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Measuring allostatic load in an urban adolescent sample: The profile and role of biomarker dysregulation in depression outcomes

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The profile and role of biomarker dysregulation in depression outcomes

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Biography

Heather Marshall completed her doctoral internship at Allegheny General Hospital where she focused on receiving training in evidence-based trauma treatment across the developmental lifespan and in working with serious mental illness in general and perinatal adult populations. She was presented with the Ester Mandelker Excellence in Psychotherapy Award from Allegheny General Hospital's Department of Psychiatry. This award was granted to Ms. Marshall for her dedication to providing evidenced based treatment and her commitment to establishing and maintaining rapport in her work with complex clinical cases during her internship year. Heather has had long-standing interest and motivation for continued learning about the impact of stress and trauma on mental health outcomes in both her research and clinical work. She is in the process of completing B-Tech DBT Foundational Training and is looking forward to beginning her post-doctoral fellowship position at the Chicago DBT Institute® - The Center for Mindfulness and Behavior Therapy, a DBT LBC Certified Program, in the fall of 2021. She is committed to continuing her professional growth in providing evidence-based practices developed for the treatment of complex mental health presentations and complex trauma. She approaches this work with a combination of humble curiosity, flexibility, empathy, and grit.

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Abstract

Accumulated, chronic stress exposure is well established as a precursor for allostatic load (AL). Both stress exposure and AL have been associated with depression in the existing literature. While many studies have focused on biomarkers representative of various physiological systems, a clear understanding of how physiological AL results in depression is yet unclear. Further, variability of hypo- and hypercortisolemic profiles have been associated with depression. A review of the existing literature supports hypocortisolemic profiles in relation to female depression and hypercortisolemic profiles in male depression across both adolescent and adult populations. The function of alpha-amylase (AA) dysregulation within the context of depression is even less well established. Previous research (Ali & Pruessner, 2012) has suggested a ratio of AA area under the curve with respect to ground (AUCg) over cortisol (CORT) AUCg, termed AOCg, as an indicator of the asymmetry between CORT and AA, and therefore, the hypothalamic pituitary adrenal (HPA) axis and sympathetic nervous system (SNS). Research supports an imbalance between these two systems may be representative of AL. AOCg has been correlated with major event exposure and depression in an adult sample. Adolescence is a sensitive biological period, perhaps posing even greater risk for the development of depressive symptoms within the context of AL. This provides rationale for use of the AOCg metric in an adolescent sample. A review of theory on stress research by Grant and colleagues (2003) posits a model for the etiology of psychopathology from stress exposure through the examination of potential biological mediators and moderators. The current study sought to further existing information on stress accumulation, AL, and depression by testing a path model with AUCg and area under the curve with respect to increase (AUCi) metrics of AA and CORT as mediators of the relationship between major events (ME), daily hassles (DH), and violent crime exposure (VC) with self-reported depression symptoms at two time points. We hypothesized that increased AUCg/AUCi of both CORT and AA would mediate the relationship between stressors and depression symptoms. We also hypothesized biological sex and parent depression severity would moderate the relationship between the physiological stress response and depression symptoms. In addition, the current study sought to replicate findings from the Ali & Pruessner study in an adolescent sample by running multiple regression analyses to identify associations between AOCg, stress, and depression symptoms. Results of the analyses indicate AUCg/AUCi metrics of AA and CORT do not mediate the relationship between stressors and depression symptoms. Regarding sex differences, female adolescents in the current sample exhibited a blunted response to the acute stressor task compared with males and presented with significantly more depression symptoms. AOCg was not significantly associated with depression symptoms in our adolescent sample but was significantly and positively associated with VC. Follow-up analyses indicate more VC is associated with lower COAg and that more COAg predicts less depression in adolescents. In addition, the use of more disengagement coping (DC) in response to more DH led to more depression symptoms at time one and lower AUCg AA values. Greater AUCg AA values in the current study were associated with less depression symptoms at time one and greater depression symptoms at time two. These results parallel previous studies demonstrating more SNS reactivity over time with

failure to habituate contributes to changes in neurobiological processes that create vulnerability for increased depression symptoms (McCarty, 2016). The current study provided further support for the need to utilize multiple measures of the stress response to elucidate associations between specific stressor types and specific parts of the stress response system that may be most impacted. In addition, the current study added to the topic of AA measurement in adolescents by identifying an association between AA with a chronic, uncontrollable distal stressor (VC) and identifying a positive association between AA and depression symptoms over time in a community sample of adolescents.

Measuring allostatic load in an urban adolescent sample: The profile and role of biomarker dysregulation in depression outcomes

Introduction

The role of allostatic load (AL) in psychopathology and pathophysiology is well established in the literature (McEwen, 1998). However, the mechanisms by which chronic stress results in the dysregulation of specific physiological systems and how the dysregulation, in turn, plays a direct or indirect role in the development of psychopathology is less well established. Biomedical and psychology researchers alike have contributed to this topic in various ways, though many previous studies have focused on one biomarker or physiological system at a time. Recently, research has expanded into the exploration of how different physiological systems, represented by various biomarkers, function in comparison to one another in response to acute and accumulated stress (Nederhof et al., 2015; Mauss et al., 2015; Mauss et al., 2016; Slopen et al., 2014). This has been done through the exploration of an overall allostatic load index (ALI), which includes multiple biomarker measurements across various physiological systems, and more precisely through the examination of interactions between the neuroendocrine and sympathetic nervous systems.

The ALI is a representation of cumulative physiological risk comprised of one or several biomarker/s representing various physiological systems. Though the ALI method is more inclusive than the measurement of any one biomarker alone, it lacks specificity that may be acquired through the examination of the individual biomarkers/systems and interactions. Relatively little is known about the way these physiological systems work

together to promote risk for the onset of mental health problems. Further investigation of specific physiological systems and how asymmetry of their biomarker representatives interact with one another to influence mental health is needed to create a better overall understanding of this complex network of functioning.

Adolescence is an important developmental period to explore in relation to the effects of chronic and acute stress exposure on biomarker functioning because it is characterized by many physiological, social, and emotional changes. Additionally, stress exposure in adolescence is associated with increased allostatic load in adulthood (Berg, Simons, Barr, Beach, Philbert, 2017). Adolescence is a particularly sensitive period in biological development, creating circumstances that may promote increased risk for the development of psychopathology (Chaby, Zhang, & Liberzon, 2017). Changes in physiological functioning during adolescence are hailed as a major component in this risk. This may be especially true for depression in which hormonal changes in female adolescents have been related to depression symptoms (Thapar, Collishaw, Pine, & Thapar, 2012), suggesting neurobiological factors play a role in the etiology of depression.

Because of these sensitivities, study of the etiology of depression during adolescence is important. A recent study conducted by the National Institute of Mental Health (NIMH; 2019) found adolescents with depression make up of 13.3% of the U.S. population. Further, approximately 71% of these youth experience severe impairment. Depression rates are higher among female adolescents (20%) compared with their male peers (6.8%; NIMH, 2019), highlighting the importance of considering biological sex in methodology within depression studies. Additionally, Major Depressive Disorder (MDD)

is highly heritable, with rates of heritability in teens falling between 30 and 50 percent (NIMH, 2019). A review of epigenetics cites multiple gene polymorphisms within this age group as being associated with depression (Xia & Yao, 2015). Genetic vulnerability along with chronic stress has long been linked to the development of depressive symptoms, however, little is known about the specific role of stress response biomarker dysregulation in this equation beyond significant associations with stressors and depression.

Taken together, it can be concluded exploration beyond that of associations between stress exposure and resulting physiological functioning in the etiology of depression in adolescent populations is needed. The present study is designed to aid in the development of understanding how stress exposure influences the physiological stress response, and how dysregulation of the stress response system may be involved in the development of depression symptoms in adolescent populations. Specific focus will be given to the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS), through cortisol (CORT) and salivary alpha-amylase (AA) metrics. These biomarkers are well established as representative of these respective components of the stress response system. Additional elucidation of the physiological systems involved as impacted by stress exposure can aid in the possible development of prevention and intervention aimed at minimizing the effects of stress on the body and mental health in an adolescent population. Further, intervention at this period may be critical in minimizing a broad range of health outcomes across the lifespan.

Allostatic Load

Allostatic load refers to the dysregulation of physiological mechanisms of the SNS and HPA axis in response to repeated exposure to stress (McEwen, 1998). The theory of allostasis can be thought of as an expansion of homeostasis (Wilkinson & Goodyear, 2011), such that, maintaining baseline in the event of stressors is not the goal, but rather the aim is maintaining physiological stability through change to meet the demands of the environment (Sterling & Eyer, 1988). Thus, allostatic load occurs when physiological adaptation contributes to physical and/or psychological pathology.

It has been hypothesized the risk for allostatic load increases when adaptation in an earlier environment does not meet the context of stress in a later environment (Shields & Slavich, 2017). For example, previous exposure to repeated instances of events categorized as high in stress may lead to a maladaptive response when faced with everyday stressors. While these responses may have been adaptive in previous circumstances of high stress, they fail to be useful in the context of lower stress events. This pattern of reactivity can change baseline levels of physiological functioning, such that, baseline levels of biomarkers are no longer within the normal limit, but rather, consistently elevated or blunted.

Extant research provides evidence supporting allostatic load as a large contributor to pathophysiology and psychopathology. For instance, frequent increased heart rate and blood pressure due to activation of the sympathetic nervous system can damage blood vessels and arteries, leading to increased risk for heart attack (McEwen & Lasley, 2003). Further, in a study conducted by Sabbah and colleagues (2007), allostatic load was found to mediate the relationship between socioeconomic status and ischemic heart disease.

Additionally, chronic stress is associated with increased risk of upper respiratory infection (Cohen et al., 1997; Runeson-Broberg & Norback, 2014; Campisi et al., 2017).

The link between mental health consequences and allostatic load have also been explored though extant findings are highly variable. For instance, studies of the association of CORT and depression have found support for both the overproduction of CORT (hypercortisolemic) and underproduction of CORT (hypocortisolemic) as predictors of depression across adult and adolescent samples (Lopez-Duran, Kovacs, & George, 2009; Ishitobi et al., 2010; Suzuki, Poon, Papadopoulos, Kumari, & Cleare, 2014; Mazurka, Wynne-Edwards, & Harkness, 2018; Badanes et al., 2011; Burke, Davis, Otte, & Mohr, 2005). Variability in the findings suggest complexities of the relationship between stress exposure, CORT dysregulation, and depression. Reviews of the literature on CORT output both within the context of stress alone and its association with depression suggest gender as a main component of differential findings of hypercortisolemia and hypocortisolemia (Liu et al., 2017; Zorn et al., 2017; Mazurka et al., 2018). These findings underscore the need to consider gender within models including biomarker dysregulation.

The following sections will discuss the biomarkers CORT and AA in more depth, their association with the HPA axis and SNS, and existing research on these specific biomarkers in relation to stressor type and depression. Further, existing research exploring how the HPA axis and SNS function in relation to one another and what we do and don't know about these interactions will be discussed.

Cortisol and the HPA Axis

The HPA axis makes up a portion of the central nervous system and plays an integral role in the stress response. Once activated, a complex cascade of events contributes to the release of cortisol, which is a subclass of steroid hormone (Smith & Vale, 2006). The presence of elevated cortisol levels inhibits further activation of the HPA axis through a negative feedback loop through which corticotropin-releasing hormone (CRH) is decreased (Hastings et al., 2011). If inactivation of the stress response system is impaired, repeated, prolonged exposure to stress hormones occurs, leading to the wear-and-tear on physiological systems referred to as allostatic load (McEwen, 1998).

Cortisol is a widely studied biomarker of stress and has been utilized in studies to obtain diurnal profiles, basal levels, and stress reactivity. It has been established as an effective measure of stress reactivity in human subjects across various age ranges, in response to acute stressor laboratory tasks (Hostinar, McQuillan, Mirous, Grant, & Adam, 2014; Katz, Peckins, & Lyon, 2019; Goodman, Janson, & Wolf, 2017). Existing research on cortisol levels in response to repeated or prolonged exposure to stress provides support of associations between both hyposecretion and hypersecretion of cortisol and chronic stress exposure. Extant research has sought to identify explanatory factors for the variation in cortisol levels in response to adversity and/or chronic stress. A meta-analysis conducted on studies investigating differences in CORT outcomes by gender provides promising findings to support the difference between hypo- and hyper-values of CORT to be a condition of variant functioning by biological sex (Liu et al., 2017).

Other theories of variation in CORT have been posited, including pitfalls within the way researchers operationally define accumulated stress exposure across studies.

Exploring the effects of stress exposure on the adolescent stress response system within the context of biological sex, while utilizing a concrete operational definition of previous stress exposure, will increase our existing knowledge of the effects of accumulated stress during this sensitive developmental period. Stress exposure has historically been measured in a variety of ways, often with little thought to constructing an operational definition, or been presupposed to exist without measurement of stressful events. This has been the case of some studies measuring physiological biomarkers without the inclusion of life stress interview or inventory.

Further elucidation of how stress exposure impacts cortisol functioning and how this, in turn, leads to the evolution of depression symptoms is also needed. Additionally, exploration of these pathways in adolescence will be a useful addition to the literature on AL and psychopathology during this developmental period.

What We Know About CORT, Stress, and Depression

Overall, the existing literature on depression and CORT in adolescent populations supports a hypercortisolemic profile as measured in studies investigating diurnal and reactivity output. Existing literature on adult populations additionally supports hypercortisolemia. However, some variability does exist within each of these age groups dependent upon stress exposure and biological sex.

A meta-analysis utilizing studies focused on the association between CORT and depression in pediatric samples, including adolescents, provides evidence to suggest hypercortisolemia is associated with depression in this age group (Lopez-Duran, Kovacs, & George, 2009). Specifically, the authors found higher diurnal CORT output is associated with depression symptoms. Studies investigating CORT values in response to

acute stress, were lacking, with the authors noting they found three. The results of the three summarized studies, paralleled diurnal patterns, with higher values post-stressor present in adolescents who endorsed depression symptoms. Empirical investigations in adolescent samples following the publication of this meta-analysis, further support a hypercortisolemic profile being associated with depression (Harkness, Stewart, & Wynne-Edwards, 2011; LeMoult, Ordaz, Kircanski, Singh, & Gotlib, 2015).

However, even with corroborated support for hypercortisolemia in subsequent studies, variability across studies is present and appear to be context dependent. For example, one such study, provided support only in the context of mild to moderate depression severity in the context of exposure to trauma (e.g., childhood maltreatment), with moderate to severe depression severity and no trauma exposure being associated with blunted reactivity (Harkness et al., 2011). A study conducted more recently parallels findings for the significant association of depression symptoms and hyperreactivity of CORT, but only in males, with females endorsing depression exhibiting reactivity levels significantly lower than females with no depression (Mazurka, Wynne-Edwards, & Harkness, 2018).

Adult studies match patterns in those conducted with adolescents, with the majority of support existing for hyperreactivity (Suzuki, Poon, Papadopoulos, Kumari, & Cleare, 2014; Fiksdal et al., 2019), and greater diurnal CORT output in depressed individuals (Dienes, Hazel, & Hammen, 2013). Variability between hyper- and hyporeactivity in adult samples is also present, with a meta-analytic study highlighting the role of biological sex as the contributing factor in variation of profiles (Zorn, Schur, Boks, Kahn, Joels, & Vinkers, 2017). This review of existing studies on MDD and HPA-

axis functioning found, in adults diagnosed with MDD, women exhibited blunted reactivity, while men exhibited hyperreactivity in response to an acute stressor task (Zorn et al., 2017).

Concurrent findings in both adolescent and adult samples suggest males and females exhibit variation in CORT profiles as related to depression. This parallels previously discussed knowledge that male and female reactivity profiles are variable even outside of the context of depression. A meta-analysis conducted by Liu and colleagues (2017) found significant differences in CORT reactivity and recovery values for men and women during exposure to the Trier Social Stress Test (TSST), with men exhibiting higher values of CORT at both time points. Relatively few studies with adolescents have made the differentiation between sex groups on CORT values. However, given consistent support for variation of CORT metrics by biological sex in existing research in both adult and adolescent populations, it can be considered an important component in the measurement of depression etiology within the context of physiological functioning.

AA, the Sympathetic Nervous System, and Extant Knowledge About the Relationship Between AA and CORT

The sympathetic-adrenal-medullary (SAM) system is comprised of the sympathetic nervous system (SNS), a component of the autonomic nervous system (ANS), and the adrenal medulla in the brain, and is responsible for the fight-or-flight mechanisms involved in the stress response (Korte et al., 2005). The ANS produces a rapid response when triggered by a stressor leading to respiratory and cardiovascular changes that are almost immediate (Katz & Peckins, 2017). AA has been considered in a variety of studies as a biomarker associated with sympathetic nervous system activity

(Ali & Pruessner, 2012) and is well documented as a marker of stress reactivity (Nater et al., 2005; Allwood et al., 2011; Katz & Peckins, 2017).

Evidence suggests AA and CORT measure distinct mechanisms of the stress response, given the lack of association between the two biomarkers in existing research (Allwood et al., 2011). A literature review of research with human and rat subjects on the production of AA suggests the biomarker may be used as an indirect measurement of ANS activation (Nater & Rohleder, 2009). More specifically, AA has been highly correlated with norepinephrine and epinephrine reactivity levels in response to exercise, denoting it as potentially useful in measuring SNS activity. However, research exploring the association between AA and catecholamines within the context of stress reactivity has found less support, with correlations between norepinephrine and AA reaching significance inconsistently (Kang, 2010; Nater & Rohleder, 2009).

Because AA is a biomarker representative of physiological functioning unique from that of CORT, inclusion of AA as a biomarker in studies may prove useful in further elucidating the mechanisms of allostatic load and psychopathology. For instance, Allwood and colleagues (2011) found asymmetric measurement between HPA axis and ANS biomarkers to be associated with attention problems, social problems, and symptoms of anxious-depression in a sample of youth. Additional studies have also found asymmetry between AA and cortisol to be predictive. Gordis and colleagues (2008), for instance, compared a group of adolescents who reported no maltreatment with a group who experienced maltreatment (based on DCFS involvement). In the group with no history of maltreatment, AA levels measured at baseline, peak, reactivity, area under the curve with respect to ground (AUCg), and area under the curve with respect to increase

(AUCi) were correlated with corresponding levels of cortisol. In contrast, in the group of adolescents who experienced maltreatment, corresponding levels of AA and cortisol were not correlated. Additionally, the asymmetry between AA and cortisol was moderated by maltreatment status, such that, participants with higher AA peak levels in the maltreatment exposure group had lower peak CORT levels compared with the comparison group. However, in another study examining cortisol and AA reactivity in adolescents, it was found there was a significant association between reactivity and recovery metrics of AA and cortisol (Katz & Peckins, 2017).

Given findings of the aforementioned studies, within group differences of stress exposure/adversity may play a role in the asymmetry of AA and cortisol values. It is likely, specificity of contextual factors (i.e., maltreatment, or more generally, exposure to major events) play an important role in explaining conditions of asymmetry among these biomarkers. Further, differential biomarker symmetry between groups with and without a major event exposure indicate the experience of a severe major event may present itself as an imbalance between the SAM and HPA systems.

It has been proposed, for example, that dysregulation in one biomarker may change functioning in another whereby, if one system is dysregulated, it may lead to dysregulation of the other (Bauer, Quas, & Boyce, 2002). For example, if CORT levels fail to increase following stress exposure, the feedback loop is not initiated, and SAM activation remains. Additionally, asymmetry between CORT and AA may be helpful in identifying pathways of psychopathology development. A review of the literature on profiles of SAM and HPA axis functioning in children and adolescents suggests profiles of higher reactivity in both create risk for internalizing symptoms (Bauer et al., 2002).

Ali and Pruessner (2012) sought to explore the phenomenon of AA and cortisol asymmetry further by considering a ratio of AA AUCg divided by cortisol AUCg which they termed AOCg. In a study conducted with adults in two groups, one group who endorsed childhood abuse and poor parental care and one group who did not, the authors found an association between AOCg and reported chronic stress and depression. The correlations were stronger for AOCg with stress measures than either AA or cortisol alone and were only significant for AOCg and depression. This indicates AOCg demonstrates a significant association with stressful events and depression and perhaps increased sensitivity for identification of the relationship between depression and stress response dysregulation. Therefore, ratio metrics such as AOCg may be a better measure for asymmetry between and/or dysregulation of AA and cortisol coordination than either of these biomarkers measured separately. The authors note the AOCg metric can be interpreted as AA scores when controlling for CORT.

Taken together, the information regards AA as a reliable biomarker of sympathetic nervous system reactivity, indicates associations between depression and AA exist, and highlights the importance of considering asymmetry of AA and CORT values in measures of allostatic load and psychopathology.

Logically, this parallels HPA-axis reactivity and SNS reactivity which both function as part of the stress response system but play different roles in the regulation of the stress response. It will be important to include AA in future stress related studies focused on elucidating the mechanisms of allostatic load and mental health outcomes to gain a more holistic representation of how CORT and AA function together, and how this relationship fits with the development of psychopathology in the context of the HPA axis

and SNS. However, replication of the AOCg ratio should be applied in an adolescent sample to further build understanding of biomarker functioning in this developmental time period. Given other biomarkers of the stress response (i.e., CORT) have shown similar responses in adult and adolescent populations, it is hypothesized AOCg will behave similarly in an adolescent population as in the adult sample examined by Ali & Pruessner (2012). Yet, empirical support is needed to confirm this assertion.

What We Know About AA, Stress, and Depression

Limited studies exist exploring the relationship between depression and AA in adolescent samples. Of the two available, one study found no relationship between AA and depression symptoms (Katz et al., 2019) while another found a significant negative relationship between AA and depression in adolescent males with a history of trauma exposure (Vigil et al., 2010). Studies conducted with adults show associations between higher AA awakening, area under the curve with respect to increase (AUCi), and reactivity metrics in clinical samples of Major Depressive Disorder (MDD; Bauduin et al., 2018; Tanaka et al., 2012), while basal levels of AA were lower in those with depression (Cubala & Landowski, 2014). AA has been adopted as a biomarker for the stress response relatively recently, which may explain the relative absence of studies exploring AA levels and depression.

Given only two published studies are available for adolescents and studies with adults have found some support for AA being linked with depression, it is important to replicate models with the association of AA and depression to provide further information confirming a lack of association in adolescent samples, or to increase our understanding of the conditions under which this association does or does not exist. The

SAM axis is posited as a system activated in response to controllable stressors (Bauer et al., 2002; Godoy et al., 2018), therefore, a model including daily hassles along with major events may be a better method for linking stress exposure with a biomarker representing the SAM axis.

The Grant and Colleagues Model of Stress and Etiology of Psychopathology in Adolescents

Grant and colleagues (2003) proposed a model of etiology for the development of psychopathology in adolescents. The authors conducted an extensive review of the literature focused on the definition of stress, which was and continues to be highly variable within the literature. They propose a definition of stress inclusive of environmental variables (events and chronic conditions) that have the objective ability to create physical or emotional harm to an individual.

Additionally, the authors encourage the use of a model inclusive of possible mediators and moderators in the investigation of mental health etiology in adolescent populations. The model posits stress exposure, including major events, daily hassles, and chronic conditions lead to bio-psycho-social processes that mediate the relationship between stress exposure and psychopathology outcomes. It is additionally proposed diathesis and other individual level characteristics may moderate the association between the mediator and psychopathology outcomes (Grant, Compas, Stuhlmacher, Thurm, McMahon, & Halpert, 2003).

A diathesis typically denotes a genetic predisposition to a specific mental health disorder (Monroe & Simons, 1991). The latter point fits with a portion of the commonly studied diathesis-stress model, which posits vulnerability (diathesis) and exposure to

environmental factors synthesize to further increase vulnerability and likelihood for the onset of mental health disorders (Raulin & Lilienfeld, 2015, p.100). However, Grant and colleagues (2003) go beyond the diathesis-stress model in proposing environmental factors (stress) and individual-level factors (bio-psycho-social processes) as not merely variables that may interact with one another to predict psychopathology, but as pathways to the development of psychopathology.

In terms of the presented definition of a diathesis, and focusing specifically on depression, an important diathesis to consider is parent history of depression. Depression is a highly heritable disorder, with between 30 – 50 % (NIMH, 2019) of adolescents with a first-degree relative endorsing depression, developing the disorder themselves.

Environmental factors that increase vulnerability include stress exposure, while possible biological factors in this model include AL, as measured in dysregulation of CORT and AA. In a review of the literature on depression in the context of diathesis-stress, Monroe and Simons (1991) noted major life events commonly precipitate the onset of Major Depressive Disorder (MDD). This has been further supported over the course of many studies in subsequent years (Harkness et al., 2011; Dienes et al., 2013; Suzuki et al., 2014; LeMoult et al., 2015). Further, it has been posited an experience of a major life event may be compounded by the addition of daily hassles, such that the likelihood of developing depression is increased when an individual has both experienced a major life stressor within the tandem experience of many daily stressors of lower severity (Monroe & Simmons, 1991).

Given what we know about the associations between stress exposure, CORT dysregulation, and depression, the application of these variables within the context of the

etiological model of stress and psychopathology proposed by Grant and colleagues (2003) could further develop our understanding of the way in which stress translates into allostatic load and how this leads to risk for depression in adolescents.

Operationalizing stress within the current study

Studies measuring history of stress exposure have utilized a wide array of stress measures covering various stressor types, are often aggregated, and are rarely clearly defined. Theory and empirical support bolster the assertion that the experience of major events and chronic stress are important precursors for both the development of allostatic load and depression (McEwen, 1998; Monroe & Simons, 1991; Grant et al., 2003). The present study will utilize the definition of stress created by Grant and colleagues (2003) which states a stressor is any external factor (major acute event or chronic) which poses an objective physical or emotional threat to an individual.

In an effort to be at once parsimonious and comprehensive, the present study will investigate stress exposure in relation to AL and depression. To do so, observed variables will include total major events, total daily hassles, and neighborhood violence rates by geolocation. The following paragraphs will discuss why these variables were chosen.

Major events refer to stressors that are objectively major occurrences for an adolescent to experience and can include a variety of incidents including failure of a grade, intimate partner violence, a family member going to jail (Allison et al., 1999), etc. These events can be chronic or episodic in nature. Daily hassles refer to stressors that objectively include a low severity impact (i.e., difficulty with schoolwork, chores at home; Allison et al., 1999) but typically recur and can accumulate over time. Major

events and daily hassles have traditionally been included in stress research to identify accumulated exposure to impactful life events of higher and lesser severity.

Exposure to community violence is of specific interest to the current study sample given the adolescents in the sample reside in an urban area characterized by high rates of violent crime. To explicate, local police department statistics report a total of 1,587 shootings within the last 7 months along with a 41% increase in murders over the last two years, and a 22% increase in criminal sexual assault over the last year (Chicago Police Department, 2021). Variations in violent crime data exist demonstrating specific neighborhoods are differentially affected, with higher rates of violent crime occurring disproportionately within lower-income areas (Sackett, 2016). Previous studies on exposure to violence in adolescent populations have shown violence exposure is related to dysregulation of biomarkers associated with stress response functioning (Murali & Chen, 2005). Thus, adolescents residing in low-income neighborhoods are differentially impacted by community violent crime and at additional risk for development of AL. Community rates of violent crime fall within the category of chronic stress exposure and can be objectively rated as an environmental factor that has the ability to cause both physical and emotional harm. Therefore, community violence exposure fits within the operational definition of stress provided by Grant and colleagues. Further, while major event and daily hassle exposures constitute more proximal forms of stress, the examination of community violence provides an opportunity to identify the impact of a more distal stressor.

Summary

Chronic stress and allostatic load are topics that receive paramount interest as evidenced by the plethora of extant research focused on these topics. Evidence has been provided that chronic stress exposure leads to wear-and-tear on physiological systems involved in the stress response system, and this in turn has consequences for both physical health and mental health outcomes.

Much evidence exists supporting an association between stress exposure and hypercortisolism and depression in both adolescent and adult populations. Variability within the literature between hypocortisolemic and hypercortisolemic profiles is explained through variation of biomarker functioning in males and females according to reviews of the literature in both adult and adolescent populations. It has been found that males with depression exhibit hyperreactivity of CORT while females exhibit blunted CORT reactivity.

Despite a multitude of studies within this area, mediational models connecting accumulated stress exposure and depression symptoms through allostatic load have yet to be examined. A model of stress and mental health etiology proposed by Grant and colleagues (2003) provides a theoretically supported representation of how stress leads to psychopathology, while providing a concrete operational definition of accumulated stress and clear direction for incorporating bio-psycho-social and individual characteristics to be examined through mediation and moderation.

The present study seeks to expand the extant knowledge available from previous research through the application of multiple stressors, allostatic load - as measured through dysregulation of the stress response -, and biological risk in the etiology of depression. The present study will focus on reactivity values of CORT and AA. Previous

studies have contributed to evidence supporting biomarker reactivity, in response to an acute stressor task, as a more sensitive measurement for psychopathology than diurnal values (Bae et al., 2015). To test an etiological model of stress, AL, and genetic risk on depression across male and female adolescents, a structural equation model will be applied. Specifically, major events, daily hassles, and community violence rates will be entered as exogenous variables predicting AL and depression symptoms. AL will be represented by CORT and AA values, set as a mediators between accumulated stress exposure and depression symptoms. Depression will be measured by total depression symptoms as assessed by the CDI scale. Further, genetic risk, as measured by parent depression will be set as a moderator of the relationship between AL and depression. It is important to note while the present study is utilizing parent depression as a measure of potential genetic risk, parent depression may also be influential on child depression symptoms in other ways. For instance, parent depression may in itself be a stressor for adolescents and may have environmental contributions for child depression.

Additionally, Ali & Pruessner (2012) successfully tested a model utilizing a ratio of AA AUCg/CORT AUCg (AOCg) in an adult sample and found a relationship between the AOCg metric and previous major event exposure, and depression outcomes. The present study will seek to replicate this finding in an adolescent sample. This may add to the literature through confirmation that the relationship between AA and CORT, therefore, the HPA axis and SAM axis are reciprocal and allostatic load a representation of an asymmetry between the two. Additionally, it will assist us in understanding whether AOCg functioning in adolescents might represent a difference or similarity with adult populations.

Research Questions & Hypotheses

Question 1. Can the etiology of depression in an adolescent sample be explained by pathways between stress exposure, AL, and depression? If AL mediates the relationship between stress and depression, is this mediation moderated by parent depression severity? Is the relationship between AL and depression symptoms moderated by biological sex?

Hypothesis 1a. It is hypothesized major events, daily hassles, and community violence will positively and significantly predict $AUCi_{CORT}$, $AUCi_{AA,}$ $AUCg_{CORT}$, and $AUCg_{AA}$.

Hypothesis 1b. It is hypothesized that $AUCi_{CORT}$, $AUCi_{AA,}$ $AUCg_{CORT}$, and $AUCg_{AA}$ will mediate the relationship between major events, daily hassles, violent crime exposure, and child depression symptoms.

Hypothesis 1c. It is hypothesized that biological sex will moderate the relationship between $AUCi_{CORT}$ and $AUCg_{CORT}$ and child depression symptoms.

Hypothesis 1d. It is hypothesized that biological sex will moderate the relationship between $AUCi_{AA}$ and $AUCg_{AA}$ and depression symptoms.

Hypothesis 1e. It is hypothesized that parent depression severity will moderate the relationship between $AUCi_{CORT}$ and $AUCg_{CORT}$ and child depression symptoms.

Hypothesis 1f. It is hypothesized that parent depression severity will moderate the relationship between $AUCi_{AA}$ and $AUCg_{AA}$ and child depression symptoms.

Question 2. To replicate findings of Ali & Pruessner (2012) in an adolescent sample, is there a significant association between stressors, AOCg, and depression. Specifically, do daily hassles, major events, and violent crimes significantly predict

AOCg? Does AOCg significantly predict adolescent depression symptoms at time one and time two?

Hypothesis 2. It is hypothesized that AOCg will be positively associated with depression symptoms at time one and time two when controlling for biological sex, age, and history of depression symptoms (when predicting time two depression).

Methods

Participants

A sub sample of 235 (48.4% male) students recruited from four public schools in a large Midwestern city, participated in an acute stressor task as part of the larger Stress and Learning study. Participants range in age from 11 to 18 years. The sample is diverse in race/ethnicity (32.7% Latino/a, 37.6% Black/African American, 33.7% White/European-American, 11.4% Asian/Pacific-Islander, 1% Native Hawaiian/Pacific Islander, 0.5% Alaskan Native/American Indian, 5.8% Bi/Multi-racial) and annual family income level (30% $20,001 - $50,000, 22.3% $0 – $25,000, 21.4% $50,001 - $80,000, 12.3% $80,001 - $100,000, 8.2% $100,001 - $150,000, 5.9% > $150,000).

Procedure

All of the measures and protocols used in this study were approved by the Institutional Review Board at DePaul University and Northwestern University, as well as the Research Review Board at Chicago Public Schools. All adolescent participants signed written assent forms and signed consent forms were obtained from a parent/guardian of each participant. Participants came to DePaul University for a full day of data collection activities on one of five consecutive Saturdays in the fall of 2012. During the data collection day, participants were randomly assigned to one of four groups, determining

the order in which they would participate in data collection tasks and other activities. Every group started off with a check-in, orientation, and breakfast then either went on to complete 1) Life Stress Interviews, the 2) Group Public Speaking Task for Adolescents (GPST-A), or 3) surveys. Then, each group went on to complete the next two activities, in which they had not yet participated (interviews, GPST-A, surveys) or lunch and activities until all activities were completed by each group. A wrap-up and dinner was participated in by all groups directly following the completion of all tasks and activities (see Figure 1.).

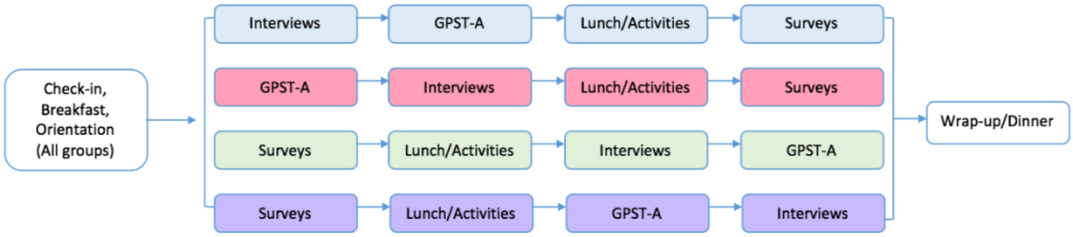

Figure 1. Data Collection Procedure by Group

Measures

Demographics. Demographic information including age, gender, race/ethnicity, income, and grade was obtained through a self-report survey.

Group public speaking task for adolescents (GPST-A). The Group Public Speaking Task for Adolescents (GPST-A; Hostinar et al., 2014) was used to expose youth to a minor stressor in vivo. The GPST-A is a modified version of the Trier Social Stress Test for Groups (TSST-G; Von Dawans, Kirschbaum, & Heinrichs, 2011). The GPST-A is an age-appropriate version of the task, for adolescents, mimicking a classroom setting rather than a business or laboratory setting as in the TSST-G (Von

Dawans et al., 2011). The overall point of the task is still the same, creating an environment of social-evaluative stress (Hostinar et al., 2014).

The GPST-A was administered in the following way: Baseline saliva samples were collected by research assistants using the passive drool method before the task start time, and mood surveys were completed by the participants. Participants were instructed to think of something that will cause them to produce more saliva (i.e., "Think about eating something sour") and fill up a vial with as much drool as possible. The vials were labeled with participant ID numbers and stored in a refrigerator after collection. Participants were given three minutes to prepare a brief speech introducing themselves to a new classroom of students which they would present in the next phase of the protocol. Five to eight participants were brought into a classroom and seated at a desk with dividers between them. Each participant presented their speech for one minute and fifteen seconds; saliva samples were taken again before the participant gave the speech and immediately after they gave the speech. The speech was videotaped and there was a 2-person judge panel at the front of the room and a researcher and research assistant seated at a table on the right side of the room (when facing front). The judges were dressed in business attire in an effort to mimic school personnel. After the speech was completed, participants were given mood surveys and debriefed; saliva samples were taken at three ten-minute intervals during the debriefing and rest period. The saliva samples were stored at −20 ◦ C in a freezer until they were sent by the research team at Northwestern University to the University of Trier in Germany for time-resolved fluorescence immunoassay (Hostinar et al., 2014).

Saliva samples. Saliva samples were taken using the passive drool method at 6 time points (-15 mins, 0 mins, 15 mins, 30 mins, 40 mins, 50 mins), stored at – 20 degrees Celsius, and underwent time-resolved fluorescence immunoassay at the University of Trier in Germany for AA and CORT. Salivary CORT was measured in micrograms per deciliter (µg/dL) and salivary AA in micrograms per micrometer (µg/mL). Cortisol measurement from the second saliva sample was used as baseline CORT and AA measurement from the second saliva sample was used as baseline AA. CORT AUC metrics were calculated using the salivary data from the second, third, fourth, fifth, and sixth time points to encompass increased from baseline back to recovery. AA AUC metrics were calculated using salivary data from the second, third, and fourth time points. This parallels best-practices outlined in the literature, which confirms reactivity of AA as being almost immediate, at 5 to 10 minutes following onset of stressor task (Nater et al., 2006), while CORT reactivity is delayed, showing up in saliva samples about 20 to 30 minutes following onset of stressor task (Allwood et al., 2011; Dickerson & Kemeny, 2004). In addition, it coincides with the trends for AUC in our data for CORT and AA measurements (see Figures 2 and 3).

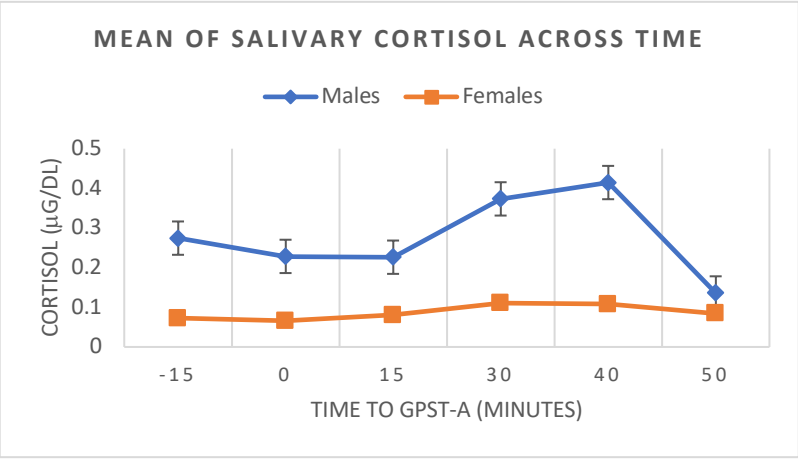

Figure 2. Mean of Salivary Cortisol Across Time

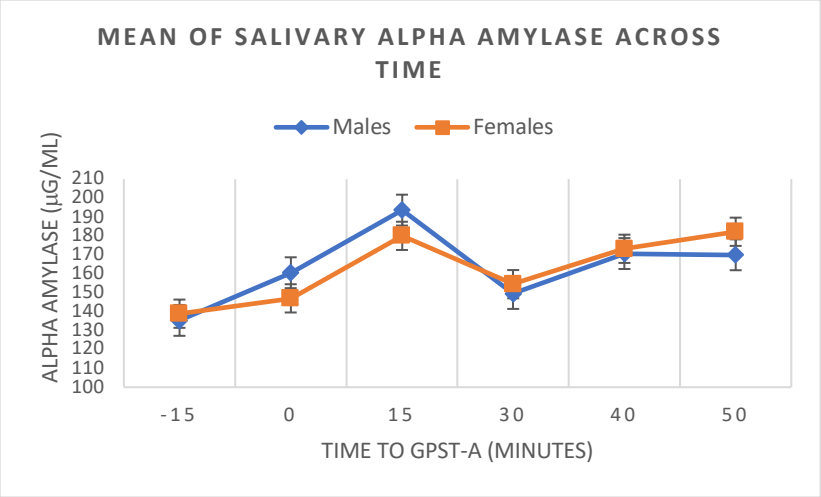

Figure 3. Mean of Salivary Alpha Amylase Across Time

AUCi and AUCg. Area under the curve with respect to increase and area under the curve with respect to ground were calculated for both salivary CORT and AA utilizing the trapezoidal method. The trapezoidal method is well established in the literature as a way to measure change of salivary biomarkers over repeated measures. The AUCg and AUCi metrics measure the change in time of data in different ways. AUCg measures the distance of data points from the ground value, while AUCi measures the distance of data points, starting with the first increase and each subsequent data point following (Pruessner, Kirschbaum, Meinlschmid, Hellhammer, 2003). Therefore, these metrics are measuring different constructs. In other words, AUCg measures total output of the biomarker over the saliva sample collection period, while AUCi is a reactive measure of the increases and decreases of the biomarker over the collection period.

AOCg. The AOCg ratio metric was calculated by dividing AUCg of AA by AUCg of CORT as defined by Ali and Pruessner (2012). This metric measures AA activity controlling for CORT.

Daily hassles, and major events. The Urban Adolescent Life Experiences Scale (UALES; Allison et al., 1999) was adapted from the Adolescent Perceived Life Events

Scale (APLES; Compas et al., 1987) as a measure with increased contextual validity for youth residing in an urban environment. Items are rated on 5-point Likert-type scale (1 = never, 5 = always) and assess stressful chronic and episodic life events across the context of school, peer, family, and personal. The total stress score is obtained by adding all scores from the measure. The measure includes two subscales: daily hassles and major events. Inter-rater reliability for derivation of the two subscales was good (.90), and test-retest reliability was acceptable (.84).

Neighborhood violence. Neighborhood violence was calculated by matching participant address to census track ID and gathering violent crime rates from the local law enforcement database by census track number.

Depression symptoms. Depression symptoms were measured utilizing the Children's Depression Inventory 2 (CDI 2; Kovacs, 2011) total score. The CDI 2 is a 28-item, self-report questionnaire from which a total score, two main scales (emotional problems, functional problems), and 4 subscales (negative mood/physical symptoms, negative self-esteem, interpersonal problems, and ineffectiveness) can be derived. Each of the 28 items includes 3 possible statements of endorsement to be chosen by the participant ranging on a Likert-type scale from 0-2 (least to most severity on item). For example, one item provides the possible choices: "I am sad once in a while" (0), "I am sad many times" (1), and "I am sad all the time" (2). Total scores range from 0 to 56, with scores of 12 and above being considered a clinically significant value. The CDI 2 was normed on 1,100 children age 7 to 17 years from 26 states within the U.S. The CDI 2 has shown good internal consistency (α = .67 to .91) and test-retest reliability (Bae, 2012).

Parent depression symptoms. A parent of participating adolescents filled out the Beck Depression Inventory (BDI-II; Beck et al., 1996), a 21-item self-report measure that assesses for the severity of depression symptoms in individuals 13 through 80 years of age. Raw scores are used to assess whether symptom severity falls within the minimal, mild, moderate, or severe range of depression.

Statistical Analyses & Results

Descriptive Statistics

All descriptive analyses were run utilizing IBM SPSS Statistical Software version 25 (2017) on a Windows computer. Bivariate correlations were run for all continuous variables at data collection time points one and two. For time one, these included age, daily hassles, major events, neighborhood violence, CORT and AA reactivity measures ($AUCi_{CORT}$, $AUCi_{AA}$, $AUCg_{CORT}$, $AUCg_{AA}$), AOCg, COAg, CDI, parent BDI score, and potential covariates for salivary cortisol and alpha amylase (e.g. hours of sleep, caffeine consumption). For time 2, this included CDI. Mann-Whitney U tests were run for biological sex and CORT reactivity, AA reactivity, AOCg, and CDI total to test for differences between males and females on these measures. Mann-Whitney U tests or Welch T tests were also run for dummy-coded (0 = No, 1 = Yes) covariates with biomarker variables. These potential covariates include taking birth control, taking ADHD medication, taking depression medication, other psychotropic medications, use of corticosteroids, and participant group.

Bivariate correlations revealed statistically significant positive relationships between time one child depression symptoms and child age ($r(306) = .25$, $p<.001$), daily hassles ($r(354) = .39$, $p<.001$), and parent depression symptom severity ($r(228) = .22$, $p =$

.001). In addition, statistically significant positive relationships were found between child depression symptoms at time two and child depression symptoms at time one ($r(179) = .51$, $p<.001$), parent depression severity at time one ($r(134) = .23$, $p = .008$), daily hassles at time one ($r(171) = .28$, $p < .001$), and adolescent age at time one ($r(150) = .21$, $p < .011$). Square root transformations were applied to correct for non-normality of data prior to running bivariate correlations. Variables transformed included AUCg metrics for CORT and AA, $AUCi_{CORT}$, COAg, AOCg, major events, and CDI at times one and two. See tables 1 and 2 for the full information on bivariate correlations among study variables and potential covariates.

The Mann Whitney U tests revealed there were no significant differences for males and females on measures of CORT reactivity (Time one $U = 5268$, $z = 1.453$, $p = 0.146$; Time 2 $U = 2226$, $z = 0.770$, $p = 0.441$), AA reactivity (Time one $U = 4039$, $z = -0.654$, $p = 0.513$; Time 2 $U = 2215$, $z = 1.386$, $p = 0.166$), and AOCg (Time one $U = 3591$, $z = -1.884$, $p = 0.060$; Time 2 $U = 1818$, $z = -0.401$, $p = 0.689$) at data collection time point one. Mann Whitney U Tests did indicate a significant difference on measures of salivary CORT for saliva samples collected at 15 minutes prior to GPST-A (S1 $U = 7751.50$, $z = 2.394$, $p = 0.017$), task start (S2 $U = 7642.50$, $z = 2.435$, $p = 0.015$), 15 minutes following the beginning of the GPST-A (S3 $U = 8179.00$, $z = 3.386$, $p = 0.001$), 30 minutes following the start of the GPST-A (S4 $U = 7224.00$, $z = 3.185$, $p = 0.001$, and 40 minutes following the start of the GPST-A (S5 $U = 5721.00$, $z = 2.183$, $p = 0.029$). There were no significant differences of CORT 50 minutes following the onset of the GPST-A (S6 $U = 2759.50$, $z = 0.520$, $p = 0.603$). Overall, the significant differences between males and females on CORT measures indicate a blunted CORT response in

female participants. See *Figure 2* for a visual representation. There were no significant mean differences for gender on saliva samples at times one through six for salivary AA. The Mann Whitney U test revealed a significant difference between males (mean rank = 132.28) and females (mean rank = 168.78) on reported depression symptoms for time one (U = 8620.500, z = - 3.645, p < .001) with no significant difference between scores for time two (U = 2366, z = -0.986, p = 0.324).

Results for Welch T-tests indicate a statistically significant mean difference on $AUCg_{AA}$, M = -36.09, 95% CI [-70.64, -1.54], $t(137)$ = -2.066, p = .041 and AOCg, M = -33.14, 95% CI [-54.66, -11.61], $t(88)$ = -3.059, p = .003 measures based on reported use of contraceptive medication with those not using contraceptives having lower mean AA readings. Statistically significant mean differences were also observed in participants using medication to treat depression symptoms, with those not using medications reporting less depression symptoms, M = -0.60, 95% CI [-0.10, -0.21], $t(311)$ = -3.011, p = .003.

Table 1. ***Means, standard deviations, bivariate correlations***

Measure	1	2	3	4	5	6	7	8	9	10	11	12	13
Time 1													
1. CDI	--												
2. ME	.05	--											
3. DH	.39**	.33**	--										
4. VC	-.07	.06	-.10	--									
5. BDI	.22**	.10	.12	.09	--								
6. $AUCi_{AA}$	-.07	.05	-.06	.05	.05	--							
7. $AUCi_{CORT}$	-.06	.10	.02	-.02	-.05	.24**	--						
8.$AUCg_{AA}$	-.22**	.09	-.07	.15	.01	.37**	.11	--					
9. $AUCg_{CORT}$	-.07	.09	-.08	-.10	-.12	.27**	.56**	.10	--				
10. AOCg	-.08	.02	.07	.20*	-.01	.03	-.25**	.67**	-.59**	--			
11. COAg	.13	-.00	.00	-.23*	.01	-.03	.26**	-.62**	.53**	-.79**	--		
12. Age	.25**	.15*	.31**	-.16**	.09	.05	.06	-.04	.15	-.00	.04	--	
Time 2													
13. CDI	.51**	.11	.28**	-.04	.23*	.06	-.14	.05	-.21	.10	-.15	.21*	--
Mean	16.87	4.61	28.51	2575.73	5.63	951.99	1.41	7370.62	5.97	1856.52	.002	14.75	5.47
SD	3.00	4.14	10.14	990.80	6.08	2290.22	4.11	4965.18	5.06	1652.11	.003	1.97	5.71

Note. *p<.05, **p<.001. Means and SDs were calculated for raw, untransformed variables. Square root transformations were applied to CDI (time 1 and time 2), ME, $AUCi_{CORT}$, $AUCg_{AA}$, $AUCg_{CORT}$, AOCg, and COAg to correct for non-normality of distributions.

Table 2. ***Bivariate correlations between biomarkers and potential covariates***

Measure	*M*	*SD*	1	2	3	4	5	6
Time 1								
1. $AUCi_{AA}$	951.99	2290.22	--					
2. $AUCi_{CORT}$	1.41	4.11	--	--				
3. $AUCg_{AA}$	7370.62	4965.18	--	--	--			
4. $AUCg_{CORT}$	5.97	5.06	--	--	--	--		
5. AOCg	1856.53	1652.11	--	--	--	--	--	
6. COAg	.001	.003	--	--	--	--	--	--
7. Coffee/tea (cups)	0.64	0.92	-.21*	-.01	.09	.06	.00	.00
8. Sleep (hours)	7.11	1.49	.05	.06	.01	.08	.08	.07
9. BMI	24.19	6.43	.03	.00	.11	.01	.17*	-.08
10. WHR	0.86	0.11	-.09	-.09	-.08	-.12	-.03	.04

Primary Analyses

Path analyses were run utilizing the Lavaan (Rosseel, 2012) package in RStudio (R Core Team, 2020) on a MacOS computer. The estimator was set to Maximum Likelihood Ratio (MLR) to correct for multivariate non-normality. It was determined data are Missing at Random (MAR) via review of missing data patterns (Rosseel, 2012), so the Full Information Maximum Likelihood (FIML) estimation was used to address missing data. The recommended critical inclusion of 200 participants (Mentzer, 1999, and Hoelter, 1983, as cited in Hoe, 2008, p. 77) was achieved in each of the path analyses tested.

Hypotheses 1a and 1b. To test hypotheses 1a and 1b, six separate models were run with AUCi metrics and AUCg metrics set as mediating variables for each individual stressor and adolescent depression symptoms. Because of variability across these physiological metrics within the literature and given they have been posited to measure reactivity differently, including both metrics in analyses for comparison has been recommended (Pruessner et al., 2003). Stressors were tested separately across models due to multicollinearity between daily hassles and major events. Major events (ME), daily

hassles (DH), and neighborhood violence (VC) collected from time one (T1) were entered as observed variables. $AUCi_{CORT}$/ $AUCg_{CORT}$ and $AUCi_{AA}$/$AUCg_{AA}$ were entered as observed variables representing AL and set as exogenous variables predicting child/adolescent depression as measured by total CDI score for both time one and time two (see *Figure 4*). Indirect effects were tested for major events, daily hassles, and neighborhood violence on adolescent depression symptoms through the mediators $AUCi_{CORT}$/$AUCg_{CORT}$ and $AUCi_{AA}$/ $AUCg_{AA}$. Biological sex and age of adolescent at time one were included as control variables and regressed onto depression at time one and time two. Biological sex was also regressed onto the mediator variables.

All estimated AUCi models included 33 free parameters on 239 observations with 5 degrees of freedom. Overall AUCi model fit indices were inflated for models examining daily hassles ($\chi^2 = 2.560$, $p = .767$, $\chi 2/df = 0.512$, CFI = 1.000, AGFI = 0.904, RMSEA = 0.000, SRMR = 0.021), major events ($\chi^2 = 1.602$, $p = .901$, $\chi 2/df = 0.320$, CFI = 1.000, AGFI = 0.922, RMSEA = 0.000, SRMR = 0.020), and neighborhood violence ($\chi^2 = 3.237$, $p = .664$, $\chi 2/df = 0.647$, CFI =1.000, AGFI = 0.913, RMSEA = 0.000, SRMR = 0.028). The fit statistics (except AGFI) of each of these models fell within the good range, however, the relationships between many of the exogenous and endogenous variables were not significant. This phenomenon can occur in path analysis/structural equation modeling when observed variables have a weak relationship (Tarka, 2018). Goodness of fit in a structural equation model is indicated by a value of the χ^2 statistic that is not significant, comparative fit index (CFI) value $\geq .95$, a goodness of fit index (GFI) of $\geq .90$, a root mean square error of approximation (RMSEA) value of $\leq .06$, and a root mean square residual value of $\leq .08$ (Lei & Wu, 2007; Hu & Bentler, 1999).

All estimated AUCg models included 33 free parameters on 239 observations with 2 degrees of freedom. Overall fit statistics for the AUCg models also appeared inflated relative to the number of significant pathways for models including daily hassles ($\chi^2 = 3.405$, $p = .182$, $\chi 2/df = 1.703$, CFI = 0.988, AGFI = 0.839, RMSEA = 0.054, SRMR = 0.031), major events ($\chi^2 = 3.066$, $p = .216$, $\chi 2/df = 1.533$, CFI = 0.987, AGFI = 0.859, RMSEA = 0.047, SRMR = 0.027), and neighborhood violence ($\chi^2 = 1.885$, $p = .390$, $\chi 2/df = 4.833$, CFI =1.000, AGFI = 0.887, RMSEA = 0.000, SRMR = 0.024). Though it was determined the overall models examined do not fit the data well, some individual paths were significant. This will be discussed below.

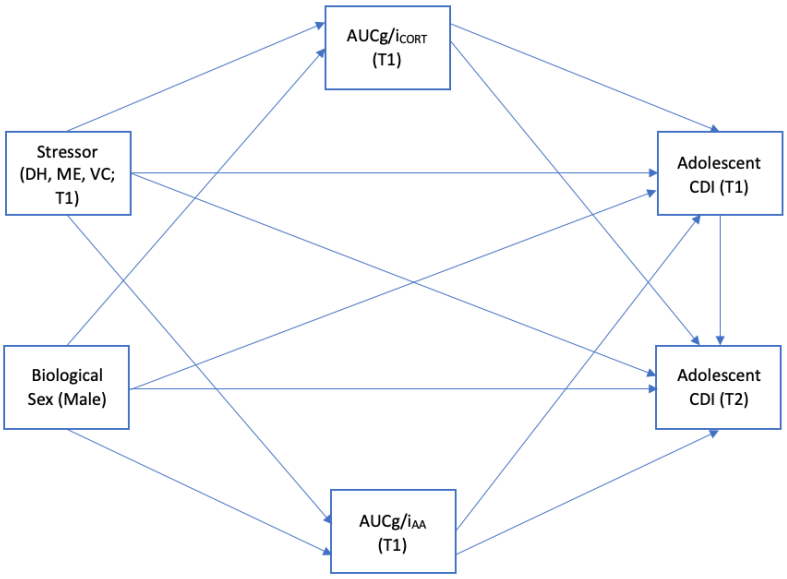

Figure 4. Model testing hypothesis 1a. *Note.* Only main variables of interest included in visual representation of model for simplicity.

There was a marginally significant association between exposure to neighborhood violence at time one and AA reactivity as measured with respect to ground ($\beta = 0.134$, $p = .058$) during the acute stressor task. The area under the curve with respect to ground

(AUCg) considers the measurements at each time point in relation to zero and most closely reflects total biomarker output throughout the course of the acute stressor task (Pruessner et al., 2003). The area under the curve with respect to increase (AUCi) considers measurements at each timepoint in relation to the prior timepoint metric and ignores the measurement from baseline to first time point measured. The latter reflecting "sensitivity of the system, pronouncing changes over time" (Pruessner et al., 2003, p. 928). Significant relationships were not found between major events ($\beta = 0.102$, $p = .218$; $\beta = 0.102$, $p = .232$) or daily hassles ($\beta = -0.070$, $p = .320$; $\beta = -0.034$, $p = .684$), and AA reactivity with respect to ground or increase or between neighborhood violence and $AUCi_{AA}$ ($\beta = 0.049$, $p = .477$). In addition, significant relationships were not found between daily hassles ($\beta = -0.061$, $p = .623$; $\beta = 0.052$, $p = .592$), major events ($\beta = 0.129$, $p = .214$; $\beta = 0.135$, $p = .090$), or neighborhood violence ($\beta = -0.107$, $p = .166$; $\beta = -0.001$, $p = .994$) and $AUCg_{CORT}$ or $AUCi_{CORT}$. Taken together, hypothesis 1a is not supported with neighborhood violence only trending towards positively predicting AA reactivity and no other stressors significantly predicting CORT or AA reactivity. See tables 3 through 8 for all parameter estimates for AUCi and AUCg models by stressor.

AA reactivity values with respect to ground ($AUCg_{AA}$) negatively and significantly predicted adolescent depression symptoms reported at time one ($\beta = -0.201$ to -0.177, $p < .01$) and positively predicted depression symptoms at time two ($\beta = 0.155$ to 0.161, $p < .05$). Neither $AUCg_{CORT}$ ($\beta = -0.081$ to -0.017, $p > .05$; $\beta = -0.125$ to -0.108, $p > .05$), nor $AUCi_{CORT}$ ($\beta = -0.062$ to -0.034, $p > .05$; $\beta = -0.121$ to -0.102, $p > .05$), nor $AUCi_{AA}$ ($\beta = -0.071$ to -0.027, $p > .05$; $\beta = 0.106$ tot 0.119, $p > .05$) predicted adolescent depression symptoms at time one or time two.

Table 3. Parameter estimates hypothesis 1a daily hassles, AUCi metric

Effect			Est.	*SE*	Standardized 95% CI *LL*	*UL*	*p*
Age	→	CDI_{T1}	0.18	0.06	0.08	0.30	<.001
Male	→	CDI_{T1}	-0.24	0.06	-0.34	-0.13	<.001
Daily Hassles	→	CDI_{T1}	0.40	0.06	0.28	0.52	<.001
$AUCi_{CORT}$	→	CDI_{T1}	-0.06	0.09	-0.24	0.11	.488
$AUCi_{AA}$	→	CDI_{T1}	-0.03	0.06	-0.14	0.08	.631
Age	→	CDI_{T2}	0.19	0.06	0.08	0.30	.001
Male	→	CDI_{T2}	0.02	0.08	-0.15	0.19	.811
Daily Hassles	→	CDI_{T2}	0.11	0.10	-0.09	0.30	.295
$AUCi_{CORT}$	→	CDI_{T2}	-0.12	0.13	-0.38	0.14	.363
$AUCi_{AA}$	→	CDI_{T2}	0.12	0.08	-0.03	0.27	.129
Male	→	$AUCi_{CORT}$	0.06	0.08	-0.10	0.22	.445
Daily Hassles	→	$AUCi_{CORT}$	0.05	0.10	-0.14	0.24	.592
Male	→	$AUCi_{AA}$	0.02	0.07	-0.12	0.17	.780
Daily Hassles	→	$AUCi_{AA}$	-0.03	0.08	-0.20	0.13	.684
Caffeine	→	$AUCi_{AA}$	-0.19	0.11	-0.41	0.02	.082

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Indirect effects were calculated for all models. Indirect effects for physiological biomarkers mediating the relationship between stressors and depression symptoms were not significant for time one or time two depression symptoms. See tables 9 and 10. Given the path models tested did not fit the data well and that indirect effects were not significant, moderated mediation by biological sex and parent depression severity were not examined.

Table 4. Parameter estimates hypothesis 1a major events, AUCi metric

Effect			Est.	*SE*	Standardized 95% CI *LL*	*UL*	*p*
Age	→	CDI_{T1}	0.28	0.06	0.16	0.39	<.001
Male	→	CDI_{T1}	-0.23	0.06	-0.35	-0.12	<.001
Major Events	→	CDI_{T1}	0.16	0.06	0.04	0.29	.009
$AUCi_{CORT}$	→	CDI_{T1}	-0.06	0.09	-0.23	0.12	.527
$AUCi_{AA}$	→	CDI_{T1}	-0.07	0.06	-0.19	0.05	.262
Age	→	CDI_{T2}	0.10	0.10	-0.10	0.30	.326
Male	→	CDI_{T2}	0.03	0.08	-0.14	0.19	.746
Major Events	→	CDI_{T2}	0.12	0.11	-0.10	0.35	.274
$AUCi_{CORT}$	→	CDI_{T2}	-0.10	0.13	-0.36	0.15	.433
$AUCi_{AA}$	→	CDI_{T2}	0.11	0.08	-0.05	0.26	.177
Male	→	$AUCi_{CORT}$	0.06	0.08	-0.10	0.21	.494
Major Events	→	$AUCi_{CORT}$	0.14	0.08	-0.02	0.29	.090
Male	→	$AUCi_{AA}$	0.03	0.07	-0.11	0.17	.689
Major Events	→	$AUCi_{AA}$	0.10	0.09	-0.07	0.27	.232
Caffeine	→	$AUCi_{AA}$	-0.21	0.11	-0.42	0.00	.049

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Table 5. Parameter estimates hypothesis 1a neighborhood violence, AUCi metric

Effect			Est.	*SE*	Standardized 95% CI *LL*	*UL*	*p*
Age	→	CDI_{T1}	0.31	0.06	0.20	0.43	<.001
Male	→	CDI_{T1}	-0.24	0.06	-0.36	-0.13	<.001
Neighborhood Violence	→	CDI_{T1}	0.02	0.07	-0.12	0.16	.740
$AUCi_{CORT}$	→	CDI_{T1}	-0.03	0.10	-0.22	0.15	.721
$AUCi_{AA}$	→	CDI_{T1}	-0.07	0.07	-0.20	0.06	.275
Age	→	CDI_{T2}	0.11	0.10	-0.09	0.30	.288
Male	→	CDI_{T2}	0.02	0.08	-0.15	0.18	.836
Neighborhood Violence	→	CDI_{T2}	-0.05	0.10	-0.24	0.14	.606
$AUCi_{CORT}$	→	CDI_{T2}	-0.12	0.14	-0.39	0.14	.369
$AUCi_{AA}$	→	CDI_{T2}	0.12	0.08	-0.03	0.27	.125
Male	→	$AUCi_{CORT}$	0.05	0.08	-0.11	0.21	.515
Neighborhood Violence	→	$AUCi_{CORT}$	0.00	0.09	-0.18	0.18	.994
Male	→	$AUCi_{AA}$	0.03	0.07	-0.12	0.17	.732
Neighborhood Violence	→	$AUCi_{AA}$	0.05	0.07	-0.09	0.19	.477
Caffeine	→	$AUCi_{AA}$	-0.20	0.07	-0.09	0.19	.477

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Table 6. Parameter estimates hypothesis 1a daily hassles, AUCg metric

Effect			Est.	*SE*	Standardized 95% CI *LL*	*UL*	*p*
Age	→	CDI_{T1}	0.18	0.06	0.07	0.29	.001
Male	→	CDI_{T1}	-0.24	0.05	-0.34	-0.13	<.001
Daily Hassles	→	CDI_{T1}	0.39	0.06	0.27	0.51	<.001
$AUCg_{CORT}$	→	CDI_{T1}	-0.02	009	-0.19	0.16	.846
$AUCg_{AA}$	→	CDI_{T1}	-0.18	0.06	-0.30	0.05	.005
Age	→	CDI_{T2}	0.12	0.10	-0.08	0.31	.257
Male	→	CDI_{T2}	0.03	0.08	-0.13	0.20	.703
Daily Hassles	→	CDI_{T2}	0.08	0.10	-0.12	0.27	.437
$AUCg_{CORT}$	→	CDI_{T2}	-0.11	0.11	-0.32	0.10	.316
$AUCg_{AA}$	→	CDI_{T2}	0.16	0.07	0.01	0.30	.032
Male	→	$AUCg_{CORT}$	0.13	0.09	-0.04	0.30	.135
Daily Hassles	→	$AUCg_{CORT}$	-0.06	0.13	-0.31	0.18	.623
Male	→	$AUCg_{AA}$	0.05	0.70	-0.13	0.14	.942
Daily Hassles	→	$AUCg_{AA}$	-0.07	0.07	-0.21	0.07	.320

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Table 7. Parameter estimates hypothesis 1a major events, AUCg metric

Effect			Est.	*SE*	Standardized 95% CI *LL*	*UL*	*p*
Age	→	CDI_{T1}	0.27	0.06	0.16	0.38	<.001
Male	→	CDI_{T1}	-0.23	0.06	-0.34	-0.11	<.001
Major Events	→	CDI_{T1}	0.18	0.06	0.06	0.30	.005
$AUCg_{CORT}$	→	CDI_{T1}	-0.08	0.10	-0.27	0.11	.402
$AUCg_{AA}$	→	CDI_{T1}	-0.20	0.06	-0.32	-0.08	.001
Age	→	CDI_{T2}	0.11	0.10	-0.09	0.31	.278
Male	→	CDI_{T2}	0.04	0.08	-0.12	0.20	.637
Major Events	→	CDI_{T2}	0.12	0.12	-0.11	0.35	.291
$AUCg_{CORT}$	→	CDI_{T2}	-0.11	0.10	-0.31	0.09	.270
$AUCg_{AA}$	→	CDI_{T2}	0.16	0.08	0.01	0.30	.041
Male	→	$AUCg_{CORT}$	0.14	0.08	-0.03	0.30	.105
Major Events	→	$AUCg_{CORT}$	0.13	0.10	-0.07	0.33	.214
Male	→	$AUCg_{AA}$	0.01	0.07	-0.13	0.15	.919
Major Events	→	$AUCg_{AA}$	0.10	0.08	-0.06	0.26	.218

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Table 8. Parameter estimates hypothesis 1a neighborhood violence, AUCg metric

Effect			Est.	*SE*	Standardized 95% CI *LL*	*UL*	*p*
Age	→	CDI_{T1}	0.31	0.06	0.20	0.42	<.001
Male	→	CDI_{T1}	-0.24	0.06	-0.36	-0.12	<.001
Neighborhood Violence	→	CDI_{T1}	0.03	0.08	-0.11	0.18	.646
$AUCg_{CORT}$	→	CDI_{T1}	-0.06	0.10	-0.26	0.14	.531
$AUCg_{AA}$	→	CDI_{T1}	-0.19	0.06	-0.32	-0.07	.003
Age	→	CDI_{T2}	0.11	0.10	-0.08	0.30	.259
Male	→	CDI_{T2}	0.03	0.08	-0.13	0.19	.707
Neighborhood Violence	→	CDI_{T2}	-0.07	0.10	-0.26	0.12	.449
$AUCg_{CORT}$	→	CDI_{T2}	-0.13	0.11	-0.34	0.09	.254
$AUCg_{AA}$	→	CDI_{T2}	0.16	0.08	0.02	0.31	.031
Male	→	$AUCg_{CORT}$	0.13	0.08	-0.04	0.29	.127
Neighborhood Violence	→	$AUCg_{CORT}$	-0.11	0.08	-0.26	0.04	.166
Male	→	$AUCg_{AA}$	0.00	0.07	-0.13	0.14	.966
Neighborhood Violence	→	$AUCg_{AA}$	0.13	0.07	-0.00	0.27	.058

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Table 9. ***Indirect Effects Hypothesis 1a AUCi metric***

Effect					Standardized *Est.*	*SE*	95% CI *LL*	*UL*	*p*
Major Events	→	$AUCi_{CORT}$	→	CDI_{T1}	-0.008	0.013	-0.033	0.017	.543
Daily Hassles	→	$AUCi_{CORT}$	→	CDI_{T1}	-0.003	0.008	-0.019	0.013	.688
Violent Crime Exposure	→	$AUCi_{CORT}$	→	CDI_{T1}	0.000	0.003	-0.006	0.006	.994
Major Events	→	$AUCi_{AA}$	→	CDI_{T1}	-0.007	0.008	-0.022	0.008	.343
Daily Hassles	→	$AUCi_{AA}$	→	CDI_{T1}	0.001	0.003	-0.005	0.007	.760
Violent Crime Exposure	→	$AUCi_{AA}$	→	CDI_{T1}	-0.004	0.006	-0.015	0.008	.553
Major Events	→	$AUCi_{CORT}$	→	CDI_{T2}	-0.014	0.017	-0.047	0.020	.418
Daily Hassles	→	$AUCi_{CORT}$	→	CDI_{T2}	-0.006	0.014	-0.033	0.020	.643
Violent Crime Exposure	→	$AUCi_{CORT}$	→	CDI_{T2}	0.000	0.011	-0.021	0.021	.994
Major Events	→	$AUCi_{AA}$	→	CDI_{T2}	0.011	0.012	-0.013	0.035	.378
Daily Hassles	→	$AUCi_{AA}$	→	CDI_{T2}	-0.004	0.010	-0.024	0.016	.687
Violent Crime Exposure	→	$AUCi_{AA}$	→	CDI_{T2}	0.006	0.009	-0.012	0.024	.533

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Table 10. ***Indirect Effects Hypothesis 1a AUCg metric***

Effect					Standardized *Est.*	*SE*	95% CI *LL*	*UL*	*p*
Major Events	→	$AUCg_{CORT}$	→	CDI_{T1}	-0.010	0.014	-0.038	0.017	.463
Daily Hassles	→	$AUCg_{CORT}$	→	CDI_{T1}	0.001	0.006	-0.010	0.012	.858
Violent Crime Exposure	→	$AUCg_{CORT}$	→	CDI_{T1}	0.007	0.013	-0.018	0.032	.594
Major Events	→	$AUCg_{AA}$	→	CDI_{T1}	-0.020	0.018	-0.056	0.015	.264
Daily Hassles	→	$AUCg_{AA}$	→	CDI_{T1}	0.012	0.012	-0.011	0.036	.299
Violent Crime Exposure	→	$AUCg_{AA}$	→	CDI_{T1}	-0.026	0.018	-0.060	0.009	.145
Major Events	→	$AUCg_{CORT}$	→	CDI_{T2}	-0.014	0.015	-0.043	0.015	.333
Daily Hassles	→	$AUCg_{CORT}$	→	CDI_{T2}	0.007	0.015	-0.023	0.036	.661
Violent Crime Exposure	→	$AUCg_{CORT}$	→	CDI_{T2}	0.013	0.017	-0.019	0.046	.423
Major Events	→	$AUCg_{AA}$	→	CDI_{T2}	0.016	0.014	-0.012	0.044	.267
Daily Hassles	→	$AUCg_{AA}$	→	CDI_{T2}	-0.011	0.012	-0.034	0.012	.345
Violent Crime Exposure	→	$AUCg_{AA}$	→	CDI_{T2}	0.022	0.016	-0.010	0.053	.181

Note. T1 = First data collection time point, T2 = 6-month follow-up. Estimates are standardized.

Hypothesis 2. IBM SPSS Statistical Software version 25 (2017) on a Windows computer was used to test hypothesis 2. To test hypothesis 2, hierarchical multiple regression models were run to test 1) the contribution of stressors in predicting AOCg, and 2) the contribution of AOCg in predicting depression symptoms at times 1 and 2. In the first regression analysis, daily hassles, major events, and violent crime exposure were regressed onto AOCg while controlling for biological sex and age of adolescent. The full model was not statistically significant $R^2 = 0.061$, F (5,107) = 1.382, $p = .237$; adjusted $R^2 = .017$. The addition of violent crime exposure as a predictor for AOCg, however, did result in a significant change in R^2 ($\Delta R^2 = 0.040$, $\Delta F(1,107) = 4.570$, $p = .035$). An increase in violent crime exposure significantly predicted an increase in AA reactivity with respect to ground while controlling for CORT ($\beta = 0.204$, $p = 0.035$). See table 11.

Table 11. ***Hierarchical multiple regression with stressors predicting AOCg at Time 1***

	AOCg							
	Model 1		Model 2		Model 3		Model 4	
Variable	B	*β*	B	*β*	B	*β*	B	*β*
Constant	40.57*		39.77*		39.89*		25.92	
Age	0.04	0.00	-0.18	-0.02	-0.18	-0.02	-4.44	0.01
Biological sex	-4.63	-0.13	-4.61	-0.12	-4.61	-0.12	0.11	-0.12
Daily Hassles			0.14	0.07	0.14	0.08	0.18	0.09
Major Events					-0.11	-0.00	-0.70	-0.03
Violent Crime Exp							0.00*	0.20
R^2	0.016		0.021		0.021		0.061	
F	0.869		0.762		0.567		1.382	
ΔR^2	0.016		0.005		0.000		0.040	
ΔF	0.869		0.555		0.002		4.570*	

Note. N = 180. *p<.05, **p<.001. Square root transformation applied to AOCg and Major Events to adjust for non-normality.

In the next regression analysis, variables were regressed onto adolescent symptoms of depression at time one. Covariates of adolescent depression (age, biological sex, and taking depression medication) were included in step one as control variables. AOCg was added in step two to assess whether the addition of AOCg improved the

explanatory power of the model. The full model two was statistically significant R^2 = 0.141, F (3,124) = 6.205, p = .001; adjusted R^2 = .109. The addition of AOCg as a predictor for adolescent depression symptoms did not, however, result in a significant change in R^2 (ΔR^2 = 0.011, ΔF(1,123) = 1.516, p = .221). Results of the analysis confirmed a positive association between age and adolescent depression symptoms (β = .253, p =.003) and fewer depression symptoms in males (β = -0.225, p = .009). A statistically significant association between AOCg and adolescent depression symptoms at time one was not found (β = -0.104, p = .221). See table 12.

Table 12. ***Hierarchical multiple regression with AOCg predicting adolescent depression sx at Time 1***

	Adolescent Depression Symptoms T1			
	Model 1		Model 2	
Variable	B	*β*	B	*β*
Constant	3.50**		3.58**	
Age	0.04*	0.25	0.04*	0.25
Biological sex	-0.15*	-0.21	-0.16*	-0.23
DepMeds	0.45	0.14	0.44	0.14
AOCg			-0.00	-0.10
R^2	0.131		0.141	
F	6.205*		5.052*	
ΔR^2	0.131		0.011	
ΔF	6.205*		1.516	

Note. N = 180. *p<.05, **p<.001. Square root transformation applied to AOCg and CDI to adjust for non-normality.

The regression analysis run with adolescent depression symptoms at time two as the outcome variable included the same control variables with the addition of depression symptoms at time one. The full model was statistically significant, R^2 = 0.304, F (4, 61) = 5.237, p < .001; adjusted R^2 = .246. The addition of AOCg as a predictor for adolescent depression symptoms at time two did not, however, result in a significant change in R^2 (ΔR^2 = 0.021, ΔF(1, 60) = 5.237, p = .186). Biological sex (β = 0.038, p = .734) and age at time one (β = 0.072, p = .521) did not significantly predict depression symptoms at time 2. Self-reported adolescent depression symptoms at time one

significantly predicted adolescent depression symptoms reported at time 2 (β = 0.491, $p <$ .001). See table 13 for the full details of the regression model.

Table 13. ***Hierarchical multiple regression with AOCg predicting adolescent depression sx at Time 2***

	Adolescent Depression Symptoms T2			
	Model 1		Model 2	
Variable	B	*β*	B	*β*
Constant	-4.95*		-5.51*	
Age	0.04	0.08	0.04	0.07
Biological sex	0.04	0.02	0.09	0.04
$DepMeds_{Time1}$	1.31	0.13	1.31	0.13
CDI_{Time1}	1.54**	0.47	1.59**	0.49
AOCg			0.01	0.15
R^2	0.283		0.304	
F	6.021**		5.237**	
ΔR^2	0.283		0.021	
Δ*F*	6.021**		1.791	

Note. N = 180. *p<.05, **p<.001. Square root transformation applied to AOCg and CDI to adjust for non-normality.

Supplemental Analyses

Follow-up analyses were run to better understand the unexpected findings that: daily hassles, major events, and violent crimes were not significantly associated with stress response reactivity. It was also surprising to find that AOCg was not significantly associated with stressors or depression symptoms in the current sample given previous findings of strong associations among the AOCg metric, a history of chronic stress, and depression symptoms in the Ali & Pruessner (2012) study. As described previously, there is a relative dearth of studies focused on the AA metric in the context of depression symptoms in adolescents. It is possible this gap in the literature is reflective of publication bias. Perhaps these studies have been completed, for example, and did not yield significant results similar to our own study, precluding the results from being published in peer reviewed journals. Contrary to AA, studies examining CORT in relation to stress exposure and depression symptoms in adolescents are more prevalent within the literature, and the findings provide support for significant associations between CORT metrics, stress exposure, and depression symptoms within this developmental age

group. Ali & Pruessner (2012) suggested that the COAg metric allows for the examination of CORT while controlling for AA. Reflection on these points provides good reason to consider the overall CORT output when controlling for AA metric (COAg) in an adolescent sample.

To test these hypothesized interpretations of the unexpected findings, first hierarchical regression analyses were run to test whether daily hassles, major events, and violent crime exposure significantly predict stress response reactivity as measured by COAg. A hierarchical regression model was also run to see whether COAg significantly predicts depression symptoms at time one and time two in our sample. When controlling for participant age and biological sex, violent crime exposure negatively and significantly predicted COAg (β = -0.226, p = 0.019). See table 14.

Table 14. ***Hierarchical multiple regression with stressors predicting COAg at Time 1***

	COAg							
	Model 1		Model 2		Model 3		Model 4	
Variable	B	*β*	B	*β*	B	*β*	B	*β*
Constant	0.03		0.03		0.03		0.05*	
Age	0.00	0.03	0.00	0.04	0.00	0.04	0.00	0.00
Biological sex	0.01	0.13	0.01	0.13	0.01	0.13	0.01	0.12
Daily Hassles			0.00	-0.01	0.00	-0.01	0.00	-0.03
Major Events					0.00	0.00	0.00	0.03
Violent Crime Exp							-0.00*	-0.23
R^2	0.018		0.018		0.018		0.067	
F	1.010		0.672		0.499		1.548	
ΔR^2	0.018		0.000		0.000		0.049	
ΔF	1.010		0.014		0.000		5.659*	

Note. N = 180. *p<.05, **p<.001. Square root transformation applied to COAg and Major Events to adjust for non-normality.

When controlling for age, biological sex, and depression medication, COAg did not significantly predict depression symptoms at time one (β = 0.152, p = .071). COAg did, however, negatively, and significantly predict adolescent depression symptoms at time 2 (β = -0.230, p = .037). See tables 15 and 16.

Table 15. ***Hierarchical multiple regression with COAg predicting adolescent depression sx at Time 1***

	Adolescent Depression Symptoms T1			
	Model 1		Model 2	
Variable	B	*β*	B	*β*
Constant	3.50**		3.44**	
Age	0.04*	-0.21	0.04*	0.25
Biological sex	-0.15*	0.25	-0.16*	-0.23
DepMeds	0.45	0.27	0.45	0.14
COAg			2.29	0.15
R^2	0.131		0.153	
F	6.255*		5.609	
ΔR^2	0.131		0.023	
ΔF	6.255*		3.322	

Note. N = 180. *p<.05, **p<.001. Square root transformation applied to COAg and CDI to adjust for non-normality.

Table 16. ***Hierarchical multiple regression with COAg predicting adolescent depression sx at Time 2***

	Adolescent Depression Symptoms T2			
	Model 1		Model 2	
Variable	B	*β*	B	*β*
Constant	-4.95*		-5.12**	
Age	0.04	0.08	0.04	0.08
Biological sex	0.04*	0.02	0.12	0.05
$DepMeds_{Time1}$	1.31	0.13	1.25	0.12
CDI_{Time1}	1.54**	0.47	1.67**	0.51
COAg			-11.24*	-0.23
R^2	0.283		0.334	
F	6.021**		6.006**	
ΔR^2	0.283		0.050	
ΔF	6.255*		4.545*	

Note. N = 180. *p<.05, **p<.001. Square root transformation applied to COAg and CDI to adjust for non-normality

Another possible explanation for the unexpected results is suggested by the findings that daily hassles and $AUCg_{AA}$ were each significantly associated with adolescent depression symptoms at time one but not with each other. This pattern suggests there may be an additional step missing from the process of daily hassle exposure, to physiological stress response functioning, to depression symptoms. One such potential variable suggested by the literature is coping. Specifically, disengagement coping strategies have been linked to increased physiological arousal (O'Malley & Waters, 2018) and increased depression symptoms over time in adolescence (Seiffge-

Krenke & Klessingere, 2000). Disengagement coping includes the use of avoidant coping strategies, denial, and wishful thinking. Prior research has found disengagement coping partially mediates the relationship between a variety of types of stress exposure and depression symptoms in adolescents (Sontag & Graber, 2010). Further, avoidant coping, denial, and wishful thinking are widely known within clinical settings to allow decreased opportunity for emotion processing, anecdotally increasing the risk for both emotion dysregulation and increased depression symptoms. Therefore, a path model was tested in which disengagement coping mediates the relationship between daily hassle exposure and $AUCg_{AA}$, and daily hassle exposure and adolescent depression at time one. In addition, the model explored whether $AUCg_{AA}$ mediates the relationship between disengagement coping and adolescent depression symptoms. See *Figure 5*.

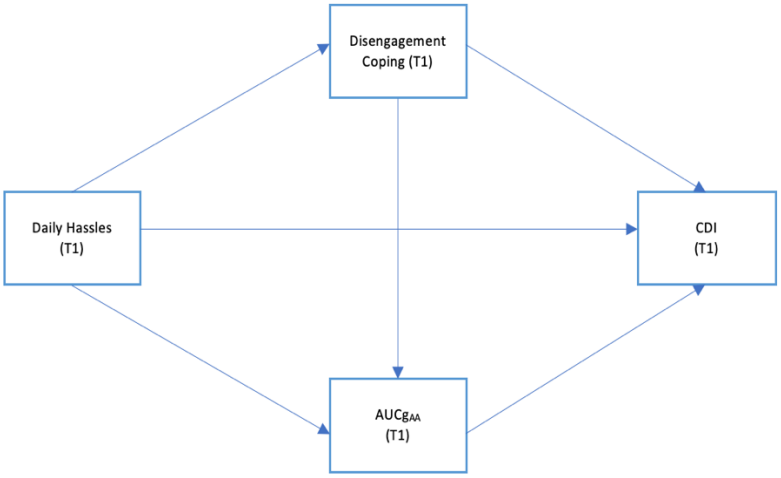

Figure 5. Path model testing role of disengagement coping; *Note.* Only main variables of interest included in visual representation of model for simplicity.

Overall model fit was good as indicated by several fit indices (χ^2 = 11.978 *(p* =.152), $\chi 2/df$ = 1.497, CFI = 0.976, TLI = 0.937, RMSEA = 0.035, SRMR = 0.030). See table 17 for a full list of parameter estimates in the model.

Table 17. ***Parameter estimates for coping model time 1***

Effect			Standardized 95% CI				
			Est.	*SE*	*LL*	*UL*	*p*
Age	→	CDI_{T1}	0.15	0.05	0.05	0.25	.003
Male	→	CDI_{T1}	-0.36	0.10	-0.55	-0.17	<.001
Depression Meds	→	CDI_{T1}	1.17	0.27	0.64	1.70	<.001
Parent Depression	→	CDI_{T1}	0.10	0.05	-0.01	0.20	.080
Daily Hassles	→	CDI_{T1}	0.24	0.05	0.14	0.34	<.001
$AUCg_{AA}$	→	CDI_{T1}	-0.16	0.08	-0.31	-0.00	.044
Disengagement Coping	→	CDI_{T1}	0.22	0.05	0.12	0.32	<.001
Daily Hassles	→	Disengagement Coping	0.28	0.06	0.16	0.39	<.001
Parent Depression	→	Disengagement Coping	0.16	0.07	0.02	0.29	.025
Taking birth control	→	$AUCg_{AA}$	0.86	0.35	0.17	1.56	.014
Parent Depression	→	$AUCg_{AA}$	0.07	0.08	-0.09	0.23	.410
Daily Hassles	→	$AUCg_{AA}$	0.00	0.07	-0.14	0.14	.979
Disengagement Coping	→	$AUCg_{AA}$	-0.24	0.08	-0.38	-0.09	.002

Note. T1 = First data collection time point. Estimates are standardized.

Use of disengagement coping in response to daily hassles at time one significantly predicted adolescent depression symptoms at time one as indicated by significant indirect effects (β = 0.060, *SE* = 0.021, *p* = .004). In addition, use of disengagement coping in response to daily hassles predicted less overall AA reactivity output (β = -0.065, *SE* = 0.024, *p* = .007). These findings suggest disengagement coping significantly mediates the relationship between daily hassles and adolescent depression symptoms and daily hassles and total AA reactivity output. Overall AA reactivity output was not found to mediate the relationship between disengagement coping and adolescent depression symptoms (β = 0.037, *SE* = 0.021, *p* = .074). However, AA reactivity was associated with fewer depression symptoms at time one within the path model (β = -0.157, *p* = .044). See table 18 for a list of indirect effects tested in the model.

Table 18. ***Indirect effects for coping model time 1***

Effect					Standardized 95% CI				
					Est.	*SE*	*LL*	*UL*	*p*
Daily Hassles	→	Disengagement Coping	→	CDI_{T1}	0.060	0.021	0.019	0.101	.004
Daily Hassles	→	$AUCg_{AA}$	→	CDI_{T1}	-0.000	0.011	-0.022	0.021	.979
Daily Hassles	→	Disengagement Coping	→	$AUCg_{AA}$	-0.065	0.024	-0.112	-0.018	.007
Disengagement Coping	→	$AUCg_{AA}$	→	CDI_{T1}	0.037	0.021	-0.004	0.078	.074

Discussion

A large body of research exists on allostatic load in the context of chronic stress exposure. The majority of the literature supports asymmetric profiles across the HPA and SAM axes as a representation of dysregulation in the stress response system. Less understood is the role of stress response asymmetry in the development of psychopathology, especially within adolescent populations. Developmental research has posited hormonal changes that occur within adolescence present an increased risk for the development of psychopathology (Chaby et al., 2017). More specifically, there is an association between hormonal change associated with puberty and depression symptoms in female adolescents (Thapar et al., 2012).

Models of stress and the etiology of psychopathology in adolescents (Grant et al., 2003) posit biopsychosocial processes mediate the relationship between stress exposure and psychopathology outcomes, while genetic risk may moderate this mediated relationship. The present study hypothesized AL as measured by hyperreactivity of CORT and AA, would mediate the relationship between stress exposure and self-reported depression symptoms in an adolescent sample. A main purpose of the current study is to add to the literature on this topic, by examining the relationship of metrics representative of two main systems involved in the physiological stress response, the HPA-axis and SNS. We sought to examine whether hyperreactivity of these systems impacts functioning in the context of specific types of stress and depression symptoms.

Extant literature in adolescent populations suggests that HPA-axis dysregulation, in general, precedes depression in adolescence (Guerry & Hastings, 2011) and more specifically, various studies have supported hyperreactivity of CORT in adolescents to be

associated with depression (Lopez-Duran et al., 2009). To our knowledge, only two studies have examined SNS activity as measured by AA in relation to adolescent depression. One such study compared adolescents with and without a history of trauma exposure and found a negative relationship between resting AA and depression symptoms in both groups, trauma exposed and those with no trauma exposure, but for males only (Vigil et al., 2010). This study, however, included only one saliva sample pre-survey and one saliva sample post survey and did not include any measures of reactivity. A more recent study by Katz and colleagues (2019) found that in a community sample of adolescents, AA reactivity was not significantly related to depression symptoms. Thus, little has been confirmed regarding the relationship between AA reactivity in adolescent depression. The hypothesis of the current study that hyperreactivity of AA would be significantly related to more depression symptoms in adolescence is rather exploratory. It does not escape our attention that this hypothesis contradicts some of the extant findings on the topic. However, many prior animal studies have shown that in response to chronic stress, a process of sensitization can occur within the SNS (McCarty, 2016). When sensitization to stress occurs, the physiological response to a repeated stressful event is heightened. It has been suggested the overall reactivity output may be increased in the context of heightened responding to stress while the reactivity response (base to peak or AUCi) can present as blunted due to a ceiling effect (Funke et al., 2015).

Finally, the current study hypothesized genetic risk would increase the strength of the relationship between AL (as measured by AA and CORT reactivity output) and depression symptoms in adolescents.

Expected Findings

Consistent with what we know about depression rates across biological sex in adolescents, female adolescents in the current study reported significantly more depression symptoms than male adolescents during time one. This difference parallels national prevalence rates of adolescent depression that report a larger percentage of females compared with males develop a major depressive disorder (NIMH, 2019). Literature has suggested these differences may be attributed to the specific hormonal changes in females during adolescence (Thapar et al., 2012).

It was hypothesized that more exposure to specific stressors would be associated with an increase in depression symptoms. The current study found more daily hassle exposure and more major event exposures were significantly associated with an increase in self-reported adolescent depression symptoms. Longitudinal research examining daily hassles in the context of adolescent depression has found increased daily hassle exposure to be associated with a Major Depressive Disorder diagnosis at follow-up. Further, this association was not found to differ by baseline exposure to major negative life events (Asselmann, Wittchen, Lieb, & Beesdo-Baum, 2017). Overall, this information suggests more exposure to both daily hassles and major events may play a significant role in the development of adolescent depression.

Further, it was hypothesized that increased stress exposure would lead to increased reactivity during an acute stressor task, and that this response would indicate hyperreactivity of the stress response system. Broadly, significant relationships between stressors and the physiological stress response were only found for violent crime exposure and this relationship was only significant for the COAg metric. These findings

will be discussed in greater detail in the unexpected findings section as the relationships found did not match our specific hypotheses.

Unexpected Findings

Extending our knowledge of the function of AL in relation to stress and depression.

The findings of the current study did not support AL as a mediator of stress and depression symptoms in our adolescent sample. The current study utilized AUCg and AUCi of AA and CORT to represent AL of the SNS and HPA axis, respectively. Out of the stressors examined in relation to depression symptoms, only daily hassles and major events at time one were significantly related to depression symptoms and only time one depression symptoms. SNS activation in response to an acute stressor task was also significantly related to depression symptoms at time one. However, there was no direct relationship between daily hassles or major events and the SNS response for time one and SNS reactivity did not significantly mediate the relationship between these stressors and depression symptoms at time one. In addition, there were no significant relationships between stressors and HPA axis reactivity as measured by the AUCg and AUCi metrics and depression symptoms at either time one or time two.

It should be noted, in the context of AL, adolescent females in the current study exhibited a blunted CORT reactivity response in comparison to their male counterparts as indicated by the means of individual sample collections two to five during the acute stressor task. Female scores from baseline (sample two) to peak (sample five) sample collections were all significantly lower compared with males. There was no significant difference for the recovery value (sample six). Previous studies have found, in healthy adolescents, there are no significant differences between CORT slope values by

biological sex (Mazurka et al., 2018) suggesting the blunted reactivity for female adolescents in the current study represents dysregulation of HPA axis functioning. There are no significant differences between males and females in the current study on daily hassle, major event, or violent crime exposure. Females, however, reported significantly more depression symptoms than male participants suggesting the possibility that more depression symptoms account for the blunted CORT reactivity response. There were no significant differences, however, on mean values of these individual CORT measures by CDI cut-off scores. It is possible this difference could be accounted for by some other biological or environmental factor not measured in the current study. For example, earlier pubertal development in adolescent females has been found to moderate hyporeactivity of the CORT response in the context of subsequent depression (Colich et al., 2015). Future studies examining CORT should consider including a measure of puberty such as the Tanner Staging questionnaire (Marshall & Tanner, 1968) given development of the HPA-axis is on-going in adolescence and changes in functioning due to development are likely. Additionally, because our acute stressor task was a social stress paradigm, it is possible that a history of repeated exposure to social stress in adolescent females within our sample has resulted in habituation to acute social stressors. Habituation occurs when an individual is exposed to the same type of stressor repeatedly and develops an attenuated physiological response to that stressor over time (McCarty, 2016).

Contrary to what we saw in the individual physiological measurements across the stressor task, there were no significant differences in AUCg or AUCi values for either CORT or AA between males and females. There were also no significant differences on the COAg or AOCg measures between males and females. There were significant

differences on the $AUCg_{AA}$ measure by CDI clinical cut-off score. Adolescents meeting criteria for clinical depression symptoms based on the community cut-off criteria ($\geq$ 19; Kovacs, 2003), had significantly lower $AUCg_{AA}$ values than those who did not meet criteria. This suggests adolescents, in the current study, with clinical levels of depression exhibited a blunted overall AA output in response to the acute stressor task. These findings parallel the significant negative relationship found between $AUCg_{AA}$ and time one self-reported depression symptoms in the path analyses.

Within the existing literature on stress and stress response dysregulation, there has been evidence to support that asymmetry in corresponding CORT and AA metrics indicates dysregulation among the HPA-axis and SNS. Gordis and colleagues (2008) directly tested this hypothesis by comparing a group of youth with a history of maltreatment to a group without maltreatment history. The authors found that within the group of children and adolescents who had experienced maltreatment, individual baseline, base-to-peak, and peak value measurements along with AUCi and AUCg metrics between CORT and AA were not significantly correlated. Within the same study, these values were significantly associated for CORT and AA in children and adolescents with no maltreatment history. The findings of their study provide support for asymmetry as a marker of dysregulation between the HPA axis and SNS in the context of trauma exposure. The current study found asymmetry between baseline, peak, and AUCg metrics for CORT and AA while AUCi and base to peak values were significantly and positively correlated. Therefore, there was symmetry of the HPA axis and SNS response to acute reactivity in our sample of adolescents while baseline, peak values, and overall output in response to an acute stressor task lack symmetry. This suggests dysregulation exists in

basal values of AA and CORT with a likely dampened or heightened output in one of these metrics.

Results of post-hoc analyses designed to better understand the findings suggest another possible way that stress and the physiological stress response may lead to depression symptoms in adolescence. Specifically, it was found that disengagement coping mediates the relationship between daily hassles and depression symptoms such that disengagement coping in the context of daily hassles was associated with more adolescent depression symptoms. Further, disengagement coping mediated the relationship between daily hassle exposure and SNS functioning such that disengagement coping in the context of daily hassles was associated with less AA reactivity. Though SNS reactivity did not mediate the relationship between daily hassles or disengagement coping and depression symptoms, there was a significant and negative association between AA reactivity and depression symptoms at time one.

AA reactivity, stress, and depression.

Taken together, these findings suggest the use of disengagement coping in the context of everyday stress contributes to blunted SNS reactivity as measured by AA. In turn, greater AA reactivity in adolescents is associated with less depression symptoms. One way to interpret this is that normal or greater response of the SNS following daily hassles may be indicative of normal stress response functioning in adolescents and, likewise, increased use of disengagement coping strategies may lead to blunted SNS reactivity in response to daily hassles.

From an evolutionary perspective, it is considered adaptive to have a response to an acute stressful event to effectively cope and proper functioning of the stress response

is needed to achieve this allostasis (McEwen, 1998). Problems arise when the activation of the stress response is prolonged, when activation of the stress response occurs frequently, or when the resources to cope with repeated stressors of the same type are not developed. When any of these situations occur, it can lead to either a dampened or heightened reactivity response to stress over time through failure of the feedback loops involved in the SNS and HPA-axis that shut off the stress response. In the current study, it is our interpretation that higher levels of AA activation represent the normal response to stress while lower levels of AA reactivity represent a dampened response to stress.

The existing literature on AA reactivity in the context of adolescent depression is minimal. A study conducted by Funke and colleagues in 2017 found that in children and adolescents diagnosed with Generalized Anxiety Disorder (GAD) and comorbid depression, AA reactivity as measured by AUCg was significantly higher compared with a healthy control group, indicating more overall AA output during an acute stressor task. The same study found the reactivity response from base levels of AA to peak levels of AA to be blunted in the patient group. Their interpretation of the findings suggested that higher levels of AA output, as measured by AUCg, and lower base to peak reactivity values indicated a ceiling effect and blunted reactivity. To our knowledge, ours is the first study to examine the relationship between AA reactivity as measured by AUCg and depression symptoms alone in a community sample of adolescents. The findings of the current study found similarities to and differences for our urban adolescent sample. Overall AA reactivity output as measured by AUCg was significantly higher in the context of less depression symptoms at time one and significantly higher in the context of more depression symptoms at time two. The findings of the current study suggest the

possibility that a higher SNS response may be protective from depression symptoms in the short-term while posing risk for increased depression symptoms in the long-term.

In the current study, a path model revealed increased use of disengagement coping strategies contributed to a decrease in total AA reactivity output in adolescent participants. This finding complements that of a study completed with a community sample of adolescents that found higher levels of disengagement coping to be associated with lower peak AA levels in response to an acute stressor task (Katz, Peckins, & Lyon, 2019). The negative association we found between AA reactivity and depression symptoms is consistent with findings from the study completed by Vigil and colleagues (2010). In their study, Vigil and colleagues (2010) found a negative relationship between AA and depression symptoms in both males who had been exposed to a natural disaster and subsequent related chronic stressors and those who had not. In the Vigil and colleagues (2010) study, however, this was not true for female participants, for whom the relationship was positively (though not significantly) trended.

A possible interpretation for the change of direction in the relationship between AA and depression symptoms from time one to time two is a failure to habituate to repeated experiences of stress. A failure to adapt to the experience of repeated stress exposure creates an increased risk for the development of depression symptoms through the disruption of complex neurobiological processes (McCarty, 2016). This in turn has an impact on the functioning of brain areas involved with behavioral response to stress. activation of AA may indicate a blunted response, which is associated with more depression. Substantial prior evidence supports this theory (Won & Kim, 2016), suggesting that prolonged SNS activation can lead to inflammation and subsequent

neurodegeneration, making the brain susceptible to depression through altered structure and functioning of areas associated with emotional processing. The process of neurodevelopment is on-going into early adulthood (Benes, 1998) which may make adolescents especially susceptible to altered neurological functioning associated with these processes.

Overall, the findings of the current study contribute to the literature on AA reactivity in relation to depression among adolescents. Though higher levels of AA reactivity were found to be associated with less time one depression symptoms and more depression symptoms at six-month follow-up, AA did not mediate the relationship between specific stressors and depression symptoms in the current sample of adolescents. This suggests that AA plays a different role in the relationship between daily stressors and depression. Some researchers have suggested, for example, that SNS activity may moderate the relationship between HPA axis functioning and depression (Vigil et al., 2010).

CORT reactivity, stress, and depression

While we are traversing a relative frontier by examining AA reactivity in relation to adolescent depression, CORT has been much more widely studied in this context. The most unexpected finding in this study was the lack of association between CORT and depression symptoms. The association between CORT and depression symptoms has been more widely studied in adolescent populations than the association between AA and depression. Variability regarding the relationship between CORT and depression does exist within these studies, though numerous studies examining CORT reactivity in the context of adolescent depression have found that higher CORT reactivity values are

associated with increased depression symptoms (Lopez-Duran et al., 2009). Sometimes, however, this has only been the case in adolescent males (Mazurka et al., 2018) or under more specific conditions such as maltreatment history in the context of mild to moderate depression severity (Harkness et al., 2011).

More recent studies have started to provide additional support for a blunted CORT reactivity response in the context of adolescent depression. For example, a recent study found lower peak CORT values following an acute stressor task to be associated with more depression symptoms in adolescence though they did not find an association between measurements leading up to the peak or in the recovery phase (Katz et al., 2019). One study provided support for a flatter reactivity slope when depression onset occurs shortly after a stressful life event (Mazurka et al., 2016). As noted previously, one likely reason for variability across the existing literature is due to the way CORT reactivity is measured. The reactivity of CORT can be measured in a variety of ways including baseline, peak, reactivity/recovery levels, or with the AUCg and/or AUCi metrics.

The only CORT measurement in the present study significantly related to adolescent depression symptoms was the COAg metric. The COAg metric measures the total CORT output in response to the acute stressor task while controlling for the total AA output. Ratio metrics are widely used in the measurement of other biomarkers representing interrelated physiological systems within biological research (Ali & Pruessner, 2012). To our knowledge, ours is the first study which has examined the COAg metric in an adolescent sample in the context of depression symptoms. Our findings suggest the COAg metric is a more sensitive measure of the relationship between CORT and adolescent depression symptoms given it was the only metric for

which a significant association was identified. This hypothesis needs additional empirical support in future studies focused on the comparison of AUCg and AUCi metrics of AA and CORT alone with the AOCg and COAg metrics.

In addition to measurement differences between this study and others, sample differences might also help explain the different pattern of findings (Harkness et al., 2011). Prior studies examining differences between groups with clinical levels of depression versus healthy controls, for example, have found dissimilarity in CORT functioning between these groups. For instance, Mazurka and colleagues (2018) found female adolescents with clinical levels of depression have significantly blunted CORT reactivity compared with both adolescent females without depression and adolescent males with depression. In addition, they found adolescent males with clinical depression have significantly higher reactivity than adolescent males without depression and adolescent females with depression. Likewise, a meta-analysis conducted by Lopez-Duran and colleagues (2009) found significantly greater CORT reactivity in adolescents with depression compared with healthy controls. However, this information was limited by number of studies (three) looking at acute psychological stressor tasks. The current study included a community sample of adolescents. Though 22.4% of our sample met clinical cut-off criteria for depression symptoms, the numbers were too low to make a comparison between depressed/non depressed groups of adolescents and to compare biological sex differences between depressed/non-depressed groups. Most studies that have found these differences, for example, have compared an age and gender matched healthy control group (e.g. no clinical levels of depression or other mental health concerns) of adolescents with a group of adolescents endorsing clinical levels of

depression. Having equal numbers of participants within the control and depressed groups and controlling for potential comorbidities within participant selection allows for adequate statistical power and more specificity in examining potential group differences.

Another point of interest within the current study was the lack of association between the stressors of major events and violent crimes with the $AUCg_{CORT}$/$AUCi_{CORT}$ metrics. Prior studies have shown significant associations between major events/trauma and stress response functioning (Harkness et al., 2011; Bunea, Szentágotai-Tătar, & Miu, 2017). One goal of the current study was to test the association between stressor type and stress response functioning, given a wide array of studies in the extant literature have assumed the impact of accumulated stress rather than including measures of stress accumulation. The findings of the current study suggest that accumulated major event exposures are not predictive of HPA-axis functioning.

One possible interpretation of this unexpected finding is that focusing on major event exposure presents too broad of a stressor category. For instance, within the measure of major events used for this study, exposure to a variety of types of events were assessed including different types of loss, exposure to parental substance use, intimate partner violence, etc. Some of these events could be considered trauma exposure while others, though they may be large scale events, such as switching schools/moving, would not fit the criteria for a trauma. The specificity of these various stressor types may or may not have more bearing on the connected physiological stress response than the accumulation or total of these. A recent study, for instance found that different types of childhood maltreatment were associated with differential functioning of the HPA axis in adults with MDD and comorbid mental health disorders (Mayer et al., 2020) and that these

differences were responsible for the variability in HPA axis functioning within adults in the study rather than depression. Findings such as these suggest specific types of stress may have a different effect on the HPA-axis such that combining them may mask specific effects.

Of the stressors examined within this study, number of violent crimes by census track was the most specific. Violent crime exposure, in the current adolescent sample, was associated with both total CORT output in response to an acute stressor task when controlling for total AA output (COAg) and total AA output when controlling for CORT (AOCg). Specifically, increased exposure to violent crimes by census track for the current sample of adolescents was associated with lower COAg values and higher AOCg values. This is notable, suggesting that chronic exposure to violent crimes may lead to a blunting of the CORT response and hyperactivation of the AA response. Recent studies provide support for hypocortisolemic profiles in the context of increased community violence exposure (Joos, McDonald, & Wadsworth, 2019) and violence exposure in general (Peckins et al., 2020) in adolescents residing in urban environments. As noted previously, blunted reactivity of the CORT response and overactivation of the AA response is indicative of HPA-axis and SNS dysregulation in the context of chronic exposure to stress and carries with it important neurodevelopmental consequences.

AOCg/COAg metric in an adolescent sample.

Of interest, the associations between CORT and AA reactivity as measured by the COAg/AOCg metrics with stress and depression in the current study varied from those in the study by Ali & Pruessner (2012). Their study with adults found AOCg only but not COAg to be a more sensitive measure to early life adversity exposure and to have a

stronger association with depression symptoms. One possible explanation for the difference between their findings and ours is the type of stress measured. Ali & Pruessner (2012) examined a population of adults falling in low and high groups for early parental care. They examined stress response functioning in the context of exposure to childhood maltreatment and/or neglect and chronic stressors experienced in adulthood across these two groups. Those in the low care group and those with a history of childhood maltreatment and/or neglect had significantly higher levels of AOCg output in response to an acute stressor task. The current study did not assess for childhood maltreatment/neglect specifically, and the only stressor that had a significant association with COAg and AOCg was exposure to community violence. The findings of Ali & Pruessner (2012) suggest the combination of childhood abuse and/or neglect without the proper support of a caregiver, specifically, impacts SNS functioning as measured by AA. The findings from the current study suggest that on-going, systems level stressors like community violence impact both the functioning of the HPA axis as measured by CORT and the SNS as measured by AA. These differences are in line with the discussion in a previous section regarding how specificity of stress may have a differential impact on the functioning of the stress response.

In the context of the current study, blunted activation of the HPA-axis provides risk for potential over-compensation of other biological systems that may increase the risk for inflammation and structural changes within the brain (McEwen, 1998). In addition, the over-activation of SNS as measured by AOCg may indicate sensitization to stress or over activation of the SNS due to failure of the negative feedback loop in the context of low magnitude responding of the HPA-axis. While a change in stress response

activation may be adaptive in the context of repeated exposure to chronic, uncontrollable systems level stress, in the short-term, there are possible long-term consequences to physical and mental health.

Another important difference between the Ali & Pruessner (2012) study and the current study is in the association between the AOCg/COAg metrics and depression symptoms. The current study found COAg to significantly predict adolescent depression symptoms at time 2 data collection while Ali & Pruessner (2012) found AOCg to be significantly and positively correlated with adult depression symptoms with no significant association between COAg and depression symptoms. The current study did not find a significant association between AOCg and adolescent depression symptoms at either time one or time two. There are no other studies that we are aware of, to date, that have investigated the relationship between the COAg metric and depression symptoms in adolescents. However, the negative association between CORT and depression symptoms in the current study is consistent with prior research that found a blunted CORT response to be associated with increased depression symptoms (Mazurka et al., 2018; Zorn et al., 2017; Vigil et al., 2010). These studies utilized various metrics in their measurement of CORT. A meta-analysis conducted by Zorn and colleagues (2017) found participants with a current diagnosis of MDD to have lower AUCi CORT values in comparison with healthy controls, however, they found no significant difference between AUCg CORT among those with MDD and healthy controls. Two of the studies cited above who found lower CORT to be associated with depression have found this to be true, specifically, for females (Zorn et al. 2017; Mazurka et al., 2018), while CORT levels for depressed males were significantly higher compared with healthy controls (Zorn et al., 2017) or with

depressed females (Mazurka et al., 2018). Again, this highlights the important role of gender in the study of HPA activity and depression symptoms found in multiple studies.

Synthesis of the information presented.

The processes of the biological stress response system are complex and given the variability within the literature on the topic, it appears the trajectory to dysregulation and subsequent depression requires multifaceted examination. The current study did not support stress response dysregulation as a mediating pathway to depression symptoms from exposure to daily hassles, major events, and exposure to community violence in adolescence. The current study did, however, support a connection between blunted CORT reactivity through the COAg metric and increased depression symptoms over time. It also did find support for the connection between higher SNS response with more depression symptoms over time. Finally, disengagement coping mediated both the relationship between daily hassles and adolescent depression symptoms as well as daily hassles and the SNS response.

Limitations

Several limitations exist for the current study. First, the data were collected from a community sample limiting the ability to make comparisons between adolescents with clinical levels of depression and those without. Second, the methodology of the current study focused only on reactivity metrics and did not include diurnal patterns of CORT and AA. Third, a follow-up path analysis was conducted with cross-sectional data, all collected at time one and while causal mechanisms were hypothesized, caution should be utilized in their generalization until findings are confirmed with longitudinal data.

As discussed in the above sections, generalization when studying mental health symptoms is limited with community samples. The percentage of adolescents in our

sample to meet cut-off criteria for clinical levels of depression symptoms was higher (22.4%) than overall rates of adolescent depression in the U.S. (13.3%; NIMH, 2020). However, our study design did not allow for the opportunity to make biological sex or other important comparisons across adolescents meeting clinical cut-off criteria for a depressive disorder with those who did not meet criteria for a depressive disorder. Previous research that has done this with adolescent samples have found hypercortisolemic profiles of CORT in depressed males and hypocortisolemic profiles in depressed females compared with adolescents who do not have depression. These studies, however, are limited in number and require further replication.

Differences in hyper- versus hypocortisolemic profiles in adolescent depression have also emerged dependent upon which biomarker metric is being examined. The current study focused only on reactivity metrics in the context of an acute stressor task. It is possible and likely that diurnal CORT and AA profiles in adolescents may provide different information about stress response functioning than reactivity metrics. For example, a diurnal profile of CORT measures the naturally occurring pattern of CORT output throughout the day while reactivity metrics focus on the response and recovery following exposure to an acute stressor. It is possible the diurnal pattern of CORT and the reactivity of CORT in response to an acute stressor function differently from one another in the context of depression symptoms. Future research should focus on identifying whether diurnal and reactivity patterns differ among the same samples and continue to explore why these differences exist, if they do.

The follow-up path analyses exploring the mediation of disengagement coping on daily hassles and $AUCg_{AA}$ and daily hassles and adolescent depression symptoms were

conducted on cross-sectional data. The variables included in the analyses were all from time one. The exposure of daily hassles and reflection on retrospective disengagement coping all occurred prior to the acute stressor task participation and subsequent biomarker data collected during the time one data collection day. However, hypotheses about whether stressors and coping styles preceded depression symptoms, or the other way around cannot be confirmed. Therefore, a longitudinal study measuring daily hassles, use of disengagement coping, stress reactivity data, and depression symptoms across several time points would assist in confirming (or identifying alternatives to) the results found in the current study.

Finally, though the current study attempted to include a variety of specific stress constructs (daily hassles, major events, violent crimes), even more specificity in stressor type could be useful in follow-up studies. These may include, for example, loss, specific types of relational stress, and specific types of trauma. In addition, it would be helpful to identify mediators and moderators of the relationship between specific stressors and depression symptoms in adolescents.

Strengths of the Current Study

A major strength of the current study was a diverse and representative sample of research participants for the geographic location. Adolescents and their parents were recruited from various public schools across the city and represented a wide range of race, ethnicity, and income level. In addition, while many research studies focused on the stress response system have relied primarily on cortisol data alone, the current study utilized salivary cortisol and alpha amylase to better understand the complex interrelationship between the HPA axis and the SNS. Further, the current study focused

on several types of stress - daily hassles, major events, and exposure to community violence –to gain a better understanding of the function of specific stressor types in predicting stress response functioning and depression symptoms in an adolescent population.

Conclusions and Future Directions

While there were several limitations to this study, it also provides important contributions to existing research on stress response functioning in the context of adolescent depression. The current study found adolescents with more exposure to daily hassles reported more disengagement coping and more depression symptoms. Further, utilization of disengagement coping in the context of daily hassle exposure significantly strengthened the relationship between daily hassles and self-reported depression symptoms. In addition, though daily hassle exposure was not directly associated with AA, there was a significant indirect effect of daily hassles on AA reactivity through the use of disengagement coping such that increased use of disengagement coping in response to daily hassle exposure led to a decrease in SNS activation during an acute stressor task. Increased overall AA output in response to an acute stressor task was associated with less self-reported depression symptoms at time one and more depression symptoms at time two, providing support for greater SNS reactivity over time as a risk for increased depression symptoms.

Finally, increased exposure to violent crimes within the community was associated with less overall CORT output and more overall AA output during an acute stressor task at time one. More overall CORT output as measured by COAg was associated with more depression symptoms at time two. This suggests the possibility that

ecologically distal, chronic, uncontrollable stressors may have a larger impact on HPA axis functioning above and beyond acute major stress and minor stressors. Further it suggests a blunted CORT reactivity response is associated with an increase in depression symptoms over time. Of interest, this relationship was only seen when controlling for total AA output. This suggests the metrics we choose to represent stress response functioning in relation to the types of stress we are measuring do matter.

Overall, the current study provided support for the need to continue to think critically about the metrics associated with the stress response within the context of the specific types of stress we are measuring in stress research. It also provided support for the role of stress response functioning in the context of depression symptoms in adolescents. Future studies should continue to explore additional mechanisms through which specific types of stress exposure influence the functioning of the SNS and HPA axis and how the functioning of each relates to depression in adolescence.

The current study found variability of outcomes when measuring the relationships between stress, stress response functioning, and depression symptoms based on which metric was being utilized to measure HPA axis and SNS functioning. Future research should include as many of these metrics (e.g. baseline, reactivity, recovery, AUCg, AUCi, AOCg, COAg, and diurnal rhythms) as possible. This will provide the opportunity for stress response research to further solidify connections between the various metrics of stress response measurement and specific stressor types, psychopathology, differences across the developmental life span, and differences among biological males and females. The stress response system, stress exposure, and psychopathology are all complex. The

first step to piecing together this puzzle requires collecting enough data to identify consistent patterns across these details.

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