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Few findings in psychological science are as well replicated as evidence that stress precipitates depression. A wealth of evidence supports dysregulation of two major stress response systems—the hypothalamic-pituitary-adrenal (HPA) axis and its cortisol biomarker, and the sympathetic nervous system (SNS) with its salivary alpha-amylase (sAA) biomarker—as complicit in the etiology of depression risk. Prior research points to genetic variation as one source of individual differences within these systems. Although recent work emphasizes additive approaches to genetics, almost no research has examined if additive genetic risk in the HPA axis (HPA multilocus genetic profile score; MGPS) influences responding to lab-based stress exposure. Similarly, despite neurobiological connections between the HPA and SNS, no work has tested whether additive HPA-related genetic risk influences SNS reactivity to stress, or whether vulnerability in both systems, indicated by HPA-related genetic risk and SNS hyperreactivity, might work together to predict cortisol reactivity to stress. Using a diathesis stress framework to test responding to negative evaluative psychosocial stress, I examined whether an additive HPA MGPS: 1) predicts blunted cortisol reactivity, 2) predicts heightened sAA reactivity, and 3) interacts with heightened sAA reactivity to predict blunted cortisol reactivity. Findings indicated that an HPA MGPS did not significantly moderate the relationship between stress condition and cortisol or sAA reactivity respectively. However, sAA reactivity and HPA MGPS moderated the relationship between stress condition and cortisol reactivity. Findings help explicate how individual differences across two stress responsive systems influence cortisol reactivity.

Keywords: HPA Axis, Sympathetic Nervous System, Stress, GxE interaction, alpha-amylase

THE INFLUENCE OF AN ADDITIVE HPA AXIS GENETIC VARIATION SCORE ON LAB-BASED STRESS REACTIVITY IN AN EMERGING ADULT SAMPLE

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CHAPTER I: INTRODUCTION

Major Depressive Disorder (MDD) is one of the most burdensome diseases in the world, affecting over 322 million people across their lifetime (WHO, 2017). MDD exacts such a burden in part due to its chronicity (WHO, 2017), as well as its relative ubiquity across the lifespan (Kessler et al., 2006). Strikingly, about 800,000 people with MDD die by suicide annually (WHO, 2017). There remains an urgent need to elucidate risk factors of depression. Compelling evidence demonstrates a strong association between stressful life events and the development of a major depressive episode (Hammen, 2005; Kendler et al., 1999). However, not all people who experience a stressful life event will develop depression; many remain resilient, calling for examination of individual differences in stress responses. Differences in key stress response systems, namely the hypothalamic-pituitary-adrenal (HPA) axis through blunted cortisol and sympathetic nervous system (SNS) through elevated salivary alpha-amylase (sAA), have been linked to heightened risk for stress-related psychopathology, including MDD (Ali & Pruessner, 2012; Burke, Fernald, et al., 2005; Gotlib et al., 2008; McEwen, 1998).

Additionally, dominant etiological models of stress-related psychopathology suggest that stress exposure and preexisting risk factors including genetic vulnerability act together to precipitate disorder onset, known as diathesis stress theory (Monroe & Simons, 1991). Gene by environment interaction research (GxE) is a well-used method to model diathesis stress theory (Colodro-Conde et al., 2018). Furthermore, GxE interaction research demonstrates that genetic variation in the HPA axis moderates the impact of stress exposure on risk for depression (see Normann & Buttenschøn, 2019 for a systematic review). MDD is moderately heritable, with a meta-analytic point estimate of 37% for the contribution of genetic variation (95% CI = 31 -

42%; Sullivan et al., 2000). However, like other genetically complex diseases, MDD is considered "polygenic," such that risk for depression is derived from many genes with small contributions, which contribute to risk in a primarily additive manner (Colodro-Conde et al., 2018). To better conform to these theoretical assumptions of behavior genetic risk, researchers have transitioned from focusing on single genes in GxE research to using additive variables, such as multilocus genetic profile scores (MGPS), which account for the collective effect of multiple empirically- and theoretically-identified single nucleotide polymorphisms (SNPs). Indeed, several MGPS developed using HPA-related genetic variation have been linked with depression and related outcomes (Di Iorio et al., 2017; Feurer et al., 2017; Pagliaccio et al., 2014; 2015a; 2015b ; Starr & Huang, 2019).

Despite this advance, insufficient work documents the relationship of an MGPS in the HPA axis (HPA MGPS) with cortisol reactivity, and with other related physiological outcomes. Evidence from human (Bauer et al., 2002; Gordis et al., 2008; Rotenberg & McGrath, 2016) and animal (Itoi et al., 2004; Jedema & Grace, 2004) studies suggests physiological and functional interconnectedness between the HPA axis and the SNS. Despite that genetic variants in the HPA axis are active in brain regions that also regulate the SNS (Modell, 1998), only one study has examined whether genetic vulnerability in the HPA axis influences SNS stress responses (DeRijk et al., 2006), though no previous studies have used an additive approach. Moreover, multisystem asymmetry theory (Bauer et al., 2002) asserts that SNS and HPA axis activity act together to enhance psychopathology risk. If this is the case, it may be that those with the greatest HPA genetic vulnerability *and* the highest SNS reactivity will have the most pronounced cortisol blunting in the context of stress exposure.

The present study tested the extent to which an HPA axis multilocus genetic score consisting of 14 previously studied polymorphisms moderates the relationship between stress exposure (either a lab-based negative evaluative social stress test or a non-stressful control condition) and reactivity in cortisol and sAA, respectively, in a non-depressed, emerging adult sample to elucidate risk pathways for depression risk. It further tests whether the combination of higher MGPS and sAA responses predicts blunted cortisol reactivity in the context of negative evaluative stress.

The Stress Response

In the context of acute environmental stress, threatening stimuli are processed through neuronal circuitry in the prefrontal cortex and limbic system, which importantly includes the hypothalamus (McEwen, 1998; McEwen & Sapolsky, 1995). Activation of the hypothalamus triggers a cascade of coordinated, sequential responses, starting with immediate activation of the SNS and followed by activation of the HPA axis within minutes (McEwen, 1998; Sapolsky et al., 2000). These systems coordinate to modulate stress reactivity and maintain homeostasis through secretion of catecholamines (norepinephrine and epinephrine) from the SNS, and glucocorticoids (cortisol) from the HPA axis (Sapolsky et al., 2000). Among healthy individuals, a moderate secretion of these hormones is adaptive for responding to acute stressors maintaining homeostasis, which is termed "allostasis" (McEwen, 1998). However, elevations in the SNS and HPA axis that are too frequent, large, or prolonged are thought to lead to physiological wear and tear known as "allostatic load" (McEwen, 1998; McEwen, 2004; McEwen & Sapolsky, 1995). Critically, patterns of dysregulation of the SNS and HPA axis as a result of allostatic load, are thought to contribute to enhancing risk for depression (Ali & Pruessner, 2012; Bauer et al., 2002; Gordis et al., 2008).

Sympathetic Nervous System and Salivary Alpha-Amylase

In recent years, researchers have employed collection of salivary alpha-amylase (sAA) to measure SNS activity in response to stressful stimuli as an alternative to indices such as heart rate, blood pressure, skin conductance, or plasma collection for catecholamine concentration (Nater et al., 2006; Schumacher et al., 2013). Alpha-amylase is a salivary protein produced by acinar cells and is secreted from the salivary glands and is thought to be regulated by the autonomic nervous system. Under unstimulated circumstances, salivary glands are more innervated by parasympathetic nerves relative to SNS activity. By contrast, under "stimulation" such as psychosocial stress, sAA is more innervated by the SNS (Granger & Taylor, 2020; Nater & Rohleder, 2009). Specifically, activation of the SNS in response to psychological stress stimulates beta-adrenergic receptors on salivary acinar cells in the oral cavity (Granger & Taylor, 2020).

The utility of sAA as an index of the SNS emerged from previous studies, which demonstrated that concentrations of sAA could be used to reliably predict sympathetic catecholamine levels, such as norepinephrine in response to lab-based physiological stress (Chatterton et al., 1996). As it pertains to psychological stressors, prior work demonstrates that sAA increased along with other sympathetic indicators (e.g., shortened pre-ejection period "PEP") in response to a lab-based psychological stressor (Bosch et al., 2003). Nater et al., (2006) observed concurrent increases in sAA secretions and sympathetic tone (LF/HF; a ratio of sympathetic heart rate to parasympathetic heart rate) in response to psychosocial stress (Nater et al., 2006). Finally, prior work demonstrates that healthy adult participants have elevated sAA reactivity in response to a psychosocial stress test (Balodis et al., 2010; Thoma et al., 2012). Thoma and colleagues (2012) concurrently examined sAA reactivity and plasma norepinephrine,

the primary neurotransmitter utilized by the SNS, and found that sAA reactivity positively predicted plasma norepinephrine in response to the Trier Social Stress Test (psychosocial stress test), suggesting that sAA validly indexes SNS activity during stress.

The appeal and frequent use of sAA stems from its non-invasiveness comparable to alternative methods involving blood collection (e.g., plasma) or electrophysiological methods (Nater & Rohleder, 2009; Nater et al., 2007). Its simultaneous collection with salivary cortisol also promotes feasibility while providing multisystem indices of bodily stress reactivity. Like cortisol, sAA also follows a diurnal cycle (Nater et al., 2007), however unlike cortisol, sAA reacts seconds after the onset of a stressor, and is collected sooner than peak cortisol reactivity (Schumacher et al., 2013). Taken together, previous work suggests that sAA is a feasible, viable biomarker for representing changes in SNS activity in response to lab-induced psychosocial stress.

Generally, the effect of stress exposure on sAA responses in populations at risk for depression and other stress-related psychopathology is a sparse and emerging literature. Prior studies examined the relationship between sAA and chronic stress, pointing to elevated sAA (Ali & Pruessner, 2012). Additional evidence points to dysregulated sAA reactivity in response to acute stress in samples with depressive risk factors, such as increased sAA in individuals with higher levels of trait rumination and neuroticism (Soliemanifar et al., 2018). Some previous evidence points to the contrary (Bagley et al., 2011). For example, Bagley et al., (2011) did not find differences in levels of sAA in healthy individuals with remitted depression versus controls in response to a lab-based psychosocial stressor. However, the authors note as a limitation that they collected sAA 10 minutes after the stressful task in order to prioritize obtaining peak

cortisol levels. As pointed out by the authors, the peak of sAA stress reactivity precedes peak cortisol levels (Nater et al., 2005), potentially explicating the lack of effect.

Hypothalamic-Pituitary-Adrenal Axis and Cortisol Dysregulation

Responses of the HPA axis unfold somewhat more slowly than those of the SNS, described above. Briefly, upon perception of acute threat, the hypothalamus secretes corticotrophin releasing hormone (CRH) from the paraventricular nucleus, which signals the pituitary gland to secrete adrenocorticotrophic hormone (ACTH) to the adrenal cortex, which further secretes glucocorticoids, known in humans as the stress-responsive hormone cortisol (McEwen, 1998; Sapolsky et al., 2000). This reactivity occurs superimposed on a diurnal rhythm for cortisol in which levels are higher in the morning, spike briefly upon awakening, and reach a nadir after bedtime (Granger & Taylor, 2020). The HPA axis maintains a negative feedback loop, where cortisol binds to glucocorticoid (GR) and mineralocorticoid (MR) receptors on the hippocampus and hypothalamus respectively, to either continue (if the threat is still present) or suspend cortisol secretion (McEwen, 2004). In the context of chronic, ongoing threat, there is an overextension of the HPA axis response, which manifests as dysregulated secretion of cortisol and over time, markedly dampens the HPA axis (McEwen, 1998; Sapolsky et al., 2000). Moreover, in humans, cortisol levels peak around 15-30 minutes after the threat begins, and normalize to pre-stress levels 60-90 minutes later, provided that the threat abates (de Kloet et al., 2005). Of importance, these patterns of dysregulation are implicated in the pathogenesis of depression, and therefore represent a risk factor (McEwen, 1998).

While there is clear support for dysregulated cortisol reactivity as a marker of depression risk, the pattern of dysregulation—whether cortisol is overactive (elevated) versus underactive (blunted) in response to an acute stressor—is mixed. As it pertains to healthy individuals at risk

for MDD, such as those with remitted depression, or with family members diagnosed with current MDD, some past research indicates elevated cortisol reactivity in response to an acute psychosocial stressor (Alexander et al., 2009; Höhne et al., 2014; Holsboer et al., 1995). However, a wealth of literature also paradoxically points to decreased or blunted cortisol reactivity in response to acute stress in healthy individuals at risk for depression (Ahrens et al., 2008; Burke, Fernald, et al., 2005; Morris et al., 2014). Some of the observed variability in cortisol response may be attributable to differential contextual factors such as severity of the stressor or gender. For example, previous studies examining cortisol reactivity to acute psychosocial threat in individuals with remitted depression found blunted cortisol reactivity (Ahrens et al., 2008; Morris et al., 2014), while other studies examining remitted depression found blunted cortisol reactivity in women participants only (Bagley et al., 2011), suggesting the importance of examining gender differences in cortisol outcomes.

Importantly, findings may also diverge based on stressor severity. For example, a previous study examining genetic risk of single variants associated with depression in non-depressed young adults manifested as a blunted cortisol response to a lab-based stressor, where individuals were provided negative feedback on a task to induce psychosocial threat (Avery & Vrshek-Schallhorn, 2016). A study using the same genetic variants, but using a more mild manipulation of the same lab-based stress paradigm as Avery and Vrshek-Schallhorn (2016), found elevated cortisol levels rather than blunted (Brummett et al., 2012). These divergent results suggest important contextual differences, where the severity of acute stress exposure may influence whether at-risk individuals demonstrate hyper- or hypo-reactive cortisol responses, as described in the cortisol reactivity threshold model (Vrshek-Schallhorn et al., 2018). Critically for the present work, this model hypothesizes that depression risk factors will predict heightened

cortisol reactivity to mild or moderate stressors, but blunted cortisol reactivity to more robust stressors, such as the present study's negative evaluative stress induction (discussed later). In this context, blunted cortisol could be seen as a failure to mobilize resources in response to psychosocial threat in people at risk for depression, a "giving up" response (Morris et al., 2014; Vrshek-Schallhorn et al., 2018). Thus, it is plausible that cortisol reactivity profiles would be blunted in the context of a robust, severe stressor in an at-risk sample.

Gene by Environmental Interactions and Stress Reactivity

Gene by environment (GxE) interaction models contend that stress-related psychopathology manifests through an interaction of genetic risk and an environmental stressor (Colodro-Conde et al., 2018; de Kloet et al., 2005). The theoretical framework supporting GxE interactions is known as diathesis stress theory (Monroe & Simons, 1991), which posits that environmental stressors activate a "diathesis" or biological vulnerability, and propagate risk of stress-related disorders, including depression (Colodro-Conde et al., 2018; Monroe & Simons, 1991; Schotte et al., 2006). Furthermore, diathesis stress models espouse that the effect of the interaction between the individual vulnerability and stressor may be *multiplicative* compared to their combined separate effects (Monroe & Simons, 1991). Thus, a number of GxE studies have aimed to clarify the role of candidate genes in relevant biological systems including HPA axis genes (e.g., *CRHR1, NR3C1, NR3C2, FKBP5;* discussed below) in modulating stress reactivity (Binder, 2009; Bogdan et al., 2016; Christine Heim et al., 2009).

Multilocus Genetic Profile Scores

Gene by environment interaction research has been largely characterized by identification of theoretically selected single genetic variants, i.e., "candidate genes;" however, this approach has been criticized for its small effect sizes and small sample sizes leading to low-powered tests

(Dick et al., 2015), and for failing to conform to the additive assumptions of behavioral genetics (Nikolova et al., 2011). That is, behavioral genetic models assume polygenic risk for depression, in which many genes each with small effects contribute in a cumulative, additive fashion, rather than any single genotype having a large effect as observed in conditions with simple genetic bases (Colodro-Conde et al., 2018; Wray et al., 2012). The candidate gene field has moved toward additive genetic profiles, known as multilocus genetic profile scores (MGPS)—which represent cumulative risk profiles comprised of known genetic variants in a physiological system (Bogdan et al., 2016; Nikolova et al., 2011). The use of an MGPS may increase power because the approach uses continuous genetic variables in contrast to dichotomous variables typically used in the single candidate gene approach (Altman & Royston, 2006). Moreover, when MGPS are constructed from specific neurobiological systems and used to examine intermediate outcomes, or "endophenotypes" (Hasler et al., 2004) relevant to closely related systems, this may further enhance power. Specifically, predicting intermediate outcomes in genetic studies is thought to reduce error variance because of the relatively proximal relationship between genetic variables and intermediate outcomes, as compared to diagnostic outcomes (Hasler et al., 2004).

Genes Impacting HPA Axis Functioning

I will preface a review of the previously reported MGPS and their findings with a brief introduction to the genes and single nucleotide polymorphisms (SNPs) used, as well as their attendant function, as an orientation to the reader. Genes, polymorphisms, and coding information is provided in Table 1.

CRHR1

Risk alleles include rs110402, rs7209436, rs242924, rs4792887, rs242939, rs1876828, and rs242941. The *CRHR1* gene codes for corticotropin releasing hormone receptor 1 in the

pituitary gland (Liu et al., 2006)). Polymorphisms in *CRHR1* have been associated with dysregulated cortisol levels in response to psychosocial stress (Christine Heim et al., 2009; Li et al., 2019; Ludwig et al., 2018), and with depressive symptoms (Bogdan et al., 2011; Davis et al., 2018; Christine Heim et al., 2009; Ludwig et al., 2018).

NR3C1

Risk alleles include rs41423247, rs10482605, and rs10052957. The *NR3C1* gene encodes glucocorticoid (GR) receptors which are highly expressed in the hippocampus and other areas of the brain. Specifically, GRs have a low affinity but high capacity for cortisol and play a role in HPA axis regulation by downregulating cortisol levels when they are high, usually in the context of chronic or ongoing stressors (Zhe et al., 2008). When stressors are enduring, repeated occupation desensitizes GRs and compromises its downregulating function, permitting cortisol to remain chronically elevated, creating a host of downstream, potentially harmful sequelae, such as compromised immune function. This gene has been linked to dysregulated stress reactivity (Plieger et al., 2018) and depressive outcomes in multiple studies (Peng et al., 2018).

NR3C2

Risk alleles include rs5522, rs2070951, and rs4635799. The *NR3C2* gene codes for mineralocorticoid receptors (MR), which are highly expressed in the hippocampus and assist in the inhibition of the HPA axis. As opposed to GR, MR has high affinity for cortisol and is occupied under low to moderate amounts of cortisol such as in the context of acute or early stages of threat (Zhe et al., 2008). Polymorphisms have been found to alter MR activity and enhance depression in individuals with a history of early life adversity (de Kloet et al., 2016; Vinkers et al., 2015) and influence cortisol responses in the context of lab-based stressors (Gutiérrez-Zotes et al., 2019; de Kloet al., 2011; Plieger et al., 2018). Along with altered HPA

axis activity, a previous study demonstrated altered *autonomic* activity in carriers of the rs5522 variant in response to the TSST (DeRijk et al., 2006).

FKBP5

Risk allele includes rs1360780. The *FKBP5* gene co-chaperones GR receptors. Variants of *FKBP5* are associated with modulation of GR sensitivity to cortisol and impaired negative feedback in the HPA axis system related to decreased GR sensitivity (Binder, 2009). Possessing the *FKBP5* variant suggests that there will be decreased binding of cortisol to GRs, which inhibits the negative feedback system of the stress response, prolonging HPA axis response and prolonging the "wear and tear" of GRs. For this reason, *FKBP5* is associated with risk for depression (Dam et al., 2019; Ising et al., 2019; Normann & Buttenschøn, 2019; Wang et al., 2018).

Established HPA MGPS

The extant HPA MGPS literature reviewed below has collectively used 14 genetic variants located in (or very near) the four genes (*CRHR1, NR3C1, NR3C2, FKBP5)* previously described. The authors (Bogdan et al., 2016; Di Iorio et al., 2017; Pagliaccio et al., 2014, 2015a, 2015b; Starr & Huang, 2019) adopted slightly different approaches to select candidate genes for their respective HPA MGPS. Table 2 provides a visual depiction of SNPs included previous studies of HPA MGPS, as well as SNPs included in the current study's HPA MGPS.

Pagliaccio et al., (2014, 2015a, 2015b) used an HPA MGPS comprised of 10 SNPs, including *CRHR1* (rs4792887, rs110402, rs242941, rs242939, rs1876828), *NR3C2* (rs5522), *NR3C1* (rs41423247, rs10482605, rs10052957), and *FKBP5* (rs1360780) across three studies. In Pagliaccio et al. 2014, the authors found that an HPA MGPS and early life stress interacted to predict hippocampal and amygdala volume in school-aged children, finding that children with

higher HPA MGPS and higher early life stress predicted volumes consistent with depressive profiles. The authors also found a main effect for HPA MGPS positively predicting cortisol activity, suggesting an HPA MGPS's relation to depression risk. In Pagliaccio et al., 2015a, the authors tested whether HPA MGPS and childhood stress exposure independently or interactively predicted amygdala activity to cortical areas central to emotion regulation and anxiety processes in a sample of school-aged children. They found that higher HPA MGPS and higher early life predicted poorer functional connectivity in the amygdala, indicating depression risk in those with a higher MGPS relative to children with a lower MGPS. Finally, in Pagliaccio et al., 2015, the same HPA MGPS was used in concert with stress exposure to examine amygdala and hippocampus responses to fearful versus neutral activation in school-aged children. This study revealed key individual differences in how children with higher MGPS respond to negative emotional stimuli via amygdala and hippocampus activation, where factors such as sex and pubertal status moderated MGPS and cortical responsivity. Taken together, this characterization of a novel HPA MGPS demonstrated the first relationships of this score with depression related neurobiological outcomes.

Di Iorio et al., (2017) used an HPA MGPS comprising 4 SNPs across 3 genes, including *FKBP5* (rs1360780), *CRHR1* (rs110402); and a *NR3C2* (rs5522, rs4635799) "CT" haplotype (SNPs that tend to be inherited together) to examine whether an HPA MGPS moderates the relationship between early stress and both amygdala function and volume in a college-aged sample. The authors found that individuals with a higher HPA MGPS had higher threat-related amygdala reactivity compared to those with a low HPA MGPS. The HPA MGPS in this study is smaller compared to previously discussed studies because the authors selected one SNP per gene (rather than multiple) to include in the HPA MGPS to equally weigh gene influence, and

therefore prioritized SNPs that were more well-characterized in the literature. Some SNPs of interest were also not available for microarray at the time this research was conducted and were unable to be included in the MGPS, such as the three *NR3C1* variants noted in forthcoming studies described below. Lastly, the authors used fewer *NR3C2* variants, as to their knowledge at that time, there were no other SNPs in the gene associated with HPA axis function, explicating their smaller MGPS.

Feurer et al., (2017) used a similar HPA MGPS as Di Iorio and colleagues (2017) to examine acute stress and depressive symptoms in a sample of at-risk children; however, Feurer and colleagues included two additional *CRHR1* variants in order to examine the *CRHR1* protective "TAT" haplotype, and they included a different SNP to examine an alternate *NR3C2* (rs5522, rs2070951) "CA" protective haplotype, yielding an HPA MGPS with 6 SNPs. Their study aims were to examine depressive symptoms in children of depressed mothers in the context of stress exposure (Feurer et al., 2017). The authors found that children of depressed mothers who had a higher MGPS and who experienced higher levels of interpersonal stress experienced heightened depressive symptoms compared to children with lower MGPS. Compared to Pagliaccio et al, (2014, 2015a, 2015b), Di Iorio et al., 2017 and Feurer et al., 2017; included additional SNPs in the *NR3C2, CRHR1,* and *NR3C1* genes to examine additional variants related to depression phenotypes. While Feurer et al., (2017) found evidence that the "TAT" haplotypes were protective, this has not been consistent in the literature. For example, Davis et al., (2018) demonstrated that carriers of the same TAT haplotype demonstrated more pronounced cognitive symptoms of depression, compared to those without the haplotype, urging additional examination.

Starr and Huang (2019), mirrored Pagliaccio's HPA MGPS, comprised of 10 SNPs in a sample of adolescents, and found a significant main effect of MGPS on depression symptoms. Similarly, HPA MGPS interacted with acute stress, chronic stress, and interpersonal childhood adversity, respectively, to predict depressive symptoms.

Our approach aims to utilize the aforementioned studies' HPA MGPS (Di Iorio et al., 2017; Feurer et al., 2017; Pagliaccio et al., 2014; 2015a; 2015b; Starr & Huang, 2019) by using the same SNPs as previously reported to facilitate eventual meta-analysis, per recommendations (Vrshek-Schallhorn et al., 2015). Moreover, the current study aims to address extant gaps in the literature. First, all but one of the aforementioned HPA MGPS studies use adolescent and child samples (Feurer et al., 2017; Pagliaccio et al., 2014, 2015a, 2015b; Starr & Huang, 2019a). Only one study to the best of our knowledge, employs an HPA MGPS in a college-aged sample, which examined cortical and depressive outcomes (Di Iorio et al., 2017). Emerging adulthood, defined as ages 18 to 24, represents a salient developmental period with its own unique risk factors for depression (Lisznyai et al., 2014). This is in part due to the multiple transitions that typically occur in this phase including identity formation, career development, and relationship formation (Arnett, 2000). The consistently changing life circumstances are perceived differentially among emerging adults, with some experiencing symptoms of anxiety and depression in response (Lisznyai et al., 2014). Additionally, previous studies suggest that personality traits such as neuroticism, a substantiated risk factor for depression, is highest during emerging adult years (Aldinger et al., 2014). Taken together, emerging adulthood is a salient timepoint to examine depression risk.

Second, most studies have examined depressive symptoms as an outcome, rather than stress reactivity directly, leaving a gap in understanding the mechanisms by which this genetic

vulnerability influences depressive risk. Pagliaccio et al. (2014) does examine cortisol reactivity using the Laboratory Temperament Assessment Battery, however, this examines temperament in response to both positive and negative/frustrating tasks, which can be ambiguous, rather than using an explicit negative evaluative manipulation that provides heightened psychosocial threat in addition to a putatively neutral control condition (Way & Taylor, 2010). These two gaps are critical, as HPA MGPS moderation of negative evaluative stress exposure predicting cortisol reactivity in an emerging adult sample has not yet been examined. A third gap, discussed below, pertains to probing the HPA MGPS's influence on interrelationships between multiple stress responsive systems. A final gap is to examine the extent to which proposed haplotypes of "CA" and "TAT" buffer, or protect the effects of stress exposure on cortisol reactivity.

Relationship between HPA Axis and SNS System

Most investigations of stress response dysregulation have focused on the HPA axis and SNS as independent from one another (Bauer et al., 2002), but there are at least three compelling reasons to test their joint action: basic physiology, asymmetry theory, and prior evidence from stress induction studies.

First, prior work suggests that the SNS and HPA axis are physically interconnected and coordinated in their response to stress, suggesting that it may be fruitful to examine them in tandem (Rotenberg & McGrath, 2016). The physical interconnectedness of the HPA axis and SNS is supported by animal (Itoi et al., 2004; Reyes et al., 2005; Ziegler et al., 1999) and human studies (Engert et al., 2011; Rotenberg & McGrath, 2016). Animal evidence indicates that the SNS and HPA axis are reciprocally innervated, such that hypothalamic neurons (emanating from the paraventricular nucleus) modulate activity of the locus coeruleus (LC), which secretes norepinephrine (noradrenaline), the primary neurotransmitter of the SNS (Itoi et al., 2004; Reyes

et al., 2005). Similarly, norepinephrine (NE) releasing neurons in the brainstem stimulates CRH releasing neurons in the hypothalamus, which leads to ACTH and then cortisol release. Moreover, NE axons emanate to all elements of the HPA axis system (hypothalamus, prefrontal cortex, hippocampus, and amygdala; (Goddard et al., 2010). Ziegler and colleagues (1999) compellingly demonstrated that lesioning the LC, thus reducing norepinephrine release, diminished HPA axis activity in rodent models. Physiological evidence in humans echoes that in animals. HPA axis variants have been shown to alter the structure and function of cortical brain structures that influence both SNS and HPA axis activity, such as CRH related variants in the hypothalamus (e.g., *CRHR1* gene; Modell, 1998). These studies suggest functional and structural interconnectedness between the LC (critical to the SNS) and the HPA axis and support that genetic variation in the HPA axis may also influence SNS regulation.

Second, theoretical models support examining HPA axis and SNS activity in tandem through statistical interactions, as prior work suggests that the two explain more variance in mental health outcomes considered together than considered separately (Bauer et al., 2002). Specifically, multisystem asymmetry theory (Bauer et al., 2002) suggests that the physiological interconnectedness between the SNS and HPA axis results in a pattern in which those with better mental health outcomes tend to have symmetric responding to threat from the SNS and HPA axis. By contrast, it predicts that those with adverse mental health outcomes (e.g., internalizing symptoms) will show asymmetric responding to threat in SNS and HPA axis biomarkers, such as increased cortisol in the context of low SNS activity, or low cortisol in the context of high SNS activity, in response to threat. Multiple studies across youth samples have demonstrated empirical support for multisystem asymmetry theory in children and adolescence (Gordis et al., 2008; Martinez-Torteya et al., 2017). For example, Vigil et al. (2010) found among a group of

Hurricane Katrina survivors in late-adolescence/early-adulthood, that hurricane exposure and sAA activity moderated the relation between cortisol and internalizing behaviors, such that higher sAA and lower cortisol predicted higher internalizing behaviors, demonstrating theoretical support for asymmetry theory. Moreover, Gordis et al. (2008) found support for multisystem asymmetry theory, such that maltreated children showed elevated sAA and lower cortisol in response to a modified TSST compared to non-maltreated youth. The authors conceptualize the asymmetry as resulting from habituation of the HPA axis response to chronic stress exposure (e.g., attenuated response over time), while the SNS maintains a consistent, robust response to repeated threat.

By contrast, the interaction between the SNS and HPA axis has been less frequently examined in adults. However, prior experimental stress induction models seem to support the asymmetry model. Andrews and Pruessner (2013) examined the interaction between the SNS and HPA axis activity. Healthy adults were given either an SNS inhibitor (propranolol) or a placebo prior to completing the TSST. In the propranolol condition, individuals had significantly reduced sAA and significantly *increased* cortisol compared to controls who were exposed to the same stressor, suggesting that inhibited SNS leads to elevated HPA activity. From this, the authors proposed that the SNS may serve an inhibitory role over HPA axis activity (Andrews & Pruessner, 2013). Similarly, prior work has tested an opposite approach, administering either placebo or dexamethasone, which inhibits the peripheral HPA axis response, and administering the TSST to healthy, adult volunteers (Andrews et al., 2012). The dexamethasone group, which had a blunted cortisol response to the TSST, also had increased heart rate (an indicator of autonomic activity consistent with increased SNS activity) relative to controls (Andrews et al., 2012).Taken together, these two studies suggest that the SNS and HPA axis depend upon each

other's action to respond to threat. The pattern of findings could be interpreted as SNS and HPA being mutually inhibitory, or instead, that when one insufficiently responds during threat, the other compensates with a larger response to marshal the resources necessary to face the threat.

Two predictions follow. First, if 1) healthy individuals experience elevated sAA in response to the psychosocial threat, and 2) HPA axis genetic variation predicts blunted cortisol reactivity to psychosocial threat, and 3) multisystem asymmetry theory contends that discordant patterns between HPA axis and SNS reactivity indicate dysregulation, then we predict that individuals with higher HPA-related genetic risk will demonstrate blunted cortisol and *elevated* sAA in response to negative evaluative stress. Second, if SNS activity precedes and modulates HPA axis activity in response to stress, then we would predict that, an elevated sAA response will affect HPA axis reactivity by blunting cortisol in those with higher genetic variation. Thus, the present work will also examine whether sensitivity in the HPA axis indicated by genetic risk score is modulated by reactivity level in the SNS, indexed by sAA, to predict maximally blunted cortisol reactivity under acute psychosocial threat.

Lab-Based Stress Paradigm

To reliably elicit a neuroendocrine and sympathetic response in a controlled manner, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) was developed as a brief, objective, lab-based psychosocial stress paradigm. The TSST permits collection of a range of physiological indices, including neuroendocrine markers like cortisol, and sympathetic markers like sAA (see Allen et al., 2017 for a review). Moreover, lab-based psychosocial stress induction has been used to demonstrate GxE interactions successfully as evidenced by a meta-analysis of a serotonin system genetic variant and TSST reactivity (Miller et al., 2013), and further may provide evidence for genetic risks for depression (Avery & Vrshek-Schallhorn, 2016).

The TSST consistently elicits an HPA axis response through its simulation of acute, psychosocial threat using social evaluation and unexpected performance-based tasks (Allen et al., 2017). In brief, the standard TSST devotes 5 minutes each to speech preparation, delivering an extemporaneous speech on a typically self-evaluative topic, such as a job interview, and finally conducting arithmetic problems out loud in front of an audience (Kirschbaum et al., 1993). In typical manipulations of the TSST, the audience is trained to not provide any feedback, and maintain only neutral facial expressions. The current study uses a TSST paradigm that aligns with previous work by Way and Taylor (2010), where there is an experimental, negative evaluative condition and control, neutral-feedback condition. The difference is that the audience in the negative evaluative condition are "judges" who are trained to provide negative verbal and nonverbal feedback while the participant performs each task (see Appendix A for a detailed behavioral script). Employing a control comparison condition facilitates the interpretation of condition effects on cortisol outcomes and is evident in our previous lab work (Avery & Vrshek-Schallhorn, 2016). In sum, the TSST is a useful tool for dosing acute psychosocial stress and activating key stress response systems to evaluate patterns of dysregulation.

Goals & Hypotheses

The goal of the present study is to examine whether individual differences in HPA genetic variation, as examined using an MGPS from previously studied SNPs, predict cortisol and sAA reactivity to a stressful condition versus control condition in an emerging adult sample. Consistent with diathesis-stress theory, I hypothesize that an HPA MGPS will interact with stress condition (e.g., the negative evaluative condition of the TSST, as compared to a control condition) to predict cortisol response in an emerging adult sample. Specifically, higher HPA MGPS will predict relative blunting of cortisol reactivity in the negative evaluative versus

control condition (see Figure 1). Second, I hypothesize that a higher HPA MGPS interacts with condition to predict augmented sAA in the negative evaluative condition relative to the control condition (see Figure 2). Finally, I hypothesize that an HPA MGPS will interact with sAA reactivity and condition to predict cortisol reactivity, such that in the context of the negative evaluative stress condition, higher MGPS and sAA responses will predict blunted cortisol (Figure 3).

CHAPTER II: METHOD

Participants

The present sample includes non-depressed emerging adults (N = 144; 55% female; 18-29 years) who were undergraduate students at the University of North Carolina at Greensboro. Individuals were recruited to participate in a study on genetics, lab-based stress, and stress responding. Participants were recruited from the psychology department's "Mass Screening" procedure after filling out an eligibility questionnaire or were recruited through IRB-approved flyers posted on campus. Participants were invited to participate if they were between the ages of 18-30, did not currently use any form of hormonal birth control, did not currently use nicotine, were not diagnosed with a chronic health condition, did not have a history of head trauma, were not taking steroidal or psychotropic medication, did not have a diagnosed learning disability, were not colorblind, and were not a non-native English speaker. Upon passing the initial screening, participants were screened for acceptable blood pressure. Participants with either a systolic blood pressure above 160 and/or a diastolic blood pressure above 100 (i.e., the diagnostic threshold for hypertension) were excluded as a safety precaution and to avoid confounding effects (Gu et al., 2008), as aims unrelated to the current study examined cardiovascular responses to stress.

A total of 152 participants were consented and completed the full protocol. After excluding individuals for missing more than two SNPs (n = 5) and for outliers (n = 3), a total of 144 participants were included final analyses. The average age was 19.5 years (SD = 1.94, range: 18-29) and self-reported gender was 58.1% female and 41.9% male. Regarding race and ethnicity, participants reported identifying as: 44.4% Black/African American, 39.2% White,

4.7% Hispanic/Latino/a, 3.4% Asian/Pacific Islander, 2.7% Biracial, and 5.4% Other. See Table 3 for the demographic composition of the sample.

Materials and Measures

Salivary DNA

Participants provided saliva samples for DNA extraction and genotyping via passive drooling through a straw into sterile DNAse and RNAse-free, cryogenic vials. After collection, saliva samples were stored in a freezer at -80°C. Frozen samples were shipped by courier to the University of Wisconsin Next Gen Core Lab for testing of 14 HPA genetic variants. DNA was extracted using Oragene extraction kits (DNA Genotek, Ontario, Canada).

For quality control, all allele frequencies were tested for deviations from expected genotype frequencies, consistent with Hardy Weinberg Equilibrium (HWE). Deviations from HWE are represented by a significant chi square goodness of fit test and can indicate the potential for genotyping errors, but can also arise due to racial/ethnic sample admixture. If any variants or haplotypes deviated from HWE in the full sample, I checked for deviations within individual racial/ethnic groups because expected differences by group can lead to spurious failures of HWE in racially/ethnically heterogeneous samples. In the event that variants continued to deviate from HWE within racial/ethnic groups, I excluded the variants from primary analyses.

Salivary Cortisol and α-Amylase (sAA)

Saliva was collected at 5 points throughout the study: (1) at baseline (+0 min), (2) after the instructions for the TSST were provided (+5 from baseline), (3) after the TSST was completed (+20 min after baseline), (4) after completing several computerized cognitive tasks not discussed here (+45 min after baseline), and (5) after debriefing was completed (+65 min after baseline). A tube of saliva was collected through passive drooling through a straw and into

a sterile cryogenic vial. These tubes were also stored in -80°C freezers, and then shipped to Trier, Germany for duplicate assay for cortisol and sAA at the conclusion of the study. I screened data for excessive outlying values (>M+/-3 SDs) in the combined sample for baseline samples, and within condition for remaining samples, and winsorized outliers to M+/- 3 SDs, which is customary for these biomarkers (Vrshek-Schallhorn et al., 2018).

I examined reactivity in each biomarker by constructing Area Under the Curve with Respect to Increase (AUCi), which reflects reactivity over baseline levels, emphasizing change over time while incorporating multiple time points (Pruessner et al., 2003). Once I calculated AUCi values, I reassessed them for univariate outliers within condition. If AUCi values still exceeded >M+/-3 SDs, they were excluded from analyses. As a result, two cases of sAA reactivity in the negative evaluative condition were excluded from analyses, and one case in the control condition for cortisol reactivity was excluded from analyses. Positive values of AUCi reflect the predominance of an increase from baseline, whereas negative values the predominance of a decrease in levels from baseline. I used samples 1, 3, 4, and 5 to calculate cortisol AUCi, and samples 1, 2, 3, and 4, to calculate sAA AUCi due to well-established differences in the time-courses for their reactivity (Nater et al., 2007). The second sample was a priori intended only to measure salivary alpha-amylase (sAA), following evidence of its rapid responding, and preliminary analyses show that sAA has returned to baseline on average by the 4th sample, indicating that also using the 5th sample for sAA may distort the measure of reactivity.

Socioeconomic Status

Participants reported parental education and vocational attainment necessary to compute the Hollingshead Index, an indicator of socioeconomic status (SES; Hollingshead, 1975). Scores

range from 8 to 66, with higher scores reflecting a higher SES (M = 44.56, SD = 12.78). Because SES is associated with depression risk and has been shown to influence HPA axis activity (Hoebel et al., 2017), SES was centered and included as a covariate if the initial model without covariates was significant.

Current and Lifetime MDD

Participants were screened for current MDD with the gold-standard Structured Clinical Interview from the DSM-IV (SCID). Participants with current MDD were diverted to the control condition out of an abundance of caution and were excluded from analyses involving the TSST due to non-randomization. We did not want to put participants with depression at increased risk by completing a negative evaluative TSST, plus we believed it could confound results based on meta analytic evidence (Burke, Davis, et al., 2005). Lifetime MDD was measured for use in analyses beyond the scope of the present project, and those with a history of MDD (40.3% of sample) completed the study the same as those without such history (n = 58).

Multilocus Genetic Profile Score

The current study examined the largest, most comprehensive MGPS possible with the available data (14 SNPs). To do so, I calculated an MGPS using all previously reported SNPs that are not excessively correlated with one another (> 0.7) in preliminary examination. The genes and polymorphisms include: *CRHR1* (rs110402, rs7209436, rs242924, rs4792887, rs242939, rs1876828); *NR3C1* (rs41423247, rs10482605, rs10052957); *NR3C2* (rs5522, rs2070951, rs4635799); and *FKBP5* (rs1360780). There is evidence of four haplotypes (sets of polymorphisms that tend to be inherited together) in the current study. First, there is evidence of a three-SNP haplotype in the *CRHR1* gene (rs242941, rs242939, rs1876828) forming a "GAG" haplotype (Pagliaccio et al., 2014; 2015a; 2015b). There is additional evidence of a protective

"CA" haplotype in the *NR3C2* gene (rs5522 and rs2070951; Feurer et al., 2017), and an additional *NR3C2* "CT" haplotype (rs5522 and rs4635799; Di Iorio et al., 2017). Feurer et al., (2017) and Di Iorio et al., (2017) both use the SNP rs5522 in their diverging *NR3C2* haplotypes, and therefore cannot be examined in tandem as to not double count rs5522 in the variance. Instead, I used Feurer et al.'s haplotype using the rs2070951 SNP due to its extensive characterization in the literature (de Kloet et al., 2016; Klok et al., 2011; van Dijk et al., 2017), but results with Di Iorio et al. 2017's haplotype are presented as well. Finally, a protective "TAT" haplotype in the *CRHR1* gene (rs7209436, rs110402, and rs242924) is noted in previous work (Feurer et al., 2017). Haplotypes using multiple SNPs were treated as a single marker, consistent with prior work (Feurer et al., 2017; Pagliaccio et al., 2014; 2015a; 2015b).

The coding of each variant is presented in Table 1. Across all polymorphisms, each genotype and haplotype were coded for the presence (2) or absence (0) of at-risk genotypes (indicating two or zero "risk" alleles), with 1 assigned to heterozygote "intermediate" cases if supported by biological evidence as used in prior reports (Bogdan et al., 2016; Starr & Huang, 2019). Haplotypes that are protective, such as the *CRHR1* haplotype used by Feurer et al., were coded as absence (2), intermediate as (1) and presence as (0). A score of summed polymorphisms for all 14 variants was calculated across each participant to create a MGPS (possible range of 0-16 after haplotypes are accounted for). Participants were included if missing up to 20% of genotypes per person based on previous MGPS work, and MGPS scores for those with missing SNP data were prorated by calculating their proportion of available risk variants (Vrshek-Schallhorn et al., 2015) by calculating the individual's sum of available risk scores, divided by their maximum possible total score without the missing polymorphism (i.e., 7) to

achieve a proportion score, and returned to the scale of the MGPS via multiplication by 8. Individuals (n = 5) missing more than two genotypes were excluded from analyses.

All genetic variants were assessed for Hardy-Weinberg equilibrium (HWE) using chi square tests. Chi square analyses revealed rs1360780 (*FKBP5* gene) was not in HWE in the full sample ($p < .0001$ for rs1360780). Further, because racial/ethnic heterogeneity can explain some deviations from HWE, HWE was re-assessed within racial/ethnic groups using dichotomized minority status (0 = white, 1 = minority status). The SNP rs1360780 deviated from equilibrium within each group (white $\chi^2 = 51.160$, $p < .0001$; minority $\chi^2 = 23.06$, $p < .0001$). Because the variant rs1360780 deviated from HWE within both groups, it was excluded from analyses. The final HPA MGPS scores (excluding rs1360780) were normally distributed (M = 5.40, SD = 1.40, observed range: 1 - 10.5). One-way ANOVA analyses revealed no significant difference between HPA MGPS profiles across conditions (F (1,143) = 1.250, p = .265).

Procedure

Participants were quasi-randomized to either the control or TSST condition. Most participants signed up online blind to a pre-scheduled condition. A smaller proportion of participants scheduled their session directly with a study coordinator, who did not know the personal characteristics (e.g., HPA MGPS) of the participants when scheduling, and the participants were blind to their scheduled conditions. This study was comprised of two sessions, completed in two consecutive days in most instances. These sessions were completed between 1:00 P.M. and 5:30 P.M. to reduce the influences of diurnal cortisol and sAA (Dickerson & Kemeny, 2004; Nater et al., 2007). Next, participants completed a semi-structured clinical interview about current depressive episodes, followed by a series of computerized questionnaires

on personality and life experiences. Participants completed an additional life stress interview and cognitive measures not discussed here for other aims in the larger study.

In the second session, individuals first completed several computerized questionnaires to adjust to laboratory conditions, followed by either a negative-evaluative variant of the TSST (Way & Taylor, 2010) or a putatively non-stressful control protocol. A smaller group completed an experimental third intermediate condition as part of the larger project but did not provide DNA samples; they are not included in the present study. Saliva samples were collected at five time points during the TSST as previously noted. Participants either received course credit or $30 for study completion, and all participants received $5 as an incentive for an additional cognitive task not described here.

Trier Social Stress Test (TSST)

In both conditions, participants performed similar tasks, where they were told that they were being video-recorded and were instructed to face the camera. Participants were asked to choose a slip of paper out of a box that had ostensibly different topics. Unbeknownst to the participant, all participants in the negative evaluative condition had the same speech prompt, which was to talk about their electability for a student leadership position, including their people skills, organizational skills, intellectual abilities, and reliability. Participants in the control condition were asked to speak for 5 minutes on a neutral topic, tips others could use to maintain a healthy lifestyle. Participants were given 5 minutes to prepare the speech and were asked to deliver the speech for 5 minutes. Afterwards, participants completed an arithmetic task counting backwards from 2,017 by 13's for 5 minutes. When participants made a mistake, they were asked to start again in both conditions.

In the negative evaluative condition, participants were told that they would be evaluated, and were uniformly provided negative nonverbal feedback by two trained judges. Judges in the negative condition were one male and one female research assistant who followed behavioral scripts to convey boredom and dissatisfaction with the speech (e.g., exchanging a judgmental glance with each other; behavioral script located in Appendix A). In addition, judges reminded participants to look at the camera, demanded participants continue speaking for the entire 5-minute period, or told them to go faster in the arithmetic portion, in efforts to provide stern feedback. In contrast, in the control condition, there were no confederate judges, the participants were explicitly told they would not be evaluated, and the experimenter remained in the room pretending to prepare for future sessions, but out of the participant's direct line of sight. Similarly, the experimenter was polite, but provided neither positive nor negative feedback. In the arithmetic portion, control participants were provided neutral feedback when a mistake was made and were asked pleasantly to start over to ensure similarity of tasks across conditions. These experimental conditions produced expected differences in cortisol reactivity in previous studies (Avery & Vrshek-Schallhorn, 2016; Ditcheva et al., 2018; Vrshek-Schallhorn et al., 2019).

Debriefing. Participants were fully debriefed after the TSST. Specifically, participants assigned to the experimental (negative-evaluative) condition of the TSST were told that the panel were research assistants trained to provide negative, non-verbal feedback to all participants and showing the participant the behavioral script. Participants were further told that the panel was trained to make the participant feel like they were doing a bad job, and that the feedback they gave had nothing to do with the participant's performance on the task. Last, the participants in the experimental condition were told that that they were not actually evaluated, and all were told

that no one was videotaped in either condition. All participants were told the general purpose of the study and could ask any remaining questions. Finally, participants provided documentation of debriefing and continued informed consent to use their data due to the prior deception; no participants withdrew their data at this time.

Manipulation Checks

Self-Report

After completing the TSST, participants completed several manipulation checks. They were asked to what extent they felt evaluated, and if they had felt evaluated, to what extent the evaluation was positive and negative on an online questionnaire administered through Qualtrics. I used this information to test whether conditions differed in perceived evaluation using separate one-way ANOVAs, where individuals in the experimental condition of the TSST are predicted to show increased feelings of overall evaluation and negative evaluation, but less positive evaluation, compared to individuals in the control condition.

Cortisol & α-Amylase

Cortisol and sAA reactivity as AUCi were used as an additional manipulation check. Consistent with analytic procedures in the stress literature, I calculated cortisol and sAA area under the curve with respect to increase (AUCi; Pruessner et al., 2003), which calculates cortisol and sAA reactivity, compared to their respective baseline levels. I compared cortisol and sAA levels in experimental versus control groups as a manipulation check. A single value was produced for cortisol and sAA respectively and entered in as dependent variables in separate one-way ANOVA models.

Cortisol and sAA were analyzed AUCi to capture reactivity, or change over time. As stated earlier, values >M+/- 3 SDs were winsorized to the value of the third SD value. A total of

n = 1 value was winsorized for cortisol. There were not any sAA values considered to be outliers per these guidelines. Participants in the current sample obtained cortisol reactivity AUCi scores that were normally distributed (skewness = .923, kurtosis = 1.246). Scores for sAA AUCi were also normally distributed (skewness = .726, kurtosis = .990), therefore, neither AUCi value was log transformed.

Analytic Plan

Preliminary Analyses

I present descriptive statistics of key study variables, including demographics (e.g., age, gender, SES), HPA MGPS and sAA distribution across the sample, as well as the number of participants in each TSST condition. Second, I present zero-order bivariate correlations among independent variables (HPA MGPS, sAA, TSST condition), covariates (e.g., gender, SES, race/ethnicity), and the dependent variable of cortisol. Conditions were tested for differences in gender, SES, and baseline biomarker levels. I initially ran primary analyses in the full sample comprising of all racial/ethnic groups (dummy coding minority stats, non-minority status as 0/1). If models showed a significant effect, I reran the finding in the largest homogeneous racial/ethnic subgroup of participants to examine population stratification, or the differences in allele frequencies between racial groups, which may produce spurious findings if left unchecked. Moreover, prior work has identified differences in this HPA MGPS by race/ethnicity (Starr & Huang, 2019). All primary models were examined for multivariate outliers using Cook's distance; I re-analyzed models without individuals scoring >.5 on Cook's distance to gauge influence on results as needed (re-including them if they do not influence results, and excluding them if they do influence results) and automatically excluded individuals scoring >1.0 on Cook's distance from models. After inspecting these values, I excluded no cases from analyses.

Covariates

To ensure that any significant GxE effects are not spuriously arising due to other variables that influence HPA axis and SNS activity, I first examined whether experimental groups differ by sex, race/ethnicity, and SES. I followed up all significant GxE models (initially conducted without covariates) by adding any variables that differ by condition. Consistent with previous work in GxE research, I covaried not only main effects of the covariates, but also interactions with the HPA MGPS and condition separately (e.g., Covariate x Condition), to partial these covariate effects from the GxE interaction effect (Keller, 2014). I ran models with and without these covariates to preserve power.

Predicting Cortisol and sAA Reactivity

Multiple linear regressions were used to test hypotheses with IBM SPSS v26 (IBM Corp, 2019). Models for predicting cortisol and sAA reactivity were run separately, with cortisol AUCi and sAA AUCi entered as the dependent variables. Next, for main effects, I entered HPA MGPS in the model, as well as TSST stress condition, which was dummy coded (0 = control, 1 = challenge). Then, I created a product term of condition and centered HPA MGPS and entered it in a second block. To test the third hypothesis, I entered main effects in the first block, followed by all relevant two-way interactions in the second block, and the three-way interaction of Condition x HPA MGPS x sAA Reactivity in the final block. For all significant models, I followed up by rerunning models with relevant covariates. If the effect remained, I then decomposed effects using regions of significance analyses using the Johnson-Neyman technique (Johnson & Fay, 1950) which permits probe the boundaries of significant values contributing to the interaction effect (Preacher et al., 2006). Finally, consistent with other work with MGPS (Starr & Huang, 2019), I ran post-hoc models with individual SNPs rather than the MGPS to

examine whether the HPA MGPS effect was driven by multiple, significant SNPs or the cumulative effect of the HPA MGPS.

Power Considerations

This is the first investigation in which an HPA MGPS is being used to predict cortisol or sAA reactivity in an adult sample, thus effect sizes are not thoroughly established. However, among a sample of emerging adults ($N = 112$), there was a significant interaction between a *single* genetic variant and life stress in predicting cortisol reactivity (Avery & Vrshek-Schallhorn, 2016). The sample size ($N = 144$) is somewhat larger than typical for studies of the TSST (meta-analytic mean $N = 29.58$; Dickerson & Kemeny, 2004); however, this level was selected to achieve greater power for genetic analyses, which we assumed would have the lowest power (Avery & Vrshek-Schallhorn, 2016), and to achieve similar size as other stress-induction studies involving genetics published when this study was being designed (e.g., $N = 118$, Way & Taylor, 2010). Given the sample size of the current study ($N = 144$), adequate power is expected. Moreover, sensitivity analyses for multiple linear regression for the two-way interactions with 3 predictors: 1) HPA MGPS, 2) Condition, and 3) Condition x HPA MGPS powered to .80 indicated a minimum detectable effect size of 0.0752 (R^2 deviation from zero) with alpha levels at 0.05. The sensitivity analyses was calculated using G*Power (version 3; Erdfelder et al., 1996).

CHAPTER III: RESULTS

Descriptive Statistics

Preliminary Analyses

There were no group (control vs. challenge condition) differences based on gender (F (1,142) = .000, p = 1.000) or minority status (F (1,142) =, p = .236). However, there were group differences based on SES (F (1,142) = 1.002, p = .026), such that the experimental group (M = 42.198, SD = 13.412) had a lower SES relative to the control group (M = 46.926, SD = 11.738). SES was a planned covariate, however, which addressed this potential confound. See Table 4 for all sample characteristics across TSST condition.

Manipulation Checks

Manipulation checks were completed to ensure that the TSST conditions had the expected effect on perceived evaluation, cortisol reactivity, and sAA reactivity.

Validity of TSST Stress Paradigm. As expected, one-way ANOVAs revealed significant differences across condition of overall perceived evaluation (F (1,143) = 14.429, p < .001), positive evaluation (F (1,127) = 38.131, p < .001), and negative evaluation (F (1,146) = 52.978, p < .001), such that the negative evaluative condition reported greater overall perceived evaluation, less perceived positive evaluation, and greater perceived negative evaluation (Table 5).

Biomarker Reactivity. One-way ANOVAs revealed significant differences across condition for cortisol AUCi (F (1,142) = 43.752, p < .001) and sAA AUCi (F (1,146) = 6.267, p = .013). Specifically, sAA was increased in the challenge condition (M = 9.948, SD = 14.344) compared to the control condition (M = 3.990, SD = 14.508), demonstrating an increase of sAA

over time from baseline. Similarly, cortisol was significantly increased in the challenge condition (M = 18.658, SD = 14.344) compared to the control condition where there was an observed decrease of cortisol level from baseline consistent with cortisol's natural diurnal rhythm (M = -11.375, SD = 30.711). A visualization of cortisol activity across TSST condition is presented in Figure 4. There were no significant differences in baseline cortisol (F (1,143) = .664, p = .416) or sAA levels (F (1,143) = .388, p =.535) across condition. A visualization of sAA activity across TSST condition is presented in Figure 5.

Zero-order Correlations

Zero-order bivariate correlations were examined among focal predictors, covariates, and dependent variables and are presented in Table 6.

Primary Regression Results

Interaction Effects of MGPS x TSST Condition Predicting Cortisol Reactivity

Results from regression models indicated an HPA MGPS did not significantly moderate the relationship between TSST condition and cortisol reactivity (interaction term t = .229, p = .819). A main effect emerged in TSST condition predicting cortisol reactivity as expected (t = 6.304, p < .001). No other main effects emerged. Results for this model are presented in Table 7 and a visualization of cortisol reactivity (AUCi) as a function of HPA MGPS and stress condition is presented in Figure 6.

Interaction Effects of MGPS x TSST Condition Predicting sAA Reactivity

Results from regression models revealed that an HPA MGPS not significantly moderate the relationship between TSST condition and sAA (t = -.757, p = .450). A main effect emerged in TSST condition predicting sAA reactivity as expected (t = 2.322, p = .021). No other main

effects emerged. Results are presented in Table 8, and a visualization of sAA reactivity (AUCi) as a function of HPA MGPS and stress condition is presented in Figure 7.

Interaction Effects of MGPS x TSST Condition x sAA Predicting Cortisol Reactivity

Regression models revealed significant evidence that the relationship between TSST condition and cortisol reactivity depended jointly on a 7-SNP HPA MGPS and sAA reactivity (t = 2.294, p = .023; R^2 = .0285), a three-way interaction effect. Additionally, there was a main effect such that TSST condition predicted cortisol reactivity as expected (t = 6.156, p < .001). See Table 9 for results of this model. Because groups did not differ based on gender or race/minority status, I included only SES as a covariate in the follow-up model. Once accounting for SES and its interactions with sAA, MGPS, and condition (consistent with Keller, 2014), results for the hypothesized 3-way interaction remained significant (t = 2.462, p = .015). In addition to a significant main effect of condition (t = 6.035, p < .001), SES and MGPS interacted to predict cortisol reactivity (t = -2.438, p = .016). See Table 10 for results.

For post-hoc analyses of the hypothesized effect, I utilized the Johnson-Neyman technique (Johnson & Fay, 1950) using SPSS PROCESS (Hayes, 2013) to probe regions of significance (ROS) identifying at *which* levels of genetic risk and sAA reactivity TSST condition significantly predicted cortisol reactivity. However, critical to interpretation, we are most interested in the regions in which condition does *not* result in a significant difference in cortisol reactivity, consistent with blunting in response to the negative evaluative challenge condition. This resulted in two ROS at low sAA reactivity, at ≤ -2706.63 U/ml (slightly below -1 SD from the mean) and separately at high values of sAA reactivity, ≥ 3059.21 U/ml (slightly below +1 SD above the mean). Thus, the regions that did *not* significantly differ, and could be viewed as relatively blunted were defined by the region -2706.63 to 3059.20 U/ml. To visualize results,

cortisol reactivity was plotted as a function of HPA MGPS on the X axis across three panels representing low (-1 SD), middle (mean), and high (+1 SD) sAA reactivity (Figure 8). An alternative visualization depicts cortisol reactivity as a function of four profiles (high sAA with high and low MGPS, low sAA with high and low MGPS) in Figure 9.

We also reran this finding in the largest homogenous racial/ethnic group, consistent with approaches with heterogenous samples in GxE interaction research (Starr & Huang, 2019). The largest homogenous group in our sample was Black and African American participants (44.4%). Given the reduced sample size in this analysis (n = 64), we did not focus on p-values and instead evaluated the magnitude of the beta coefficient and direction. Results were similar to those in the full sample such that the sAA x Condition x MGPS interaction term approached significance in predicting cortisol (b = .037, p = .088), which was similar to the magnitude of the beta coefficient and direction in the full sample (b = .027, p = .023). Moreover, because there were no other significant correlations or group differences between race/ethnicity and key outcomes (sAA, cortisol), we conclude that it is unlikely that population stratification influenced findings.

Finally, consistent with other work with MGPS (Starr & Huang, 2019), we ran post-hoc SNP x TSST x sAA predicting sAA to examine whether the HPA MGPS effect was driven by multiple, significant SNPs, rather than the cumulative effect of the HPA MGPS. Results indicate that only one SNP (rs4792887) was significant (t = 2.182, p = .031; all other p's > .05), and also revealed that SNPs had effects in opposite directions, suggesting that the effect of the HPA MGPS is driven by the additive, cumulative effect of several of the HPA MGPS, but that others tended to reduce the detected additive effect. Results are presented in Table 11.

Tests with Previously Established HPA MGPS

Each of the following previously established HPA MGPS were directly tested in the current study, without *FKBP5* as noted previously. This includes the HPA MGPS reported in 1) Starr & Huang, 2019, Pagliaccio et al., 2014, 2015a, and 2015b, 2) Feurer et al., 2017, and 3) and Di Iorio et al. 2017.

None of these previously established HPA MGPS moderated the relationship between TSST condition and cortisol reactivity (all *p*'s > .05) or sAA reactivity (all *p*'s > .05). See Tables 12 and 13 for results of models using Pagliaccio et al., (2014, 2015a, 2015b), and Starr & Huang (2019)'s 10-SNP MGPS predicting cortisol reactivity and sAA reactivity, respectively. See Tables 14 and 15 for results of models using Feurer et al., (2017)'s 3-SNP model predicting cortisol reactivity and sAA reactivity, respectively. See Tables 16 and 17 for results of models using Di Iorio's 6-SNP HPA MGPS predicting cortisol reactivity and sAA reactivity, respectively.

Protective Haplotype x TSST Condition Predicting Cortisol and sAA Reactivity

An additive score of the *CRHR1* "TAT" haplotype and *NR3C2* "CA" haplotype did not moderate the relationship between TSST condition and cortisol reactivity (t = -.791, p = .430), nor between TSST condition and sAA reactivity *(*t = -1.300, p =.196). See Tables 18 and 19 for results of these models.

CHAPTER IV: DISCUSSION

The current study utilized a GxE interaction framework to examine whether an additive genetic risk score influenced acute physiological reactivity to a negative-evaluative stressor versus a control in a healthy, emerging adult sample. Outcomes were measured using a multi-system approach, collecting both cortisol and sAA reactivity to index HPA Axis and SNS activity, respectively. Contrary to predictions, the current study demonstrated that the relationship between a lab-induced stressor (TSST condition) and either cortisol or sAA reactivity did not significantly depend on an additive score of genetic variants known to impact the HPA axis. However, the results of the proposed three-way interaction demonstrated that the relationship between TSST condition and cortisol reactivity depended on both an HPA MGPS and sAA reactivity, implicating joint vulnerability across *two* stress systems as a risk factor for dysregulated stress responding. First, I present the conceptualization and theoretical considerations underpinning the relationship between stress exposure, sAA, and MGPS and cortisol reactivity and implications. Following, I discuss methodological, developmental, and theoretical considerations that may partially explain the null findings of the first two primary hypotheses and unsuccessful extensions of prior MGPS.

Three-way Interaction of sAA, HPA MGPS, and Condition Predicting Cortisol Reactivity

The current study is the first to report a significant three-way interaction such that sAA (as an index of SNS activity under threat) and an HPA MGPS moderate the relationship between stress exposure and cortisol reactivity. Findings partially supported study hypotheses. Consistent with hypotheses, the difference between cortisol levels across conditions was most pronounced in those with both low sAA and low HPA MGPS. Additionally, consistent with predictions, there

were *moderate* levels of cortisol reactivity in the negative evaluative condition for low sAA and high HPA MGPS—and as such, under low sAA, higher MGPS corresponded to comparatively blunted cortisol reactivity. However, in contrast to study hypotheses, individuals with high sAA and high MGPS had pronounced cortisol elevation in the negative evaluative condition, rather than the hypothesized blunted effect, and those with high sAA and low MGPS showed relative blunting as opposed to hypothesized moderate reactivity.

That an HPA MGPS differentially predicted cortisol reactivity as function of sAA reactivity is complex and intriguing. We posit that rather than an HPA MGPS capturing "risk" exclusively for negative outcomes, that instead, it reflects a *sensitivity* score, and appears to be sensitive to SNS influence in modulating cortisol. Conditions of low sAA reactivity demonstrated that a higher MGPS was associated with relative cortisol blunting. However, under higher sAA, HPA MGPS may indicate level of sensitivity to higher SNS activation. Greater sensitivity may lead to greater coordination and symmetry between sAA and cortisol responses. Thus, when sAA is high indicating robust SNS inputs, low MGPS (i.e., less sensitivity, in this view) was associated with low cortisol reactivity to stress (an uncoordinated response), while high MGPS (i.e., more sensitivity, in this view) was associated with high cortisol reactivity to stress (a more coordinated, symmetrical response). Although speculative, this sensitivity may be more important when sAA reactivity is high (where initial predictions were not supported), as opposed to when it is low (where initial predictions were supported).

Cortisol is conceptualized as a resource-mobilizing hormone to activate physiological and psychological processes that aid in adaptive responses to threat (Sapolsky et al., 2000). By this logic, a high sAA and high MGPS may suggest symmetric, coordinated effects between the HPA axis and SNS to respond to threat. By contrast, when SNS activity is high but HPA MGPS is

low, it would follow that the individual has less sensitivity to SNS activity via reduced genetic variation and thereby produces a less robust cortisol response toward the direction of blunting. Blunted, or reduced responses have been conceptualized as a failure to mobilize resources in response to threat, or a "giving up" response which has been linked to depression risk (Morris et al., 2014; Vrshek-Schallhorn et al., 2018), perhaps hinting that an asymmetrical (low sAA/high MGPS; high sAA/low MGPS) may be less adaptive by producing less resource-mobilizing cortisol.

It is prudent to acknowledge that this conceptualization of an HPA MGPS as potentially adaptive at high or low values does not align with the initially hypothesized diathesis stress framework. If the HPA MGPS is the theorized diathesis, we would have expected to see an increased HPA MGPS predict blunted cortisol. Here, however, we observe that HPA MGPS can be associated with both blunted and elevated cortisol responses, as a function of sAA reactivity. The conceptualization of an HPA MGPS as not solely risky adds to the pool of mixed results, in which some studies finding that a higher HPA MGPS moderates environmental risk and confers increased clinical/endophenotypic profiles consistent with depression (Di Iorio et al., 2017; Pagliaccio et al., 2014; 2015a; 2015b), while others have suggested the HPA MGPS acts as a plastic, or malleable score (Feurer et al., 2017; McKenna et al., 2021; Starr & Huang, 2019) consistent with sensitivity rather than risk per se. For example, McKenna, Hammen, and Brennan (2020) recently tested a 3-SNP HPA MGPS and maternal prenatal perceived stress (self-report measure) in offspring predicting depression in a longitudinal design. Individuals who had a *higher* HPA MGPS reported significantly *higher* depressive symptoms in the context of maternal prenatal stress at age 20, but *fewer* depressive symptoms in the context of *lower* maternal prenatal stress. While sympathetic activity was not concurrently measured in McKenna

et al., (2020), it conveys an important idea that an HPA MGPS may not function solely as a risk score, but as a malleability score. The current study adds to this literature an additional potential mechanism by which an HPA MGPS score influences stress reactivity, linking it to the SNS system, though replication is also an important next step.

Additionally, results indicated that SES and MGPS interacted to predict cortisol reactivity such that high SES participants (+ 1 SD from the mean) with high MGPS had low/blunted cortisol levels. By contrast, low SES participants (- 1 SD from the mean) with a high MGPS had a high cortisol response. This did not vary as a function of stress level. No prior studies have examined SES in concert with MGPS and cortisol reactivity, and this work suggests an HPA MGPS may be sensitive to SES, though requires further examination.

HPA MGPS and Condition Failing to Predict Cortisol.

In contrast to previously published studies of HPA MGPS, the current study found that the relationship between negative evaluative threat exposure and cortisol did not significantly depend on an HPA MGPS alone in an emerging adult sample. Possible explanations for this pattern of findings may include: 1) use of heterogenous stressors in prior work (naturalistic versus lab-based), 2) stressor severity (chronic versus acute), and 3) developmental considerations.

Methodological Considerations

The current study found that an *acute, explicit negative evaluative lab-based* stressor did not moderate the relationship between an HPA MGPS and cortisol reactivity, both in the full MGPS as well as the additive protective haplotype score. To my knowledge, this was the first experiment that utilized any variant of the TSST protocol as the "E" of a GxE interaction framework with an HPA MGPS predicting cortisol reactivity in an emerging adult sample. One

prior study examined children's cortisol reactivity to a series of fun and frustrating tasks intended to evoke positive and negative affect (Pagliaccio et al., 2014), but no studies have examined more traditional lab-based psychosocial stress inductions such as the present explicit negative evaluative induction. Across all remaining previous studies of an HPA MGPS, participants (or their parents depending on age) were administered semi-structured interviews ascertaining information about adversity in the child/adolescent's life, almost exclusively naturalistic stressors. The current study extends this work by being the only to test the TSST and only one of two studies to use the HPA MGPS to examine cortisol reactivity.

This raises the question whether stressor duration (acute versus chronic) or stressor type (interpersonal versus non-interpersonal), might influence whether a significant GxE effect emerges with an HPA MGPS. Prior studies have found evidence that the HPA MGPS interacts with both chronic (Starr & Huang, 2019), and episodic (acute) stressors (Feurer et al., 2017; Huang & Starr, 2019; Starr & Huang, 2019). Interestingly, Starr & Huang (2019) demonstrated that an HPA MGPS moderated the relationship between acute and chronic, interpersonal stress on depressive symptoms, such that those with higher MGPS and increased stress exposure had increased depressive symptoms. By contrast, an HPA MGPS did not interact with non-interpersonal stress to predict depressive symptoms in the full sample. This is consistent with abundant research that emphasizes that interpersonal stress, such as stress that affects an individuals' close relationships, or availability of interpersonal relationships, is a key determinant by which stressors can lead to depression (Hammen, 2005).

Despite the ability of the present study's negative evaluative variant of the TSST to robustly induce psychosocial threat, one possibility is that the TSST may not sufficiently mimic *naturalistic* interpersonal stress, which appears to have been a critical aspect of several prior

such GxE studies. The current study adds to a growing conversation around the critical importance of delineating and testing "candidate environments" for GxE research (Dick et al., 2015; Starr et al., 2019). Specifically, previous groups have underscored that the specification of a candidate environment is equally as important as specifying gene candidates (Vrshek-Schallhorn et al., 2014), and further, that it is possible that different types of stress are relevant for different types of genetic variables (Dick et al., 2015). The current study extends this concept by documenting a lack of significant support that the HPA MGPS alone modulates cortisol and sAA responses to negative evaluative lab-based stress.

Developmental Considerations

Further, the current study is one of the few that examined an HPA MGPS in an emerging adult sample. The remaining studies were completed in samples of children (Feurer et al., 2017 and Pagliaccio et al., 2014, 2015a, 2015b) and adolescents (Huang & Starr, 2020; Starr, Dienes et al., 2019; Starr & Huang, 2019). Importantly, the impact of stress exposures on stress reactivity varies as a function of developmental stage (Crosswell & Lockwood, 2020). Specifically, childhood (i.e., prior to age five), puberty, and adolescence are considered sensitive periods for stress exposure, where prior evidence indicates that stress exposures during these periods can sensitize the HPA axis leading to lasting changes in reactivity (Christine Heim et al., 2008; Lupien et al., 2009). This is in part due to brain areas key to HPA axis regulation (e.g., prefrontal cortex, an area rich in primary cortisol receptors) not being fully developed (Crosswell & Lockwood, 2020; Lupien et al., 2009), leading to sensitization of the HPA axis (C. Heim et al., 2000). Additional evidence consistent with this notion comes from the only other study to our knowledge that examined the effect of an HPA MGPS on cortisol reactivity (Pagliaccio et al., 2014). Specifically, children with and without histories of early-life stress were exposed to a

lab-based task and provided salivary cortisol at baseline, before, and after the task. The authors observed a positive main effect of an HPA MGPS predicting augmented cortisol reactivity in children with a history of ELS. Considering developmental theories of stress sensitivity coupled with Pagliaccio et al.'s (2014) findings, this suggests that emerging adulthood may be considerably less sensitive to stress exposure relative to childhood and adolescence due to more developed stress-related brain structures, which could perhaps minimize the influence of the HPA MGPS.

Taken together, methodological considerations, namely the candidate environment used, as well as developmental considerations may have contributed to a lack of evidence that the HPA MGPS moderates the effect of negative evaluative stress versus a control on cortisol reactivity.

HPA MGPS and Condition Failing to Predict sAA Reactivity

Despite theoretical and empirical evidence of physiological interconnectedness between SNS and HPA axis activity, the current study did not find evidence that an HPA MGPS and condition interacted to predict sAA reactivity. First, we mention other work that as examined HPA genetic variation in SNS outcomes. Next, we consider how sAA may be upstream of the HPA axis, and therefore be too distal of an outcome from the HPA MGPS as a potential explanation for null findings.

Methodological Considerations

Only one study to the best of our knowledge has examined HPA axis genetic variation predicting SNS-related outcomes. DeRijk et al., (2006) found that rs5522 (which affects mineralocorticoid receptors [MR]) interacted with TSST condition to predict ANS activity (measured through heart rate), such that individuals with two copies of this SNP had elevated heart rate. This study proposed that MR's role in mediating fast membrane events in the brain

may elevate ANS activity. Beyond this, no prior work provides evidence of single or additive genetic HPA axis scores predicting SNS activity in the context of stress. The current study informs future genetic work as it concerns examining the effect of HPA axis genetic variation predicting SNS outcomes.

We predicted sAA as an outcome of the interaction of HPA MGPS and TSST condition. This was grounded in research demonstrating bidirectional influence of the HPA axis on SNS activity in response to the TSST (Andrews, D'Aguiar, & Pruessner, 2012, Andrews & Pruessner, 2013) as well as salivary bioscience methodology (Granger & Taylor, 2020). The theorized mechanism was that an HPA MGPS would affect the brain regions involved in the stress response (hypothalamus, paraventricular nucleus, hippocampus), affect cortisol, influence subsequent SNS activity, which would be reflected in sAA (not only HPA reactivity). Prior evidence contends that HPA axis mediation of sympathetic activity may unfold slowly (Sapolsky et al., 2000). It is possible that sAA as an outcome in this model may have been too distal from the mechanism of the HPA MGPS, and that the current study was able to capture sAA's modulation of HPA MGPS activity, rather than vice versa.

Limitations

While the study benefited from strengths such as using a robust lab-based stressor, a novel genetic approach, and multisystem biomarkers to objectively capture stress reactivity, there were also limitations. First, the *FKBP5* variant, rs1360780, deviated from HWE both in the full sample, and within each racial subgroup (white vs. minority status), and thus was not usable, which may have reduced the effect size and predictive ability of the MGPS. Moreover, the current study used previously established MGPS profiles for the purpose of facilitating future meta-analysis. However, it does not include all SNPs that have been found to influence HPA axis

reactivity (e.g., Arginine Vasopressin, Angiotensin Converting Enzyme genes; Normann & Buttenschøn, 2019); additionally, previously studied HPA MGPS profiles published after the present study's genotyping was conducted, utilized different SNPs of the same genes (e.g., rs6198 of *NR3C1*; McKenna, Hammen, & Brennan, 2020), thus no extant HPA MGPS is exhaustive. Finally, as the use of sAA as a marker of SNS activity is relatively new, it would have benefitted the study to have concurrent measures of gold standard SNS activity (plasma catecholamines) to concurrently examine SNS activity in response to stress exposure.

Future Research & Considerations

As studies continue examining additive genetic profiles in GxE interaction frameworks, careful attention must be applied to both the "G" and the "E". While the HPA MGPS profiles used in the current study all had roughly the same SNPs, variation in numbers of SNPs were used as well as variable inclusion of haplotypes. Research groups may have different criteria by which they qualify a SNP for inclusion in an MGPS (e.g., the SNP was found to predict intermediate and/or clinical outcomes), or may include several different SNPs of the same gene despite attempting to predict similar outcomes. Zhang and Belsky (2020) discuss the critical point of standardizing how SNPs are included in additive genetic scores, and being clear the extent to which they should be based on hypotheses grounded in biologically-plausible systems, versus from a genome-wide-association study which are atheoretical and hypothesis-free. By the same token, rigorous, judicious measuring of the environment through adoption of gold-standard, interview-based tools, parsing out acute versus chronic, interpersonal versus non-interpersonal, for example, is of critical importance for establishing GxE interaction effects.

Conclusion

Scientists have long sought to identify risk factors that clarify who is most at risk for psychopathology following a stressful life event, to attenuate the burden of stress-related disorders, like depression. The current study was the first to demonstrate that the effect of an additive genetic profile related to the HPA interacted with SNS reactivity to predict an intermediate outcome related to depression, cortisol reactivity in response to negative evaluative threat, but did not provide support that this genetic score *alone* predicted cortisol or SNS reactivity to negative evaluative threat. This advances the effort to characterize the individual differences and subsequent mechanisms that confer risk for stress-related psychopathology.

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APPENDIX A: TABLES AND FIGURES

Table 1. Single Nucleotide Polymorphism Coding and Prevalence for Current Sample

Gene	Polymorphism	Coding	N	Polymorphism Score		
				0	**1**	**2**
CRHR1	rs4792887	TT = 2, CT = 1, CC = 0	142	81	53	8
CRHR1 TAT haplotype*	rs110402 rs7209436 rs242924	Zero copies = 2 One copy = 1 Two copies = 0	124	59	63	2
CRHR1 GAG haplotype*	rs242941 rs242939 rs1876828	Zero copies = 2 One copy = 1 Two copies = 0	127	28	68	31
NR3C2 CA haplotype*	rs5522 rs2070951	Zero copies = 2 One copy = 1 Two copies = 0	139	116	22	1
NR3C2 CT haplotype	rs4635799 rs5522	Zero copies = 0 One copy = 1 Two copies = 2	138	132	6	0
NR3C1	rs41423247	GG = 2, CG = 1, CC = 0	142	12	61	69
	rs10482605	TT = 2, CT = 1, CC = 0	139	3	31	105
	rs10052957	AA = 2, AG = 1, GG = 0	133	60	64	9
***FKBP5*	rs1360780	TT = 2, CT = 1, CC = 0	135	122	5	8

*****Denotes haplotypes that are evidenced to be protective, and therefore reverse coded where 0 copies indicate additive risk.

**Gene was not in HWE, polymorphism excluded from all analyses.

Table 2. Composition of HPA MGPS Across Previous Studies

HPA MGPS	Total SNPs & Haplotypes	CRHR1	NR3C1	NR3C2	FKBP5									
		rs4792887	rs1876828[1]	rs242941[1]	rs242939[1]	rs110402[2]	rs720943[2]	rs242924[2]	rs4142324	rs1048605	rs1005057	rs5522[3,4]	rs2070951[3]	rs4713599[4]	rs1360780
Di Iorio et al. 2017	4 1 haplo.					**X**						**X**		**X**	**X**
Feurer et al. 2017	6 2 haplo.					**X**	**X**	**X**				**X**	**X**		**X**
Pagliaccio et al., 2014; 2015a; 2015b	10 1 haplo.	**X**	**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**			**X**
Starr & Huang, 2019	10 1 haplo.	**X**	**X**	**X**	**X**	**X**			**X**	**X**	**X**	**X**			**X**
Current Study	14 4 haplo.	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

[1]Part of "GAG" haplotype CRHR1 gene (rs242941, rs242939, rs1876828) used by Pagliaccio et al., 2014; 2015a; 2015b. Of note, these SNPs were tested both independently and as part of the haplotype.

[2]Part of "TAT" haplotype CRHR1 gene (rs7209436, rs110402, and rs242924) used by Feurer et al., 2017

[3]Part of "CA" haplotype NR3C2 gene (rs5522, rs2070951) used by Feurer et al., 2017

[4]Part of “TC” or “CT” haplotype NR3C2 gene (rs5522, rs4635799) used by Di Iorio et al., 2017

Table 3. Sample Demographics Across Condition

Demographics (*N = 144*)	N (%)
Gender (Female)	82 (56.9)
Race/Ethnicity	
Black or African American	64 (44.4)
White	57 (39.6)
Latino/a	7 (4.9)
Asian/Pacific Islander	4 (2.8)
Biracial	8 (5.6)
Other	4 (2.8)
TSST Condition*	72 (50)
	M (SD)
Age	19.44 (1.96)
SES index	44.56 (12.78)
HPA MGPS	6.02 (1.54)
sAA AUCi (U/ml)	1009.88 (2033.47)
Cortisol AUCi (nmol/l)	41.13 (192.75)

***** There were (*n* = 72) participants in the control condition, and (*n* = 72) in the challenge.

Table 4. Sample Characteristics Across TSST Condition

	Control	Negative Evaluative
	Mean (SD)	
Age	19.47 (1.78)	19.40 (2.13)
SES Index*	46.93 (11.74)	42.20 (13.41)
HPA MGPS (range 0-16)	6.16 (1.46)	5.88 (1.62)
Baseline Cortisol	5.40 (2.91)	5.01 (2.74)
Baseline sAA	113.03 (60.85)	120.69 (84.97)
sAA AUCi*	613.64 (1831.10)	1406.13 (2157.97)
Cortisol AUCi***	-49.05 (121.48)	131.31 (208.75)

*$p < .05$
***$p < .001$

Table 5. Manipulation Checks for Primary Variables Across Conditions

Level of Evaluation	Control Mean (SD)	Negative Evaluative Mean (SD)	One-way ANOVA
Perceived Evaluation	3.10 (1.07)	3.00(1.07)	F (1,143) = 15.921***
Positive Evaluation	3.39 (.61)	3.05 (.74)	F (1,143) = 40.842***
Negative Evaluation	1.27 (.45)	1.22 (.42)	F (1,143) = 52.127***
sAA AUCi	613.64 (1831.10)	1406.13 (2157.97)	F (1,143) = 5.6460*
Cortisol AUCi	-49.05 (121.48)	131.31 (208.75)	F (1,143) = 40.149***

* $p < .05$
*** $p < .001$

Table 6. Zero-Order Correlations Among Key Study Predictors, Covariates, and Outcomes

	1	***2***	***3***	***4***	***5***	***6***	***7***
1. Ethnicity[a]	-						
2. Gender	*.18**	-					
3. SES	*-.20**	*.08*	-				
4. TSST	*-.02*	*.00*	*-.19**	-			
5. HPA MGPS	*-.19**	*-.03*	*.01*	*-.09*	-		
6. sAA[b]	*-.08*	*-.01*	*-.11*	*.20**	*-.05*	-	
7. Cortisol[b]	*.02*	*.01*	*-.11*	*.47***	*-.01*	*.06*	-

[a]Ethnicity is dummy-coded (0 = White, 1 = Minority status)
[b]Measured in AUCi
*Correlation is significant at p < .05
**Correlation is significant at p < .01

Table 3. Primary Model 1: MGPS x Condition Predicting Cortisol Reactivity

	b	SE(b)	t-value	p-value
Intercept	-49.744	33.759	-2.323	.022
TSST Condition	**181.378**	**29.696**	**6.304**	**<.001**
MGPS (centered)	1.405	14.304	.100	.920
MGPS x Condition	4.316	18.988	.229	.819

Note. Bolded values indicate $p < .05$.

Table 4. Primary Model 2: MGPS x Condition Predicting sAA Reactivity

	b	SE(b)	t-value	p-value
Intercept	585.348	250.564	2.336	.021
TSST Condition	**784.901**	**336.589**	**2.332**	**.021**
MGPS (centered)	56.879	163.796	.347	.729
MGPS x Condition	-166.828	220.288	-.757	.450

Note. Bolded values indicate p < .05

Table 5. Primary Model 3: sAA x MGPS x Condition Predicting Cortisol Reactivity

	b	SE(b)	t-value	p-value
Intercept	-39.385	22.263	-1.769	.079
TSST Condition	**179.272**	**29.120**	**6.156**	**<.001**
sAA (AUCi, centered)	.018	.012	1.344	.181
MGPS (centered)	-7.105	15.049	-.472	.638
MGPS x Condition	3.236	19.955	.162	.871
Condition x sAA	-.018	.015	-1.189	.236
MGPS x sAA	-.013	.010	-1.358	.177
MGPS x Condition x sAA	**.027**	**.012**	**2.294**	**.023**

Note. Bolded values indicate $p < .05$.

Table 6. Primary Model 3: sAA x MGPS x Condition Predicting Cortisol Reactivity (with SES covariate)

	b	SE(b)	t-value	p-value
Intercept	-42.082	22.510	-1.869	.064
SES (centered)	.706	1.738	.406	.685
SES x sAA	.000	.001	.340	.734
SES x MGPS	**-1.891**	**.776**	**-2.438**	**.016**
SES x Condition	-1.283	2.309	-.556	.579
TSST Condition	**177.189**	**29.358**	**6.035**	**<.001**
sAA (AUCi, centered)	.018	.014	1.324	.188
sAA x MGPS	-.015	.010	-1.540	.126
sAA x Condition	-.020	.016	-1.262	.209
MGPS (centered)	.923	15.303	.060	.952
MGPS **x** Condition	-10.162	20.641	-.492	.623
MGPS x Condition x sAA	**.029**	**.012**	**2.462**	**.015**

Note. Bolded values indicate $p < .05$.

Table 7. Individual SNP x TSST Condition x sAA Interaction Terms, from Separate Models

Gene	SNP x TSST x sAA term	b	SE(b)	t-value	p-value
NR3C1	rs10052957 x TSST x sAA	.054	.030	1.798	.075
NR3C1	rs10482605 x TSST x sAA	-.015	.034	-.432	.666
NR3C1	rs41423247 x TSST x sAA	-.021	.030	-.687	.493
CRHR1	rs4792887 x TSST x sAA	**.051**	**.024**	**2.182**	**.031**
NR3C2	CA haplotype x TSST x sAA	-.034	.035	-.979	.330
CRHR1	GAG haplotype x TSST x sAA	.029	.025	1.166	.246
CRHR1	TAT haplotype x TSST x sAA	.015	.027	.560	.577

Note. Bolded values indicate $p < .05$.

Table 8. 10-SNP MGPS x Condition Predicting Cortisol Reactivity (Pagliaccio et al., 2014, 2015a, 2015b; Starr & Huang, 2019)

	b	SE(b)	t-value	p-value
Intercept	-48.003	20.431	-2.349	.020
MGPS (centered)	-3.263	8.647	-.377	.707
TSST Condition	**178.056**	**28.885**	**6.164**	**< .001**
MGPS x Condition	-.671	12.007	-.056	.956

Note. Bolded values indicate $p < .05$.

Table 9. 10-SNP MGPS x Condition Predicting sAA Reactivity (Pagliaccio et al., 2014, 2015a, 2015b; Starr & Huang, 2019)

	b	SE(b)	t-value	p-value
Intercept	596.411	239.242	2.493	.014
MGPS (centered)	53.907	101.250	.532	.595
TSST Condition	**826.332**	**338.229**	**2.443**	**.016**
MGPS x Condition	-1.932	140.592	-.014	.989

Note. Bolded values indicate $p < .05$.

Table 10. 3-SNP MGPS x Condition Predicting Cortisol Reactivity (Feurer et al., 2017)

	b	SE(b)	t-value	p-value
Intercept	-49.682	20.498	-2.424	.017
MGPS (centered)	1.577	8.990	.175	.861
TSST Condition	**177.938**	**29.066**	**6.122**	**< .001**
MGPS x Condition	-1.498	12.850	-.117	.907

Note. Bolded values indicate $p < .05$.

Table 11. 3-SNP MGPS x Condition Predicting sAA Reactivity (Feurer et al., 2017)

	b	SE(b)	t-value	p-value
Intercept	554.131	241.143	2.298	.023
MGPS (centered)	147.369	105.760	1.393	.166
TSST Condition	**873.777**	**341.951**	**2.555**	**.012**
MGPS x Condition	-137.229	151.173	-.908	.366

Note. Bolded values indicate $p < .05$.

Table 12. 2-SNP MGPS x Condition Predicting Cortisol Reactivity (Di Iorio et al., 2017)

	b	SE(b)	t-value	p-value
Intercept	-48.995	20.683	-2.369	.019
MGPS (centered)	9.888	33.097	.299	.766
TSST Condition	**175.256**	**28.961**	**6.051**	**< .001**
MGPS x Condition	-4.147	42.901	-.097	.923

Note. Bolded values indicate $p < .05$.

Table 13. 2-SNP MGPS x Condition Predicting sAA Reactivity (Di Iorio et al., 2017)

	b	SE(b)	t-value	p-value
Intercept	520.456	249.827	2.083	.039
MGPS (centered)	718.511	399.776	1.797	.075
TSST Condition	**911.742**	**349.821**	**2.606**	**.010**
MGPS x Condition	-674.948	518.196	-1.302	.195

Note. Bolded values indicate $p < .05$.

Table 14. Protective Haplotype x TSST Condition Predicting Cortisol Reactivity

	b	SE(b)	t-value	p-value
Intercept	-49.852	20.337	-2.451	.015
TSST Condition	**181.305**	**28.875**	**6.279**	**< .001**
MGPS (centered)	27.889	52.826	.528	.598
MGPS x Condition	-63.208	79.903	-.791	.430

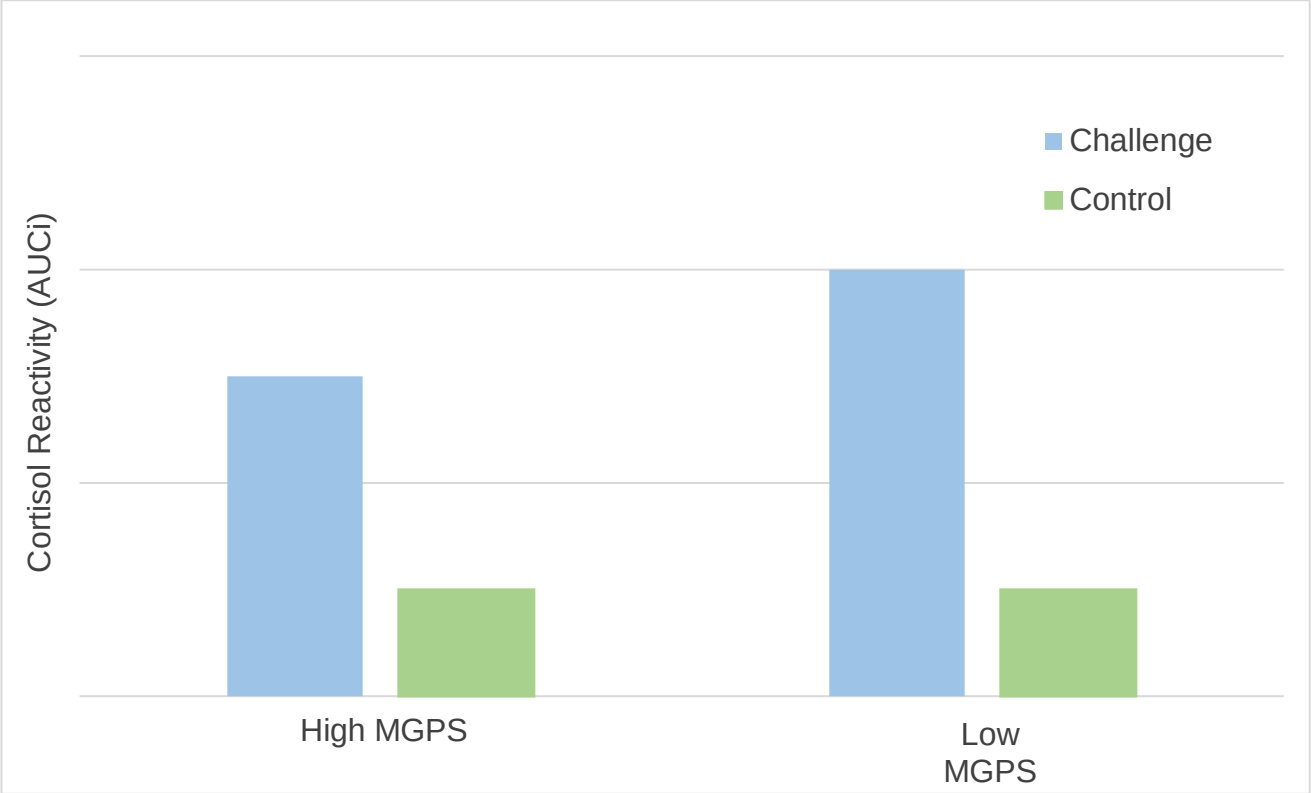
Note. Bolded values indicate $p < .05$.

Table 15. Protective Haplotype x TSST Condition Predicting sAA Reactivity

	b	SE(b)	t-value	p-value
Intercept	594.654	237.526	2.504	.013
TSST Condition	**799.953**	**337.240**	**2.372**	**.019**
Haplotype (centered)	655.876	616.974	1.063	.290
Haplotype x Condition	-1213.351	933.211	-1.300	.196

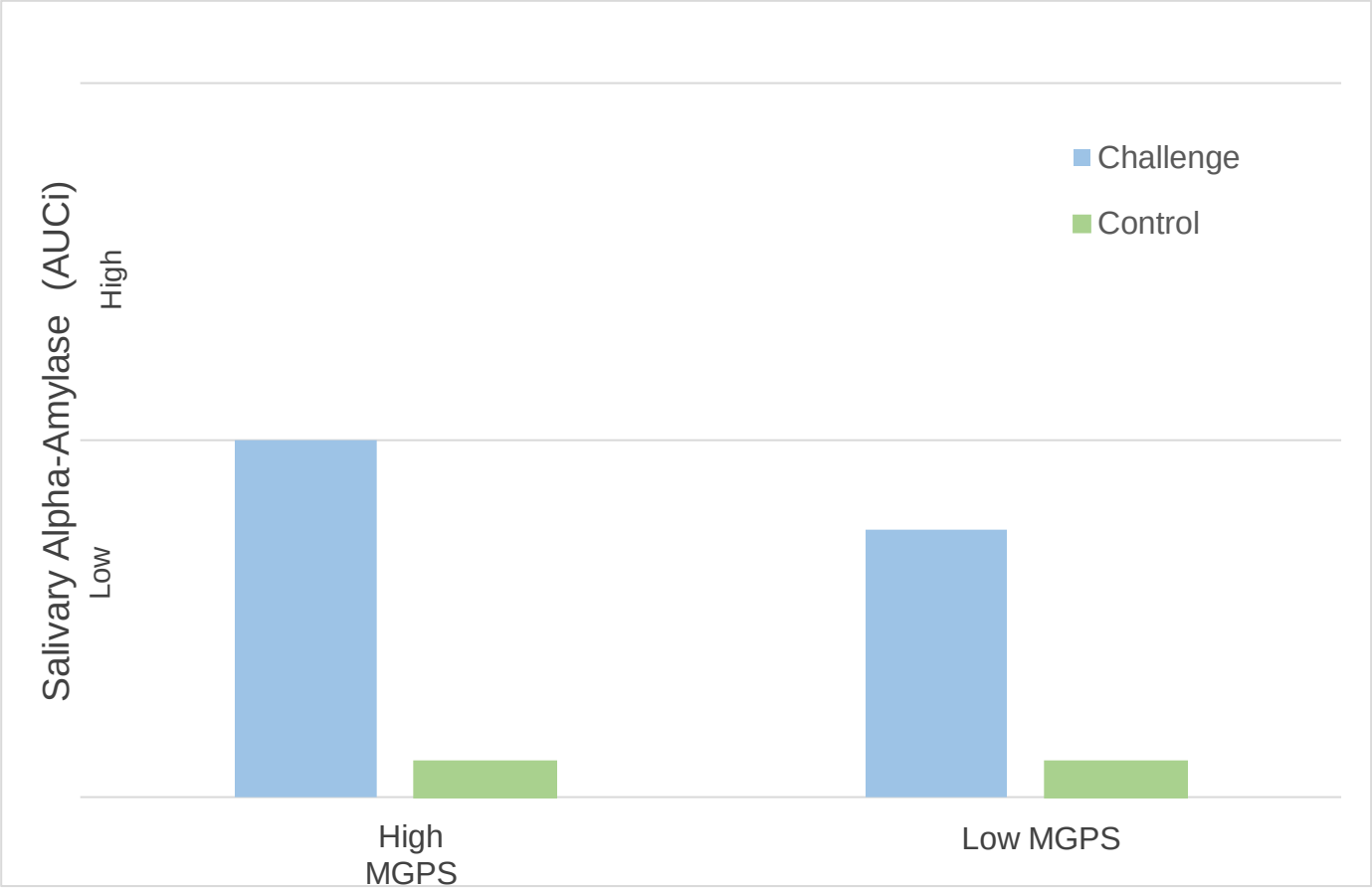
Note. Bolded values indicate $p < .05$.

Figure 1. Predicted Effect of HPA MGPS on Cortisol Reactivity by Condition



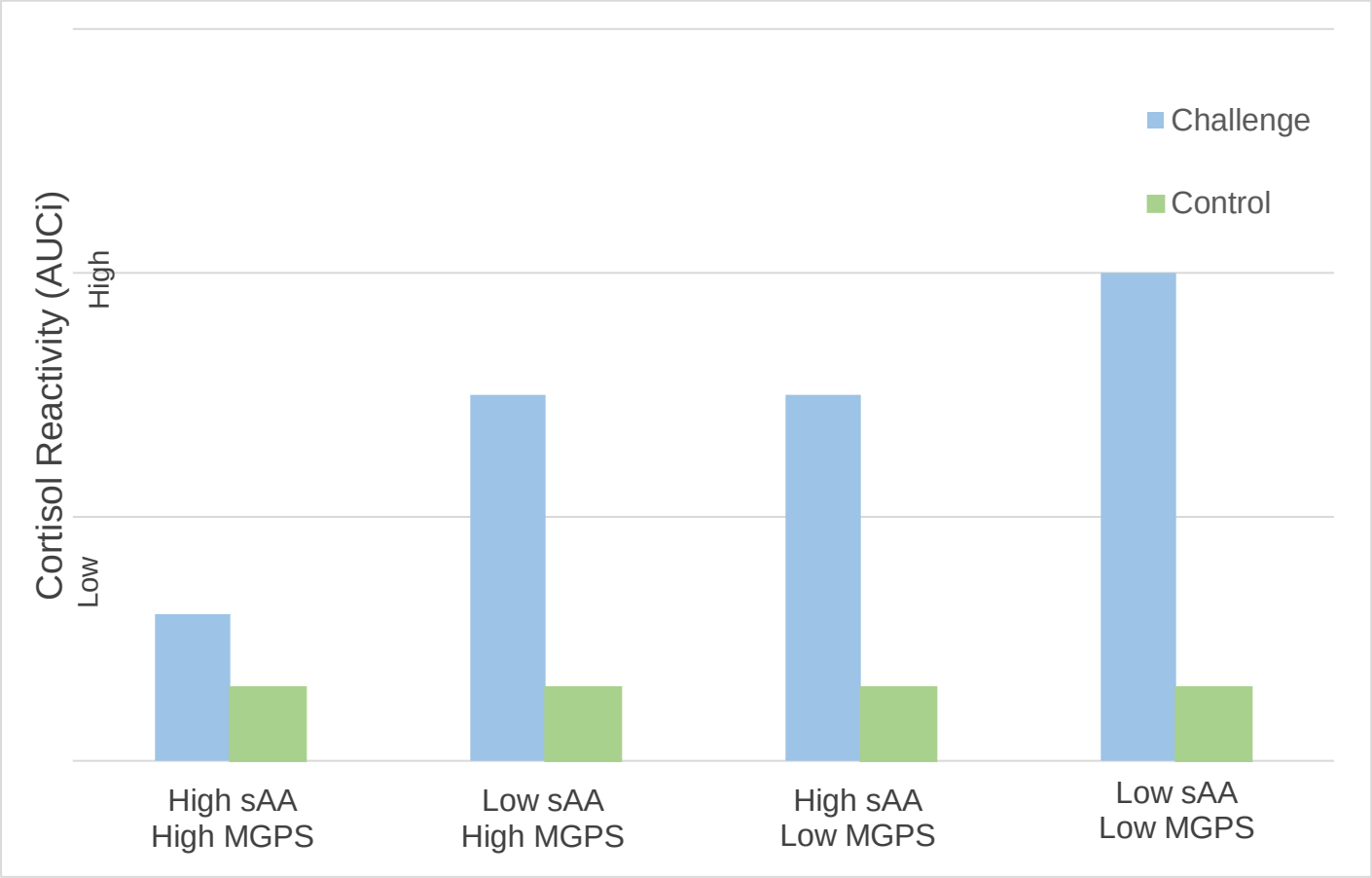
In the negative evaluative (challenge) TSST condition, cortisol will be blunted for individuals with a high HPA MGPS score, whereas normative (high) cortisol responses will be observed for individuals in the negative evaluative condition with a low HPA MGPS score. Participants in the control condition will show a decline in cortisol over the repeated samplings consistent with cortisol's diurnal rhythm which declines throughout the day, producing a negative value for AUCi.

Figure 2. Predicted Effect of HPA MGPS on sAA Reactivity by Condition



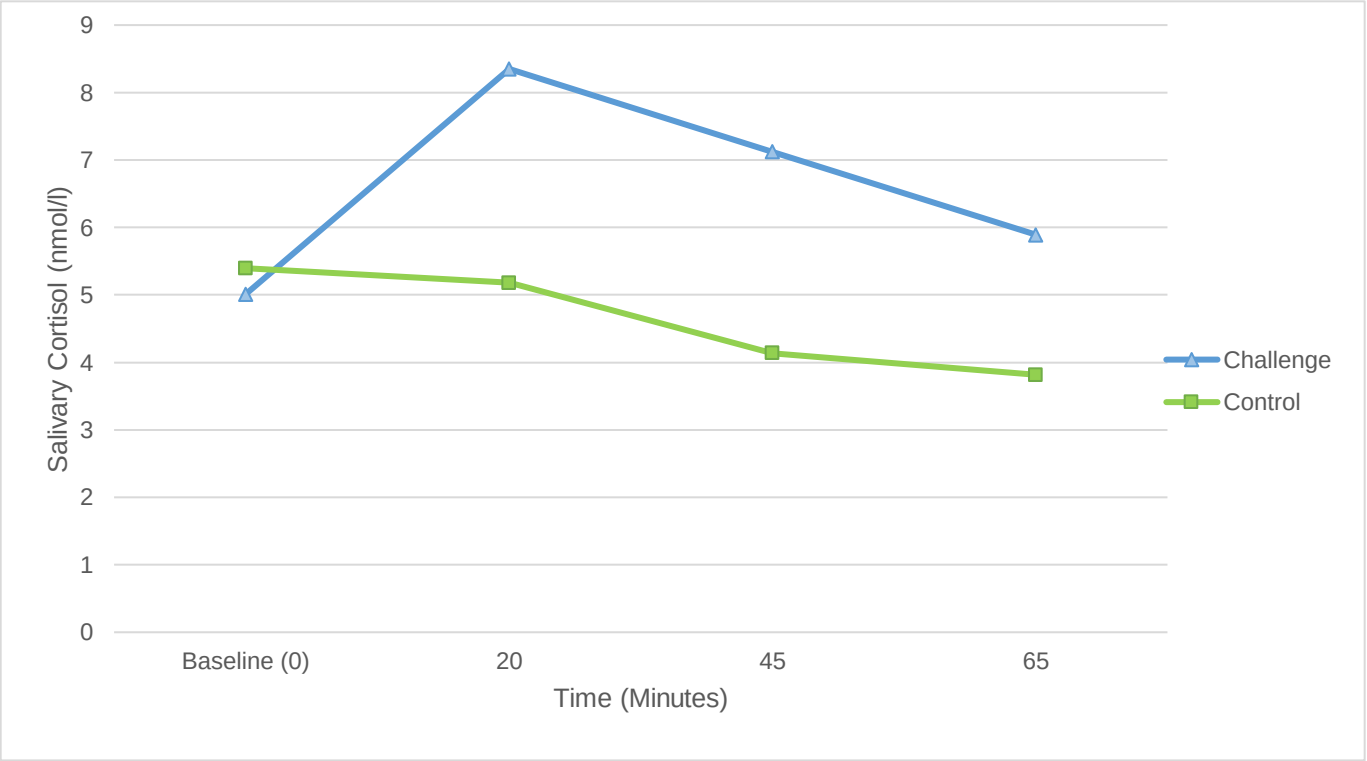
In the negative evaluative TSST condition, sAA will be relatively elevated for individuals with a high HPA MGPS, whereas individuals with low HPA MGPS will experience relatively lower sAA responses in the negative evaluative condition.

Figure 3. Predicted Effect of HPA MGPS on Cortisol Reactivity by sAA and Condition



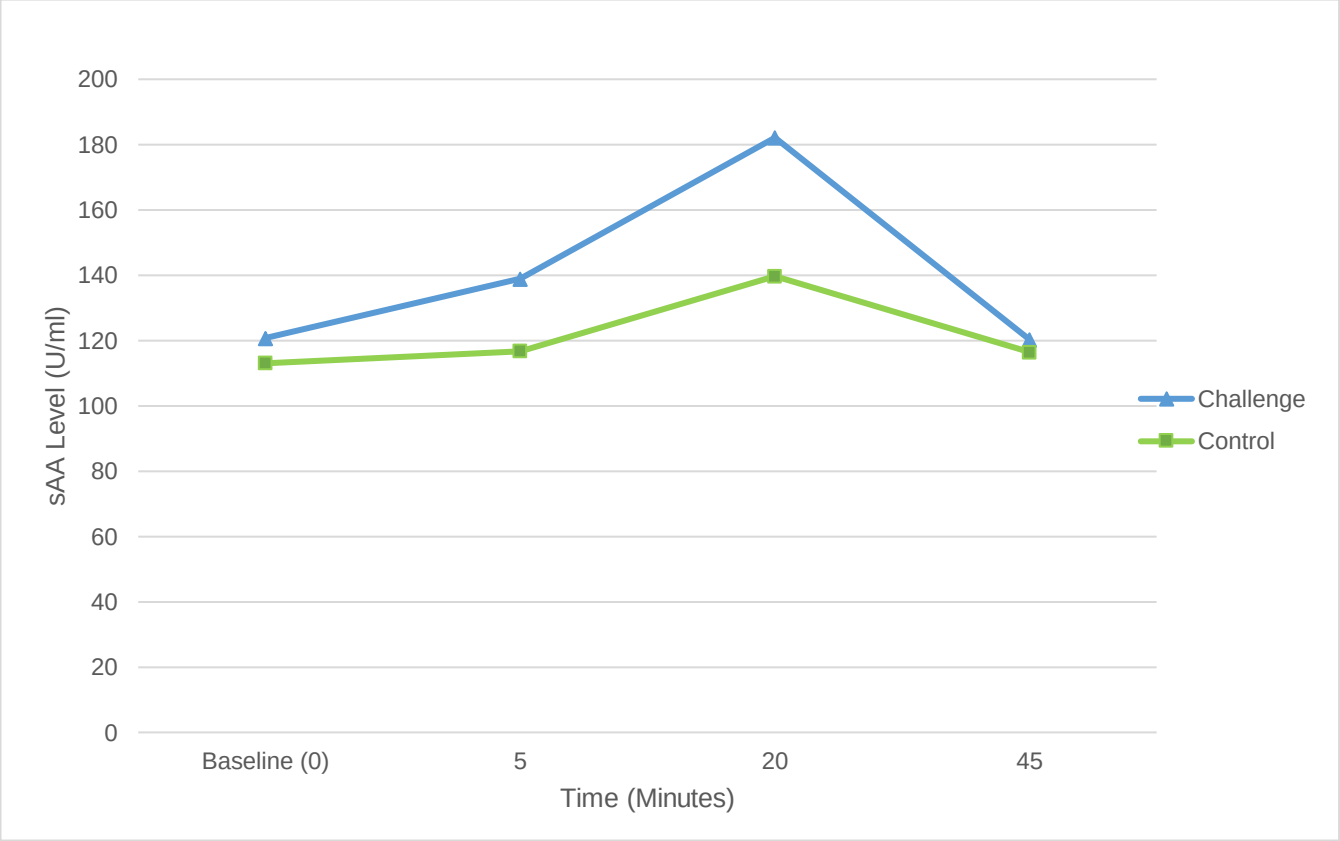
At high levels of sAA reactivity and high HPA MGPS, in the negative evaluative condition, cortisol reactivity will be maximally blunted relative to others in the same condition.

Figure 4. Cortisol Levels Across TSST Conditions



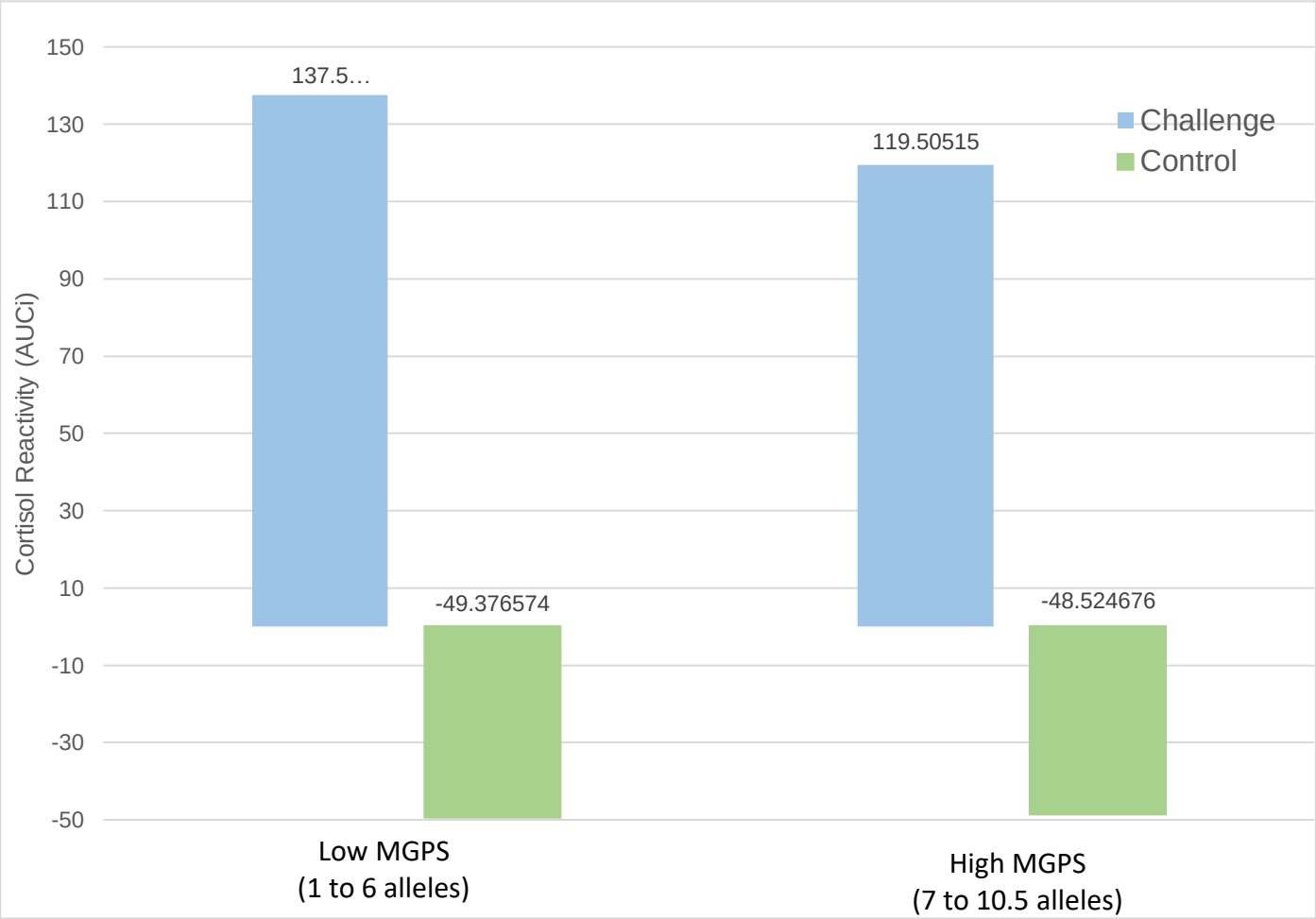
Cortisol Levels across Condition. Samples included in cortisol calculation were collected 1) Before TSST (Baseline Sample, 0 min), 2) After the 5 min TSST preparation period (20 min from baseline), 3) After the two TSST tasks (45 min from baseline) and after additional study activities (65 min from baseline). Cortisol was measured in nmol/l.

Figure 5. Alpha-Amylase Level Across TSST Conditions Before, During, and after TSST



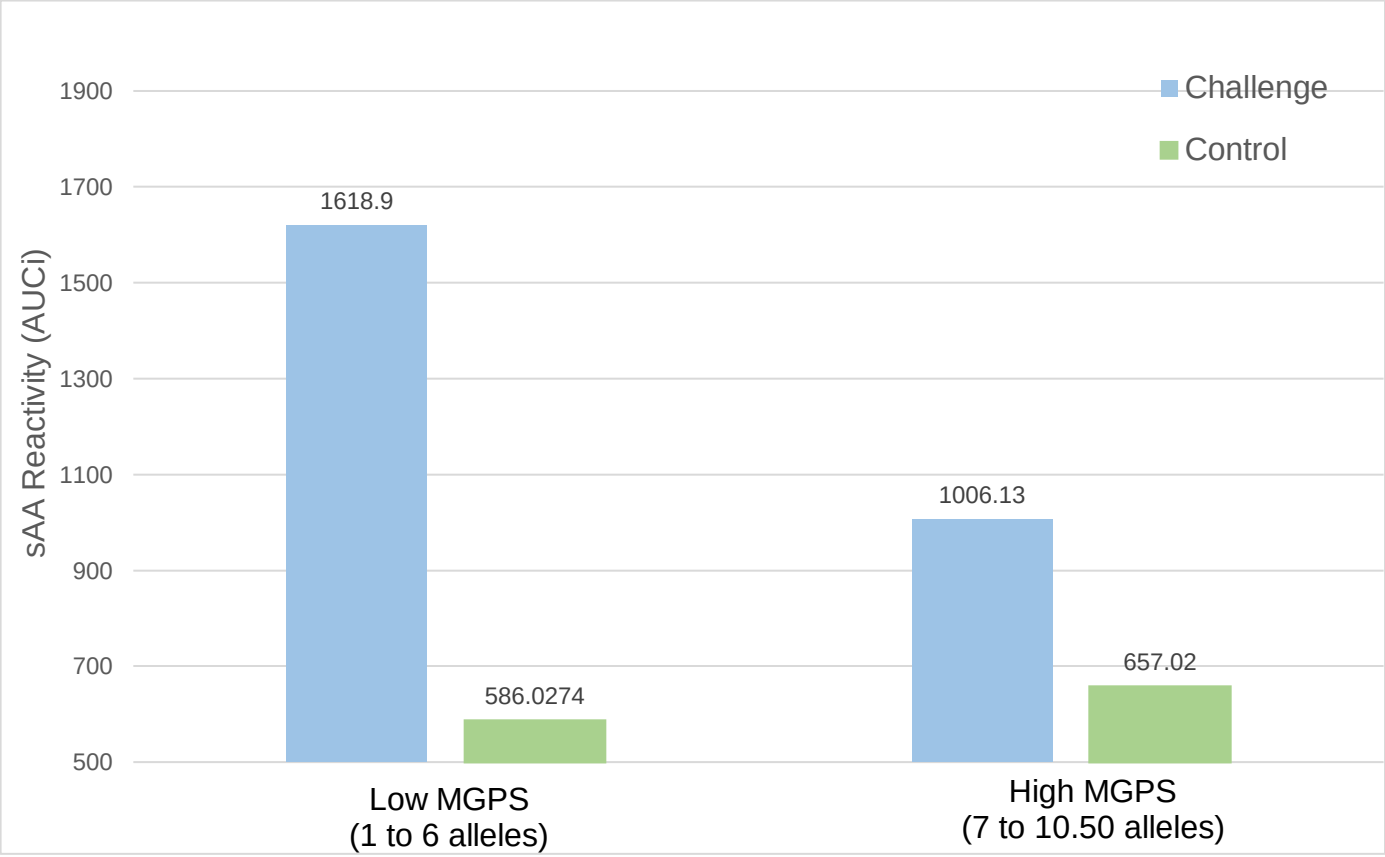
The sAA samples were collected 1) Before TSST (Baseline Sample, 0), 2) +5 minutes from baseline (before the TSST), +20 minutes from baseline (following the TSST), and +45 minutes from baseline (after the TSST and other study tasks) when average levels have returned to baseline. Salivary Alpha-Amylase was measured in U/ml.

Figure 6. Observed Effect of HPA MGPS on Cortisol Reactivity (AUCi) by Condition



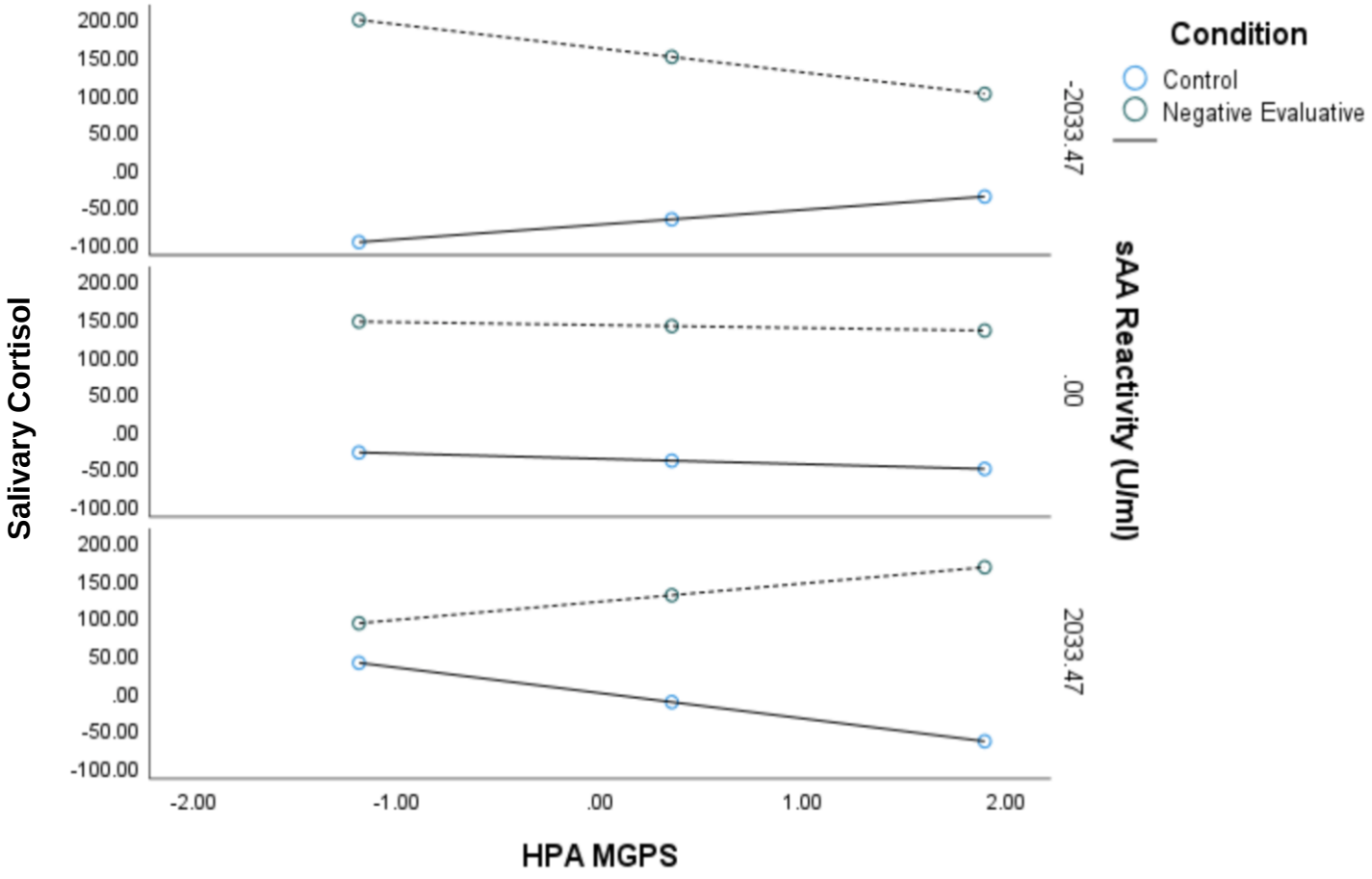
Observed effect of HPA MGPS on cortisol reactivity by Condition in Individuals with Low (*n* = 91) and High MGPS (*n* = 53). Low and High HPA MGPS groups were formed by median split. Values are mean AUCi, units of cortisol are nmol/l.

Figure 7. Observed Effect of HPA MGPS on sAA Reactivity (AUCi) by Condition



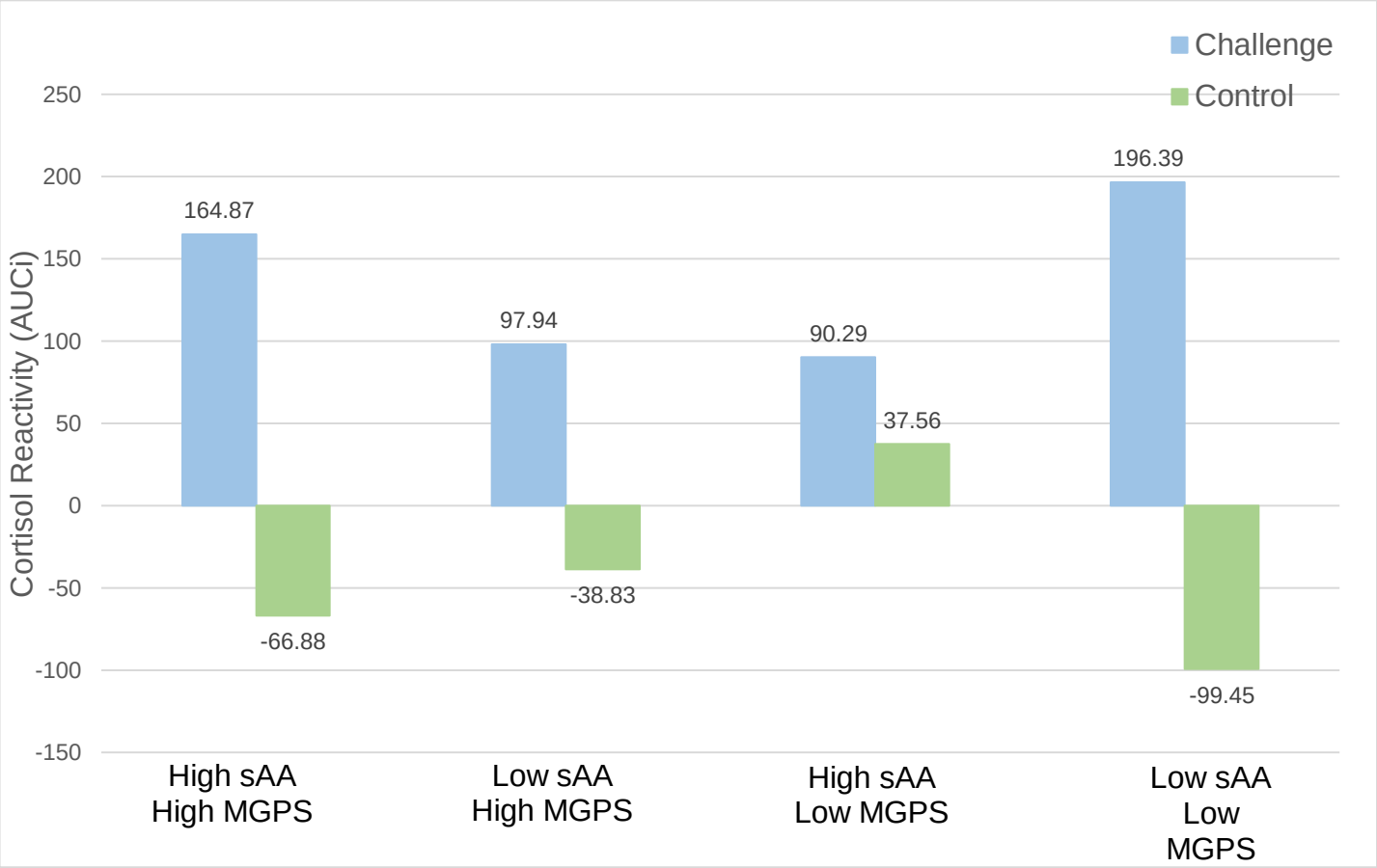
Observed effect of HPA MGPS on sAA reactivity by Condition in Individuals with Low (*n* = 91) and High MGPS (*n* = 53). Low and High HPA MGPS groups were formed by median split. Values are mean AUCi, units of sAA are U/ml.

Figure 6. Three-way interaction of sAA, MGPS, and TSST Condition Predicting Cortisol Reactivity



sAA reactivity is mean centered. Values of sAA are: -1 standard deviation below the mean (-2033.47 U/ml), at the mean (.00 U/ml), and +1 standard deviation above the mean (2033.47 U/ml). At high levels of sAA reactivity and high HPA MGPS, cortisol reactivity was robustly *elevated* in the negative evaluative condition relative to controls. At low sAA and low HPA MGPS, cortisol reactivity was robustly elevated in the negative evaluative condition relative to controls. At asymmetrical levels (low sAA/high MGPS; high sAA/low MGPS), cortisol reactivity appeared blunted in the negative evaluative condition. Johnson-Neyman regions of significance were identified at values ≤ -2706.63 U/ml and ≥ 3059.21 U/ml of sAA reactivity.

Figure 7. Observed Effect of HPA MGPS on Cortisol Reactivity (AUCi) by sAA and Condition



All high levels are +1 SD above the mean, and low levels are – 1 SD below the mean. At high levels of sAA reactivity and HPA MGPS, cortisol reactivity was elevated in the negative evaluative condition relative to controls. At low sAA and HPA MGPS, cortisol reactivity was elevated in the negative evaluative condition relative to controls. At asymmetrical levels (low sAA/high MGPS; high sAA/low MGPS), cortisol was blunted in the negative evaluative condition.

APPENDIX B: CHALLENGE CONDITION BEHAVIORAL SCRIPT

Speech portion:

Both confederates begin with a mildly pleasant facial expression and neutral to interested body language, e.g., sit up and slightly lean forward in your chair

Administer all directions with a firm, stern tone of voice.

Possible timing in speech	**Confederate 1 (dissatisfied)**	**Confederate 2 (bored)**
0:00	Scribble notes on your paper	Slump shoulders & posture
0:30	Furrow brow with slightly confused look	Quiet sigh of fatigue
1:00	Continue scribbling	Stare into space
1:30	Look more confused	Play with hair
2:00	Shuffle papers	Slight eye roll
2:30	Look at other confederate and shrug shoulders as if to ask, "what do you think?"	Look at other confederate and slightly shake head "no"
3:00	Subtle grimace; rub the bridge of your nose	Cross arms, squirm
3:30	Make a conspicuous X mark on your papers	Look at your watch briefly
4:00	Glance at your phone then put it away	Widen eyes and breathe in and out deeply
4:30	Exchange dissatisfied glance with another confederate	Exchange dissatisfied glance with other confederate
5:00	Tap fingers on table	Fidget with fingernails

Arithmetic portion: Conspicuously make tally marks on your paperwork for errors/restarts. Maintain dissatisfied or bored body language and stern tone of voice.