | Left Putamen ^ | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Moderator: Value** | **b** | **t** | **b** | **t** | **b** | **t** | **b** | **t** | **b** | **t** |
| *Simple Slopes Effects for Life Events* | | | | | | | | | | |
| **Low GPS: 4.314** | 0.010 | 6.005*** | 0.011 | 5.673*** | -0.008 | -3.980*** | -0.009 | -3.678*** | 0.007 | 3.245** |
| **Mean GPS: 5.508** | 0.001 | 1.071 | 0.003 | 2.077* | 0.001 | 0.367 | -0.001 | -0.776 | 0.002 | 1.447 |
| **High GPS: 6.701** | -0.008 | -3.993*** | -0.006 | -2.573* | 0.009 | 3.859*** | 0.007 | 2.310* | -0.003 | -1.179 |
| *Simple Slopes Effects for Genetic Profile Scores* | | | | | | | | | | |
| **Low LE: 5.518** | 0.067 | 4.664*** | 0.059 | 3.584*** | -0.068 | -4.001*** | -0.057 | -2.776** | -0.009 | -0.490 |
| **Mean LE: 15.917** | -0.011 | -1.178 | -0.014 | -1.283 | 0.006 | 0.489 | 0.010 | 0.723 | -0.050 | -4.325*** |
| **High LE: 26.315** | -0.089 | -5.196*** | -0.086 | -4.409*** | 0.079 | 3.882*** | 0.077 | 3.116** | -0.091 | -4.355*** |

*Simple slope effect of life events (LE) are presented on the top half and simple slope effect of genetic profile scores (GPS) are presented on the bottom half of the table. Simple slopes are presented at low (mean - 1SD), mean, and high (mean + 1SD) of the moderator. Unstandardized simple slope effects (b) and their associated t-value are controlling for all covariates in main regression (Table 4.3) are presented. Simple slope effects were isolated using the PROCESS tool for regions showing significant GPS x LE interactions. ^ The putamen showed a significant interaction effect but this was not observed at the whole-brain level. * p<0.05, **p<0.01, ***p<0.001*

**Supplementary Table 4.9: Hierarchical Regressions of Connectivity Predicting Outcomes at Follow-up**

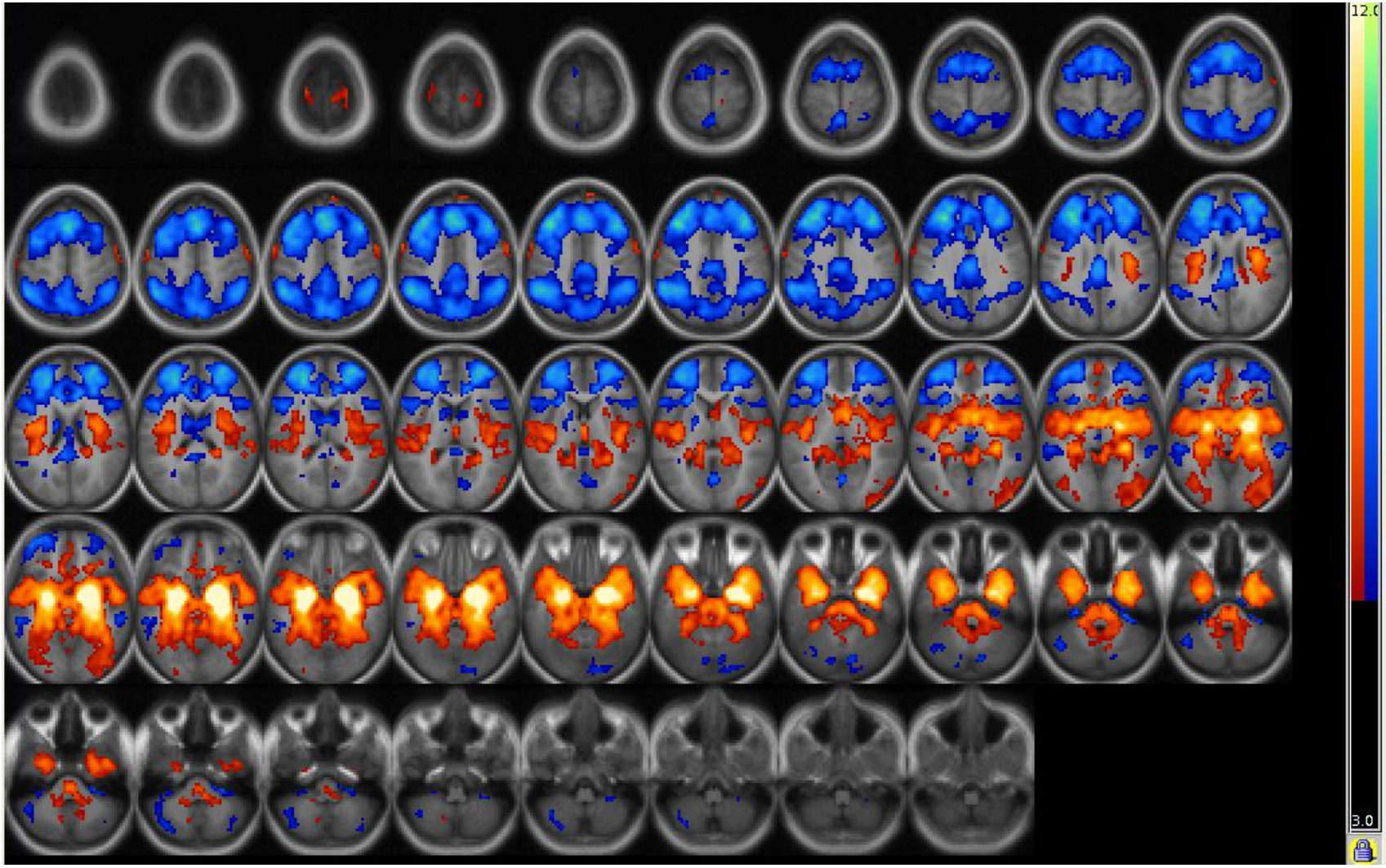
| | | **Parahippocampal Gyrus - Emotion Regulation** | | **Post-Central Gyrus-Emotion Regulation** | | **Postcentral Gyrus-Externalizing Symptoms** | | **Anterior Cingulate-Externalizing Symptoms** | | **Anterior Cingulate-Anxiety Symptoms** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Step** | **Predictor** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** | **β** | **t** |
| **1** | **Connectivity** | 0.010 | 0.099 | **-0.194** | **-1.992*** | -0.051 | -0.515 | 0.029 | 0.282 | **0.226** | **2.303*** |
| **2** | **Connectivity** | -0.015 | -0.190 | **-0.178** | **-2.298*** | 0.092 | 1.239 | -0.033 | -0.440 | **0.075** | **0.756** |
| | Concurrent Scores | **0.593** | **7.174***** | **0.587** | **7.319***** | **0.684** | **8.831***** | **0.666** | **8.707***** | **0.378** | **3.937***** |
| | Months between Scan and Follow-up | -0.038 | -0.361 | -0.035 | -0.340 | -0.069 | -0.710 | -0.066 | -0.677 | -0.008 | -0.065 |
| **3** | **Connectivity** | -0.006 | -0.059 | **-0.239** | **-2.612*** | **0.052** | 0.606 | -0.013 | -0.155 | -0.006 | -0.056 |
| | Concurrent Scores | **0.577** | **6.141***** | **0.539** | **5.981***** | **0.610** | **6.518***** | **0.598** | **6.498***** | **0.409** | **3.932***** |
| | Months between Scan and Follow-up | -0.014 | -0.116 | -0.051 | -0.451 | -0.062 | -0.594 | -0.068 | -0.652 | -0.028 | -0.219 |
| | Sex | 0.139 | 0.789 | 0.213 | 1.244 | **-0.278** | **-1.68#** | -0.268 | -1.609 | 0.004 | 0.021 |
| | Ethnicity | 0.178 | 0.829 | **0.375** | **1.71#** | -0.054 | -0.276 | -0.018 | -0.097 | 0.067 | 0.286 |
| | GPS | -0.010 | -0.092 | 0.110 | 0.997 | 0.154 | 1.537 | **0.177** | **1.92#** | -0.040 | -0.353 |
| | Life Events | 0.001 | 0.012 | 0.021 | 0.238 | 0.076 | 0.821 | 0.093 | 0.932 | **0.234** | **2.105*** |
| | GPS x Sex | 0.192 | 0.997 | 0.114 | 0.616 | -0.007 | -0.039 | -0.018 | -0.105 | -0.133 | -0.637 |
| | LE x Sex | -0.221 | -1.242 | -0.216 | -1.270 | **-0.274** | **-1.71#** | **-0.274** | **-1.671#** | -0.292 | -1.452 |
| | GPS x Ethnicity | -0.112 | -0.486 | 0.012 | 0.058 | -0.025 | -0.136 | 0.003 | 0.017 | -0.055 | -0.241 |
| | LE x Ethnicity | -0.047 | -0.224 | 0.049 | 0.241 | 0.077 | 0.418 | 0.096 | 0.524 | 0.170 | 0.747 |
| | GPS x LE | 0.074 | 0.472 | 0.149 | 1.170 | **0.206** | **1.777#** | 0.218 | 1.908# | 0.163 | 1.090 |

*Standardized regression coefficients (β) and their associated t-values are presented for hierarchical regression models. Connectivity showing relations with concurrent symptomology or emotion regulation skills were tested as predictors of that outcome at follow-up in Step 1. Concurrent scores and months between scan and follow-up were added in Step 2. All other predictors were controlled for in Scan 3. Effects of connectivity are shaded gray. Effects with p<0.10 are in bold. GPS= genetic profile scores, LE= life events. # p<0.10, * p<0.05, **p<0.01, ***p<0.001*



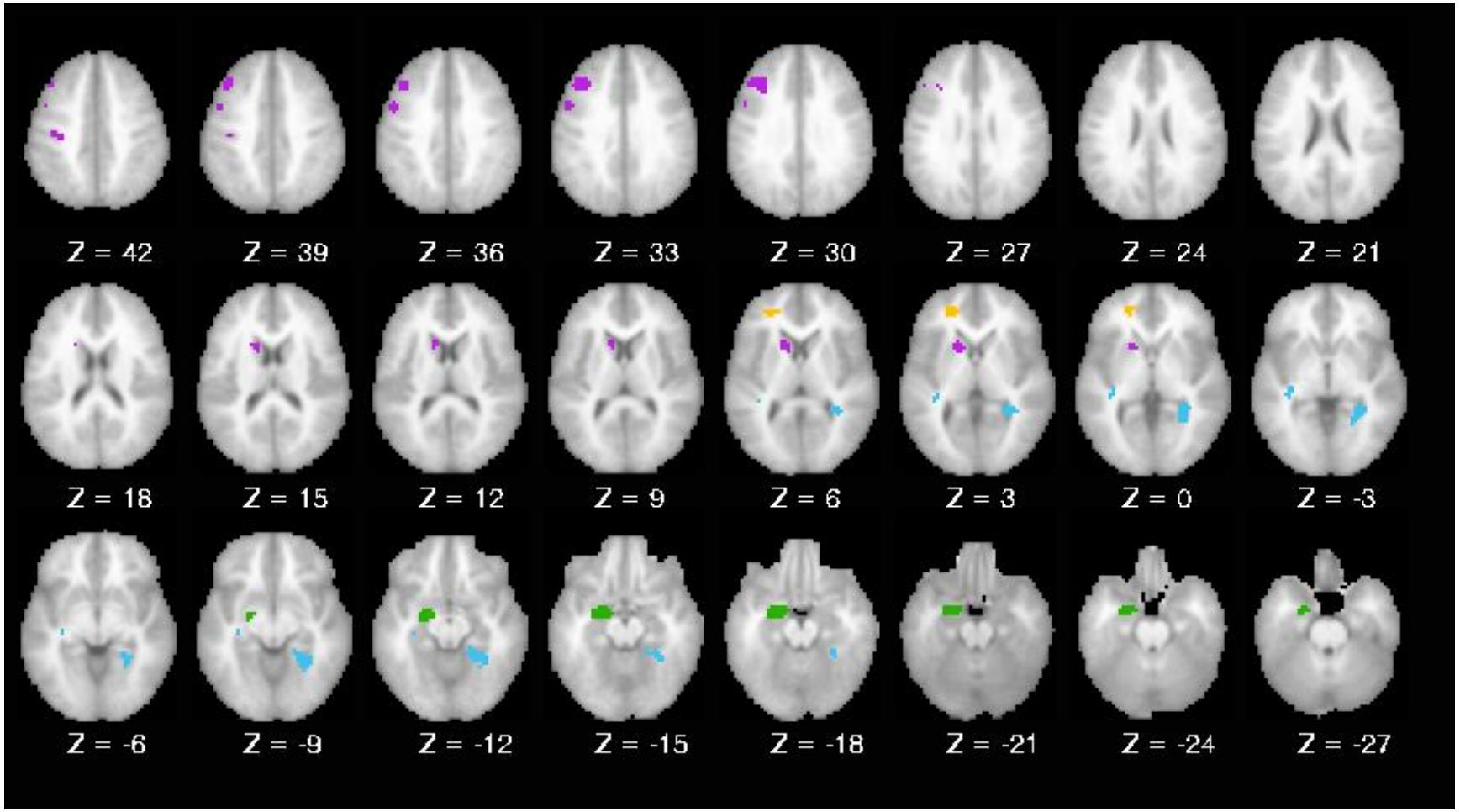

**Supplementary Figure 4.1: Normative Left Amygdala Connectivity Patterns**

*This figure presents resting state connectivity patterns with the left amygdala. Specifically, values indicate z-statistics for the whole-brain one-sample t-test indicating regions that show significant connectivity with the left amygdala thresholded at z>3 and >17 voxels.*




**Supplementary Figure 4.2: Normative Right Amygdala Connectivity Patterns**

*This figure presents resting state connectivity patterns with the right amygdala. Specifically, values indicate z-statistics for the whole-brain one-sample t-test indicating regions that show significant connectivity with the right amygdala thresholded at z>3 and >17 voxels.*




**Supplementary Figure 4.3: Regions Showing Significant Effects of Interest**

*This figure displays axial slices through the regions showing significant effects of interest from the whole-brain regressions and the left amygdala seed (green). Effects of life events are in yellow, effects of genetic profile scores are in blue, and regions showing a genetic profile score x life events interaction are in purple.*

# Chapter 5: Discussion

## 5.1 Summary

The goal of the current work was to test whether HPA axis genetic variation and early life stress predicted or interacted to predict cortisol reactivity and the structure and function of the amygdala and hippocampus in children, with the conceptual aim of elucidating potential stress-related mechanism by which genetic and environmental risk can lead to internalizing disorder pathology. As described in Chapter 2, we first created HPA axis genetic profile scores, which summed variance across multiple variants previously related in the literature to depression and/or cortisol function. As indicative of their construct validity, these profile scores predicted elevated stress cortisol levels in children at preschool-age. Further, greater early stressful life events experience predicted blunting in cortisol. These two stress-related factors then interacted to predict both left amygdala and left hippocampus volumes at school age. The effects of stress and genetic factors were partially mediated by cortisol, which negatively predicted brain volumes. In Chapter 3, we showed that these factors also predicted responses to negative emotional stimuli. First, increasing stressful life events exposure predicted elevated left amygdala reactivity to fearful vs. neutral faces. Genetic profile scores interacted with pubertal status and sex to predict activity in the left and right amygdala and hippocampus. Particularly, elevated genetic profile scores predicted elevated responses to fearful faces among pubertal girls and predicted elevated responses to neutral faces in pubertal boys. In Chapter 4, we found that stress exposure and genetic factors interacted to predict

alterations in left amygdala resting state functional connectivity. Specifically, increasing stress exposure predicted weakened connectivity with the left anterior cingulate while increasing genetic profile scores predicted weakened connectivity with left postcentral gyrus and left putamen. These factors also interacted such that children with the greatest environmental and genetic risk load showed the weakest connectivity between the amygdala and the left caudate, left inferior frontal gyrus, left middle frontal gyrus, and right parahippocampal gyrus.

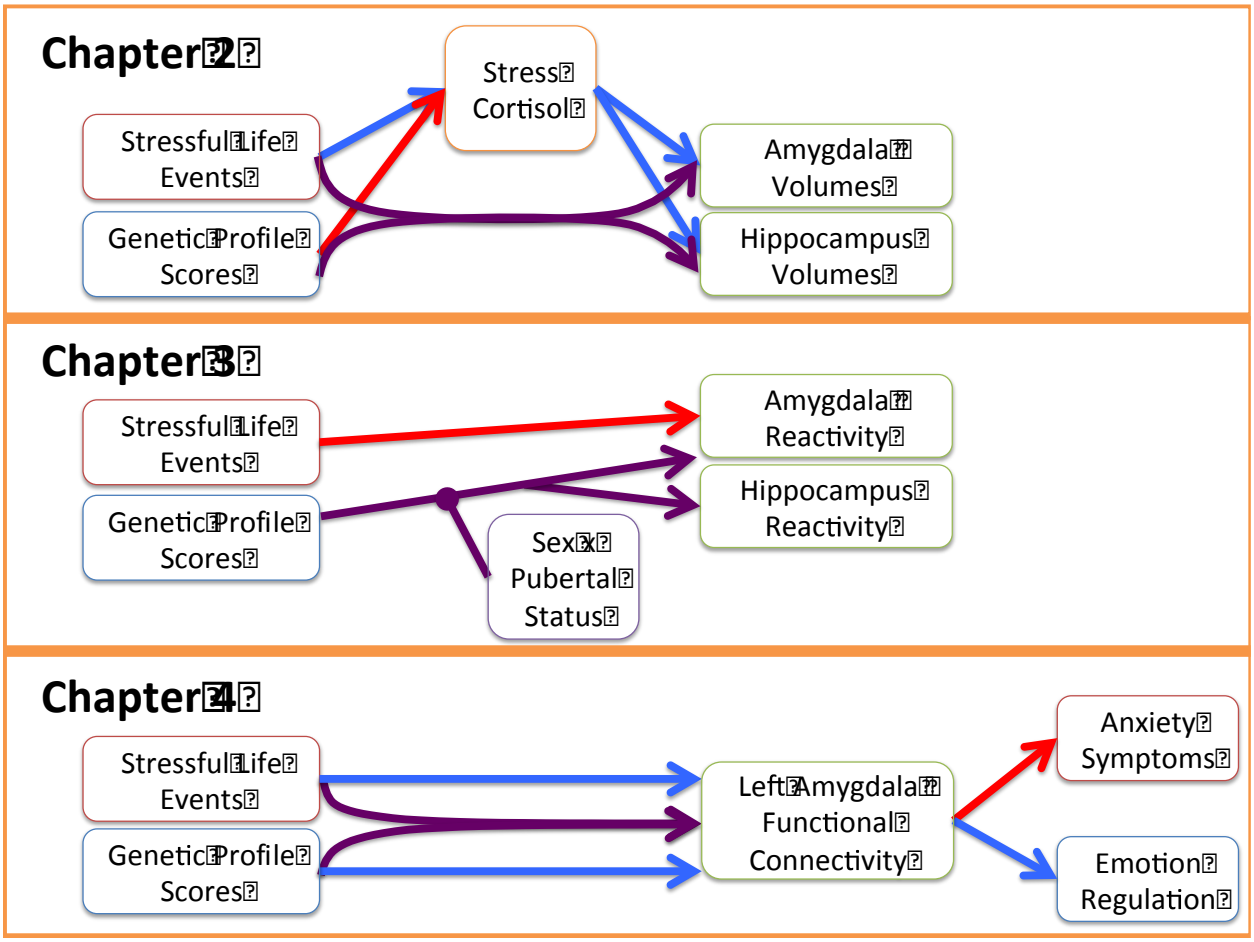



**Figure 5.1: Summary of Current Results**
Results from Chapters 2-4 are diagrammed here. Blue arrows indicate negative relationships, i.e. cortisol predicted smaller volumes and stressful life events predicted *weaker* functional connectivity. Red arrows indicate positive relationships, i.e. stressful life events predicted greater amygdala reactivity to fearful-neutral faces and *stronger* connectivity predicted greater anxiety. Purple arrows indicate interaction effects. Section 5.1 presents a further summary in text.

**5.1.1 Consistency**: These results clearly implicated stress-related mechanisms in the development of individual differences in hippocampus and amygdala structure, function, and connectivity. All three neural outcomes (and cortisol reactivity) were predicted by childhood stress exposure and/or HPA axis genetic variation. By and large, these results indicated that greater stress exposure and greater genetic risk predicted worse outcomes, e.g. amygdala and hippocampal hyper-reactivity to negative emotions and weakened amygdala connectivity, as would be expected in adults with trauma or depression.

The gene x environmental interaction predicting amygdala and hippocampal volumes indicated that greater experience of stressful life events predicted small volumes among individuals with low genetic profile scores but predicted larger volumes among those with high profile scores. While this was somewhat unexpected, given that greater stress exposure early in life predicted *blunted* cortisol, these results indicated that children with the highest cortisol levels showed the smallest volumes, i.e. those with high GPS but low stress exposure. This was confirmed further by our mediation results. Though complicated by this inverse relationship between stress and cortisol, the negative relationship between cortisol and hippocampal volume is consistent with prior literature (e.g. Lupien et al., 2005; Starkman, Gebarski, Berent, & Schteingart, 1992; Starkman et al., 1999). Further, results also typically find that early effects of stress are not observable on the hippocampus until adulthood in humans (Woon & Hedges, 2008) or animals (Isgor, Kabbaj, Akil, & Watson, 2004); whereas, our results potentially indicate that examining genetic moderation of early life stress may reveal these alterations earlier in development. On the other hand, the interpretation of our results regarding amygdala volumes were less

clear given the mixed current literature on relationships between amygdala and stress or depression. There are some studies finding smaller amygdala volumes in depression (e.g. Keller et al., 2008; Sacher et al., 2012), but these conflict with animal studies showing increased dendritic arborization in the amygdala in response to stress (e.g. Vyas, Mitra, Rao, & Chattarji, 2002). Further, there is some suggestion that effects of stress on the amygdala may vary across development (Tottenham, 2009). Particularly, stress has been suggested to induce amygdala hypertrophy in children but hypotrophy in adults. This is consistent with our finding of greater volumes with increasing stress exposure among children with high genetic risk but not with relationships to cortisol. Though, other work has related that cortisol administration to smaller amygdala volumes (E. S. Brown, Woolston, & Frol, 2008). Further work will be needed to identify whether effects of this sort of gene x environmental interaction on amygdala volumes are developmentally specific. Nonetheless, the current results indicate some degree of internally consistent, identifying related effects of stress-related factors on amygdala and hippocampal volume, function, and connectivity.

**5.1.2 Stress Effects**: Interestingly, we found relatively few *main* effects of environmental and genetic risk factors on neural outcomes whereas most effects were interactions between the two. Specifically, we only observed main effects of stress on left amygdala reactivity to negative faces and left amygdala connectivity with the ACC and we only observed main effects of genetic profile scores on connectivity with left postcentral gyrus and left putamen. The remaining effects were all interactions between genetic and environmental risk. The pattern of these interactions indicated that the effects of stress on the brain are likely most detrimental, e.g. weakening connectivity, in those with genetic

profiles conferring a more active HPA axis (note that the current cortisol results indicate higher stress cortisol with higher genetic profile scores, but do not allow us to disentangle effects of genetics on cortisol reactivity vs. regulation). Interestingly, increasing stress exposure showed a potentially beneficial effect, e.g. strengthening connectivity, in those with lower genetic profile scores. While our index of stress included both stressful and traumatic life events, we did not have a large enough sample size facing serious adversity/trauma, e.g. abuse, to test whether genes moderated severe trauma similarly to more common stressors. Further, our assessment of stressors did not include a metric of chronicity or duration, thus leaving a further open question of how genetics might differentially moderate effects of acute vs. chronic stress on the brain. Nonetheless, we found that counts of stressful life events were highly correlated with counts of traumatic life events, i.e. individuals experiencing more stress also experienced more traumatic events, and further, counts of stressful life events were better predictive of amygdala reactivity than traumatic events (Chapter 3).

**5.1.3 Sex Effects**: Another interesting issue to consider is that sex might moderate effects of stress. Particularly, we found that while genetic profile scores significantly predicted cortisol in preschoolers, this effect was stronger among females (Chapter 2). It is currently unclear what mechanism this might implicate. Particularly, it is unclear whether this is a potential biological sex difference at play this early in development or whether a more social force is at play. It is also possible that male and female children were responding to the lab stressor differently leading to differences in our index of stress cortisol, though we did not observe a main effect of sex predicting cortisol. Additionally, we found an interaction between sex and genetic profile scores (and pubertal

status) predicting limbic reactivity (Chapter 3). Here, we found that genetic profile scores differentially predicted greater reactivity to fearful faces among pubertal girls but to neutral faces among pubertal boys. This could again implicate either biological and/or social factors leading to sex differences in the effect of genetic risk. Of note, the interaction with pubertal status, which was specific to pubertal development rather than chronological age, might suggest a more biological/hormonal driver. Future work could help to elucidate these potential mechanisms. Finally, it should be noted that none of the regions showing altered connectivity identified in Chapter 4 for a relationships with life stress and/or genetic risk showed an interaction with sex. Yet, we did not examine regions that interacted with sex at the whole brain level. Interestingly, post-hoc examination of these contrasts did not reveal large sex-moderated effects of life stress or genetic profile scores: one region of left superior temporal gyrus showed a life events x sex interaction (showing a stronger positive relationship between genes and connectivity among females).

**5.1.4 Laterality**: Additionally, it is interest to note the consistent left lateralization across most of the observed effects. Particularly, life stress and genetic profile scores interacted to predict left amygdala and left hippocampus volumes, where no effects significantly predicted right side volumes. Next, while genetic profile scores interacted with sex and pubertal status to predict amygdala and hippocampal reactivity bilaterally, life events exposure only predicted left amygdala reactivity. Finally, these stress-related factors only predicted altered functional connectivity with a left amygdala seed (and mostly left side regions) despite global connectivity patterns being extremely similar for the left and right amygdala. Neuroimaging studies often find left-lateralized amygdala response to emotional stimuli (for meta-analyses, see Baas, Aleman, & Kahn, 2004; Sergerie, Chochol, &

Armony, 2008). Several theories have been suggested to explain this either as differential functioning of the left and right amygdala or as a methodological confound. Particularly, the right amygdala has been suggested to respond quickly and globally to stimuli while the left amygdala may provide more emotion-specific decoding (Adolphs, 2003). Further, the right amygdala may also habituate to stimuli more rapidly (Breiter et al., 1996). These differences could explain the lateralization of fMRI results, i.e. the temporal resolution of BOLD imaging would make it more difficult to observe rapid, less sustained responses and habituation would decrease signal averaged across trials. Relatedly, this laterality may be a function of the task examined, for example, there is evidence that the right amygdala responds more robustly to masked stimuli than the left amygdala (Costafreda, Brammer, David, & Fu, 2008; Morris, Friston, Büchel, & Frith, 1998). On the other hand, there is also suggestion that the preponderance of left-lateralized results could be an artifact of scanning procedures, e.g. phase-encoding direction (K. A. Mathiak, Zvyagintsev, Ackermann, & Mathiak, 2011). Unfortunately, there is much less evidence in the literature on lateralized effects in volume or connectivity. Particularly, even evidence regarding the direction of volumetric differences in the amygdala with stress exposure and MDD are quite mixed, making it difficult to reach more nuanced conclusions about laterality. Finally, there is little prior evidence in the literature to examine regarding laterality of stress-related differences in amygdala connectivity, particularly as examination of left vs. right amygdala seeds varies by study. For example, one study examining amygdala connectivity in adolescents reported similar stress-related effects for both the left and right but only display the left (Burghy et al., 2012) while another examining psychophysiological

interactions after maternal deprivation only present result with a right amygdala seed (Gee et al., 2013).

## 5.2 Inter-correlations Among Neural Measures

While stress-related mechanisms are implicated in individual differences in amygdala and hippocampus structure, reactivity, and connectivity, it is unclear whether this represents a common/overlapping mechanism of change or whether different mechanisms independently affect these different brain outcomes. Relatedly, the inter-correlations between structure, function, and connectivity remain an open question; this is of particular note as, for example, structure could be hypothesized to mediate effects of stress on reactivity. As discussed in Chapter 4, one other important hypothesis is that alterations in typical amygdala reactivity and thus co-activation with other regions would lead to alterations in functional connectivity. Thus, one might expect that amygdala reactivity to negative emotions could mediate effects of stress and/or genetic risk on later connectivity patterns. To aid in understand these relationships, Tables 5.1, 5.2, and 5.3 present the inter-correlation among structure, function, and connectivity for a subset of 96 white and African American children who had good quality data available on all three measures and split by sex (males N=51, females N=45).

**5.2.1 Structure-Function Correlations**: Table 5.1 presents the structure-function correlations in this subsample of children. A first clear hypothesis is that the volume of any given region would correlate with its activity. Prior work has suggested a potential negative relationship between amygdala volume and responsivity to emotional

faces in adolescents with bipolar disorder (Kalmar et al., 2009). It has also been suggested that early life stress may associate with both larger amygdala volumes and greater reactivity in pediatric samples but the inter-correlation between these factors has not been explicitly tested in this work (Tottenham et al., 2010). The current data suggests that amygdala volume (residuals controlling for WBV) and reactivity to fearful vs. neutral faces are uncorrelated. Table 5.1 also shows correlations between left amygdala volume and reactivity to sad vs. neutral faces and fearful, sad, and neutral faces (each vs. baseline); where all of these correlations were similarly non-significant. Splitting the sample by sex, we did find one significant structure-function correlation for the amygdala where larger left amygdala volumes predicted greater reactivity to neutral faces among girls. Interestingly, the opposite relationship was found for boys; though this relationships did not reach significance, sex did significantly moderate the relationship between volume and reactivity to neutral faces ($t=2.763$, $p=0.007$). This is reminiscent of the differences in relationships between genetic profile scores and neutral face activity among pubertal children in Chapter 3 and may relate to sex differences in the perception of neutral faces. Thus, future work will be needed to examine whether there is truly a sex-specific relationship between amygdala volume and function. Alternatively, this may be a developmentally specific effect or may be specific to the neuroimaging task that we examined. Particularly, other measures of amygdala response to emotional stimuli or during emotional regulation could potentially reveal stronger structure-function correlations. Conversely, examining the volume of the amygdala may not be capturing structure on the level of analysis of relevance for understanding amygdala function, e.g.

neuron-level structure/connectivity may be more relevant but below the resolution of MRI or the volume of amygdala substructures may be more informative of amygdala function.

On the hand, we found significant negative relationships between left hippocampal volume and reactivity to fearful vs. neutral faces in the whole subsample and for the right hippocampus as well. As few studies often highlight the role of the hippocampus in response to emotional stimuli and none to our knowledge have related this response to hippocampal volume, this effect is novel in the literature and should be probed further in the future. As hippocampal volume and reactivity were predicted by different interaction effects, it would be difficult to test whether, for example, volume mediates effects of genetic risk on reactivity. We did examine whether volume mediated effects of cortisol on reactivity, given a negative relationship between preschool-age stress cortisol levels and school-age left hippocampal volumes, but did not find evidence of a significant mediation effect (though the sample size was reduced to 59 as not all children had cortisol data).

Finally, we noted that right hippocampal volume was negatively correlated with left amygdala reactivity to fearful vs. neutral faces, particularly due to a positive correlation with neutral face reactivity. This effect replicated prior work in a subset of children from the PDS examining whole brain correlations between hippocampal volume and reactivity to emotional faces (Suzuki et al., 2012). Among females, neutral face reactivity in the left amygdala was positively correlated with all four brain volumes. As this type of relationships has not been examined in other prior studies to our knowledge, more work will be needed to further clarify the significance of these inter-correlations. Particularly, these results could implicate hippocampal structure (and/or structural connectivity with the amygdala) in moderating amygdala reactivity or, alternatively, hippocampal structure

could simply serve as a strong marker of stress effects of the brain, i.e. stress could lead to correlated alterations in hippocampal structure and amygdala function without any actual causal interactions between these two outcomes.

**Table 5.1: Structure-Function Correlations**

| | All Participants (N=96) | | | | Males (N=51) | | | | Females (N=45) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **L. Amyg Vol.** | **R. Amyg Vol.** | **L. HC Vol.** | **R. HC Vol.** | **L. Amyg Vol.** | **R. Amyg Vol.** | **L. HC Vol.** | **R. HC Vol.** | **L. Amyg Vol.** | **R. Amyg Vol.** | **L. HC Vol.** | **R. HC Vol.** |
| **LA - FN** | -0.067 | -0.188 | -0.176 | **-.221*** | 0.147 | 0.090 | -0.103 | -0.085 | -0.258 | **-.392**** | -0.278 | **-.387**** |
| **RA FN** | 0.071 | -0.010 | -0.116 | -0.118 | **.307*** | 0.249 | -0.073 | -0.024 | -0.145 | -0.184 | -0.185 | -0.227 |
| **LHC - FN** | -0.019 | -0.150 | **-.265**** | **-.238*** | 0.096 | 0.049 | -0.260 | -0.186 | -0.129 | **-.297*** | -0.284 | **-.303*** |
| **RHC - FN** | 0.041 | -0.091 | **-.219*** | **-.232*** | 0.127 | 0.148 | -0.200 | -0.164 | -0.027 | -0.240 | -0.260 | **-.308*** |
| **LA - SN** | 0.013 | -0.173 | -0.134 | -0.178 | 0.214 | -0.066 | -0.119 | -0.117 | -0.222 | **-.297*** | -0.157 | -0.277 |
| **LA - F** | 0.022 | 0.024 | -0.038 | -0.004 | -0.098 | -0.010 | -0.037 | 0.036 | 0.149 | 0.043 | -0.038 | -0.066 |
| **LA - N** | 0.061 | 0.168 | 0.156 | **.216*** | -0.232 | -0.091 | 0.051 | 0.119 | **.319*** | **.363*** | **.296*** | **.343*** |
| **LA - S** | 0.102 | 0.032 | -0.004 | 0.039 | -0.015 | -0.185 | -0.165 | -0.064 | 0.192 | 0.160 | 0.168 | 0.142 |

Structure-function correlations are presented for a subset of 96 white and African American children with good-quality structural, functional, and connectivity data. All values represent Pearson's correlations for the full subsample and split by sex. Rows correspond to functional activity for the left amygdala (LA), right amygdala (LA), left hippocampus (LHC) and right hippocampus (RHC) in response to fearful vs. neutral faces (FN), sad vs. neutral faces (SN), fearful faces vs. baseline (F), neutral faces vs. baseline (N), or sad faces vs. baseline (S). Columns correspond to the volumes (vol.) for these four regions. Structure-function correlations for a given region are shaded gray, i.e. left amygdala structure with left amygdala function. Correlations significant at $p<0.05$ are in bold. *$p<0.05$, **$p<0.01$

**5.2.2 Structure-Connectivity Correlations**: Table 5.2 also presents correlations between amygdala and hippocampal volumes and left amygdala connectivity. To our knowledge, this type of relationship has not been reported previously in the literature. We find no evidence of significant structure-connectivity correlations at the whole group level and only two sex-specific correlations: left amygdala volume negatively correlated with left amygdala-MFG connectivity in males while left hippocampal volume negatively correlated with left amygdala-ACC connectivity among females. We again did not find evidence that structure might mediate stress-related effects on connectivity but the subsamples were quite small when splitting by sex and thus these tests were underpowered. Further, it is important to note that this table only includes correlations with regions showing altered connectivity related to stress and/or genetic risk. There may be other regions that were not identified here relationships between connectivity and volume (but not stress-related effects).

**Table 5.2: Structure-Connectivity Correlations**

| | All Participants (N=96) | | | | Males (N=51) | | | | Females (N=45) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **L. Amyg Vol.** | **R. Amyg Vol.** | **L. HC Vol.** | **R. HC Vol.** | **L. Amyg Vol.** | **R. Amyg Vol.** | **L. HC Vol.** | **R. HC Vol.** | **L. Amyg Vol.** | **R. Amyg Vol.** | **L. HC Vol.** | **R. HC Vol.** |
| **R. Parahippocampal Gyrus** | -0.077 | 0.003 | -0.135 | -0.105 | -0.163 | -0.230 | -0.165 | -0.094 | 0.014 | 0.190 | -0.086 | -0.122 |
| **L. Caudate** | 0.019 | 0.050 | 0.082 | 0.072 | 0.000 | 0.046 | 0.128 | 0.116 | 0.029 | 0.041 | 0.025 | 0.013 |
| **L. Putamen** | 0.091 | 0.070 | -0.044 | 0.002 | 0.100 | 0.060 | 0.045 | 0.079 | 0.074 | 0.062 | -0.177 | -0.107 |
| **L. MFG** | -0.150 | -0.053 | 0.085 | 0.007 | **-.293*** | -0.123 | 0.076 | -0.005 | -0.022 | 0.003 | 0.101 | 0.026 |
| **L. IFG** | -0.180 | -0.077 | 0.045 | 0.074 | -0.217 | 0.006 | 0.083 | 0.075 | -0.130 | -0.107 | -0.016 | 0.096 |
| **L. Postcentral Gyrus** | 0.067 | 0.111 | 0.001 | 0.077 | 0.131 | 0.200 | 0.022 | 0.105 | 0.000 | 0.032 | -0.021 | 0.035 |
| **L. ACC** | -0.139 | 0.009 | -0.109 | -0.119 | -0.189 | -0.008 | 0.073 | 0.006 | -0.084 | 0.036 | **-.389**** | -0.270 |

Structure-connectivity correlations are presented for a subset of 96 white and African American children with good-quality structural, functional, and connectivity data. All values represent Pearson's correlations for the full subsample and split by sex. Rows correspond to functional connectivity values between the noted region and the left amygdala. Columns correspond to the volumes (vol.) for these four regions. Structure-connectivity correlations between left amygdala activity and left amygdala connectivity are shaded gray. Correlations significant at $p<0.05$ are in bold. *$p<0.05$, **$p<0.01$

**5.2.3 Function-Connectivity Correlations**: Table 5.3 shows the relationships between amygdala reactivity at the first scan wave with connectivity at the second scan wave. First, examining the hypothesized relationships between left amygdala reactivity and left amygdala connectivity, we generally found that greater left amygdala reactivity to fearful vs. neutral faces was positive correlated with left amygdala connectivity with regions showing typically positive connectivity (parahippocampal gyrus and caudate) and was negatively correlated with amygdala connectivity with regions typically showing negative connectivity (MFG, IFG, and postcentral gyrus). Interestingly, this seemed to be largely driven by relationships with amygdala responses to neutral faces; greater response to neutral faces negatively predicted connectivity with regions showing positive connectivity and positively predicted connectivity with regions showing negative connectivity. Thus, greater responsivity to neutral faces predicts weaker connectivity with the amygdala (among these regions identified as showing stress-related alterations). We also saw potentially analogous relationships with the right amygdala and bilateral hippocampal reactivity predicting alterations in left amygdala connectivity with these regions. Finally, examining these relationships by sex revealed that these significant relationships were generally consistent among boys and girls but were stronger among girls. Again, these results will need to be further probed in future research to parse the source/significance of these effects, particularly with examination of the longitudinal relationships between function and connectivity. Specifically, elevated reactivity to emotional stimuli across development could be predicted to either 'exercise' emotional circuitry allowing for the development of adaptive functional connectivity (strong positive connectivity with subcortical regions and strong negative connectivity with frontal

regulatory regions) or excessive reactivity could be predicted to lead to maladaptive disruptions in functional connectivity. Examining reactivity to fearful-neutral faces would suggest results more in-line with this adaptive 'exercise' of the system idea whereas examining neutral face activity would support the hypothesis that excessive reactivity may lead to the development of disrupted connectivity. Thus, this must be probed further to ascertain whether these difference scores are predicting meaningful mechanistic effects or whether they are driven by just the neutral face condition, which then could be more informative on its own. Particularly, excessive reactivity to emotionally neutral stimuli may lead to maladaptive connectivity with the amygdala.

**Table 5.3: Function-Connectivity Correlations**

| All Participants (N=96) | Parahipp. | Caudate | Putamen | MFG | IFG | Postcentral | ACC |
|---|---|---|---|---|---|---|---|
| **LA - FN** | **.263**** | **.216*** | 0.167 | **-.204*** | **-.241*** | **-.247*** | 0.135 |
| **RA FN** | 0.198 | 0.159 | -0.011 | **-.279**** | -0.177 | **-.246*** | 0.065 |
| **LHC - FN** | **.273**** | **.250*** | **.232*** | -0.097 | -0.132 | **-.206*** | 0.073 |
| **RHC - FN** | **.204*** | 0.151 | 0.193 | -0.189 | -0.126 | **-.283**** | 0.078 |
| **LA - SN** | 0.139 | 0.149 | 0.091 | -0.068 | -0.066 | **-.212*** | -0.106 |
| **LA - F** | 0.148 | 0.102 | 0.026 | -0.014 | -0.164 | -0.015 | **.208*** |
| **LA - N** | **-.220*** | **-.232*** | **-.232*** | **.254*** | 0.200 | **.331**** | -0.056 |
| **LA - S** | -0.078 | -0.094 | -0.076 | 0.189 | 0.122 | 0.095 | -0.154 |
| **Males (N=51)** | **Parahipp.** | **Caudate** | **Putamen** | **MFG** | **IFG** | **Postcentral** | **ACC** |
| **LA - FN** | 0.230 | 0.204 | -0.008 | -0.102 | -0.088 | -0.228 | 0.074 |
| **RA FN** | 0.057 | 0.071 | -0.152 | -0.061 | 0.010 | -0.044 | 0.146 |
| **LHC - FN** | 0.225 | 0.114 | 0.172 | 0.097 | -0.142 | -0.134 | 0.051 |
| **RHC - FN** | 0.117 | -0.016 | 0.137 | 0.066 | -0.104 | -0.127 | 0.174 |
| **LA - SN** | 0.129 | 0.135 | 0.151 | 0.098 | -0.048 | -0.113 | 0.006 |
| **LA - F** | 0.085 | -0.030 | -0.039 | 0.069 | -0.162 | -0.124 | 0.122 |
| **LA - N** | -0.139 | -0.221 | -0.072 | 0.142 | -0.039 | 0.182 | 0.027 |
| **LA - S** | -0.004 | -0.163 | 0.261 | .303* | -0.147 | 0.005 | -0.001 |
| **Females (N=45)** | **Parahipp.** | **Caudate** | **Putamen** | **MFG** | **IFG** | **Postcentral** | **ACC** |
| **LA - FN** | **.300*** | 0.217 | .316* | -0.275 | **-.354*** | -0.293 | 0.209 |
| **RA FN** | **.363*** | 0.254 | 0.149 | **-.483**** | **-.390**** | **-.425**** | -0.025 |
| **LHC - FN** | **.334*** | **.399**** | **.309*** | -0.285 | -0.137 | -0.271 | 0.093 |
| **RHC - FN** | **.302*** | **.315*** | 0.263 | **-.410**** | -0.169 | **-.408**** | -0.021 |
| **LA - SN** | 0.150 | 0.156 | 0.001 | -0.236 | -0.061 | **-.349*** | -0.233 |
| **LA - F** | 0.230 | 0.249 | 0.100 | -0.095 | -0.156 | 0.091 | **.322*** |
| **LA - N** | **-.304*** | -0.232 | **-.368*** | **.333*** | **.387**** | **.488**** | -0.150 |
| **LA - S** | -0.143 | -0.038 | **-.344*** | 0.108 | **.323*** | 0.169 | -0.286 |

Function-connectivity correlations are presented for a subset of 96 white and African American children with good-quality structural, functional, and connectivity data. All values represent Pearson's correlations for the full subsample and split by sex. Rows correspond to functional activity for the left amygdala (LA), right amygdala (LA), left hippocampus (LHC) and right hippocampus (RHC) in response to fearful vs. neutral faces (FN), sad vs. neutral faces (SN), fearful faces vs. baseline (F), neutral faces vs. baseline (N), or sad faces vs. baseline (S). Columns correspond to the connectivity between the left amygdala and the right parahippocampal gyrus (parahipp.), left caudate, left putamen, left MFC, left IFC, left postcentral gyrus, and left ACC. Function-connectivity correlations between left amygdala activity and left amygdala connectivity are shaded gray. Correlations significant at $p<0.05$ are in bold. *$p<0.05$, **$p<0.01$

## 5.3 Predicting Clinical Outcomes

As our genetic profile scores were created based on prior relationships with depression (and related phenotypes) and given relationships between early life stress and psychopathology, it is important to consider how these factors and related neural alterations relate to clinical outcomes. In the current sample, we found that stressful life events exposure was significantly higher among those with experience of MDD, anxiety disorders, or externalizing disorders (Supplementary Table 3.3) and predicted later anxiety symptomology (Supplementary Table 4.9) whereas genetic profile scores were not related to diagnostic outcomes. We may have been underpowered to find associations with genetic profile scores, they may be better predictive of adult pathology, or only certain SNPs may be relevant for predicting diagnostic outcomes (i.e. profile scores included SNPs based on prior work examining a variety of related outcomes, not just MDD experience). Relationships with later pathology or with specific SNPs could be probed further in the PDS.

In addition, we noted some relationships between diagnostic status and the neural outcomes of interest. In Chapter 3, we noted that amygdala and hippocampal responses to fearful-neutral faces were elevated among children with a history of anxiety disorders (Supplementary Table 3.11), as would be expected from prior literature. In Chapter 4, we noted that alterations in left amygdala connectivity mediated effects of stress-related risk factors on anxiety symptomology and emotion regulation skills. Particularly, left amygdala-postcentral gyrus connectivity mediated the association between genetic profile scores and improvements in emotion regulation skills over development (Figure 4.3A), i.e. stronger negative connectivity predicted better emotion regulation skills at a ~1 year follow-up.

Furthermore, we found evidence for a significant indirect effect (in a serial mediation model) where greater life events exposure predicted weaker amygdala-ACC connectivity (i.e. less negative) which predicted higher concurrent anxiety which in turn predicted higher future anxiety (Figure 4.3B). Thus, connectivity and current symptoms likely shared variance in predicting future symptomology. On the other hand, we did not find associations between amygdala and hippocampal volumes and diagnostic status (Supplementary Tables 2.7-2.10), though again this may become more apparent with later longitudinal assessments or these effects may be more related to stress than any particular diagnosis. This will be important to examine further in future work; particularly, it would be interesting to examine the developmental specificity of these relationships with diagnostic outcomes and to test whether these neural measures can be useful predictors of clinical course.

## 5.4 Limitations

As discussed in the prior chapters, one limitation to the current work was the coding of the genetic profile scores and stressful life events variable. Particularly, without a means of optimizing the weighting of the individual SNPs or life events, we opted to use an additive weighting scheme. While this does not capture the possibility of epistatic interactions or differential impact of different types of stressors, this approach was the most conservative for the current work given weak priors. We have presented SNP-wise relations with cortisol and brain outcomes to aid in potentially optimizing SNP weighting in future studies. The issue of weighting life stressors is also difficult; some prior work has

attempted to examine weighting of stressors but often finds weighting not to be beneficial beyond unweighted scores (e.g. Chiriboga, 1977; Cleary, 1981; Zimmerman, 2010). Given our current assessment, the low base rate of experiencing many of the stressors makes the covariance among events low and thus it is difficult to reliably examine the clustering/factoring of events. As with the SNP data, optimizing weights with an independent sample or weighting events by the reported impact could be helpful. For the current work, our summary measures appears to be a good proxy for general stress exposure as it correlates with family income, for example.

The characteristics of the PDS sample were a strength of this work but also limited certain analysis approaches. Particularly, as the sample was largely recruited based on the presence of elevated depressive symptomology at preschool age, many of the children developed psychopathology (many prior to the scanning waves). This limited our ability to test, for example, whether alterations in brain outcomes predicted first onset of psychopathology or mediated effects of stress or genetic risk on onset as relatively few children who had not experienced pathology through the time of scan then had onset within the several years after the first scan. In addition, as children exhibited a variety of psychopathologies, we would be limited in our ability to make claims about diagnostic-specific effects. Yet, as the goal of this work was to examine relationships between commonly occurring individual differences in stress exposure, genetic risk, and brain outcomes, this was not a major concern. Particularly, we find that these relationships are likely not disorder-specific and underlie more transdiagnostic change in these brain measures; stress-related mechanism likely confer risk to most type of pathology. Further, while we informed our hypotheses based on prior work in depressed samples, these prior

results likely represent one end of a continuously distributed variable rather than a depression-specific effect.

Finally, it should be noted that there were some limitations regarding the cortisol measure used. Particularly, we found that children generally had elevated cortisol levels at the first collection in the lab, perhaps indicating anxiety over coming to the novel lab environment. Thus, we did not have a baseline measure necessary to truly examine stress-induced changes in cortisol levels before and after the lab stress paradigms. Additionally, not all children with MRI data had useable cortisol data, limiting our sample size and thus power to relate cortisol to later brain outcomes. Finally, Chapter 2 examined cortisol as a *statistical* mediator of effects of life stress and genetic variation on brain structure, but it should be noted that the assertion that cortisol is an actual mediating step in this mechanism relies on temporal precedence to suggest the directionality of effects. Alternatively, for example, it is possible that amygdala and hippocampal volume could in fact be mediating effects of stress-related factors on cortisol reactivity instead (volume measured at school age could correlate with and serve as a marker of structure at preschool age). Future work would benefit from longitudinal assessment of both cortisol and brain outcomes to further examine the (direction of) relationships and as well as allowing children to acclimate to the lab setting to acquire a true baseline measure of cortisol.

## 5.5 Future Directions

As the current work poses the importance of stress-related mechanisms in the development of hippocampus and amygdala structure, function, and connectivity, this

raises a variety of additional questions that can be explored in future work. Particularly, it will be important to not only replicate these findings in an independent sample but to examine whether life stress and genetic risk predict and interact to predict change in these outcomes over time and over development. As later longitudinal waves of imaging are being collected for the PDS, this issue can be examined further in the current sample, i.e. do these stress-related factors predict altered neurodevelopmental trajectories from childhood into adolescence? Additionally, the PDS sample (or others) could be used to further probe whether these stress-related neural alterations predict later onset of psychopathology or altered trajectories of (mal)adaptive emotional development and additionally to test whether neural measures mediate effects of life stress or genetic risk on these outcomes.

Further, regarding development, results from Chapter 3 implicate pubertal changes as a potential moderate of genetic risk. Thus, longitudinally examining changes in sex hormones through the pubertal transition could help elucidate these mechanism, i.e. to test whether increases in specific hormone levels alter sensitivity to genetic or stress effects. This could be examined in later waves of the PDS where hormone data is available. Additionally, it would be important to examine other potential mechanisms related to puberty, e.g. changes in social stress/support. This type of longitudinal assessment through puberty could also help to examine the developmental specificity of the current results. It would be important to test whether these effects on structure, function, and connectivity in children are the same/different as effects in adolescence and adulthood. Further, it would be interesting to examine changes in these effects over time on, for example, hippocampal

structure, which has been suggested to not show observable effects of childhood stress until later in development.

Additionally, future work would benefit from examining the effects of these stress-related factors on other fMRI measures of emotional functioning. Examination of habituation to emotional stimuli, response to masked stimuli, emotion regulation, or other tasks could help to elucidate the specificity of the current effects and to elaborate the potential mechanism. For example, this could give insight into whether these stress-related mechanisms are altering intrinsic amygdala and hippocampal reactivity or regulatory mechanisms. This could also have implications for our understanding of alterations in functional connectivity. Additionally, it would be important to examine the emotional specificity of effects, i.e. are alterations in responsivity to fearful, neutral, or sad faces indexing the same or different effects of stress and does this change across development?

As noted in the limitations section, different types of cortisol measures could help inform different aspects of the stress-related effects on the brain. For example, effects of childhood stress and HPA axis genetic variation could have specific or general effects on a variety of cortisol outcomes, like basal cortisol, reactivity to stressors, down-regulation after stressors, waking cortisol response, or diurnal rhythms. For example, one could hypothesize that increased total cortisol levels, regardless of the source of the increase, would lead to alterations in structure whereas changes in stress reactivity might lead to more specific alterations in emotional responsivity. Thus, assessing a variety of measures could help to clarify if a particular facet of the HPA axis is contributing most to impairments, which would aid in targeting future research and interventions. Further, it would be of interest to examine whether genetic profile scores (or particular genes) are

most predictive of specific cortisol outcome to further refine our mechanistic understandings of these genetic factors, i.e. *CRHR1* polymorphism might be more related to cortisol reactivity whereas *NR3C1* polymorphisms may be more related to regulation.

Finally, examining a new, independent sample would be important to both replicate and optimize our genetic profile scores. The SNP-wise relationships with cortisol presented in Chapter 2, for example, could be used to optimize the relative weightings of SNP effects for testing in a new sample. Additionally, it would be interesting and important to examine how these HPA axis genetic variants interact with other systems. For example, BDNF has been suggested to confer protective effects against stress-related effects on the hippocampus (McEwen, 2008) and thus *BDNF* polymorphisms may be of interest as moderators of stress-related effects (e.g. Carballedo et al., 2013). Other genes, like *PERIOD1* and others involved in circadian rhythm regulation, have been suggested to impact cortisol function (e.g. Olbrich & Dittmar, 2012) and thus could also be of interest as potential moderators. Finally, it would be important to examine stress-related epigenetic changes to the HPA axis genes of interest as this would be an important mechanism unexamined in the current work by which environmental risk could confer alterations in gene expression or by which risk is inherited inter-generationally.

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