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Asymmetry between Behavioral and Physiological Responses to Stress in Children and the Role of Depressive Symptoms

by

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Abstract

Research indicates that asymmetry between behavioral and physiological indicators of stress is associated with impairment in physical and psychological health, which may be particularly problematic for child development. The present study examines the impact of depressive symptoms on the relation between behavioral and physiological responses to stress in a sample of 49 youth (ages 9-12). During exposure to an in-lab psychosocial stressor, two laboratory personnel coded participant stress behaviors. These behaviors were later categorized into factors through an exploratory factor analysis. To assess for reactivity in physiological stress response systems, saliva samples were collected at baseline and following the stressor task. Sympathetic nervous system (SNS) reactivity was indexed by salivary alpha-amylase (sAA). Additionally, participants completed self-report questionnaires to assess for depressive symptoms. The two-way interaction between physical stress behaviors and depressive symptoms accounted for unique variance in sAA reactivity. Results suggest that children with fewer depressive symptoms exhibit greater alignment between behavioral and physiological responses to stress. Future research should continue to examine factors that contribute to behavioral and physiological asymmetry among children. Additionally, research may benefit from the inclusion of indicators of parasympathetic activity.
Asymmetry between Behavioral and Physiological Responses to Stress in Children and the Role of Depressive Symptoms

Healthy individuals evidence symmetry between behavioral and physiological responses to stress, such that increases in physiological arousal are aligned with increases in behavioral arousal (Newton & Contrada, 1992; Derakshan, Eysenck, & Myers, 2007). However, there are subsets of individuals who evidence asymmetry between these domains. The most problematic type of asymmetry is low behavioral distress accompanied by high physiological reactivity (Derakshan et al., 2007). This form of asymmetry may prolong exposure to sympathetic activation of the autonomic nervous system (ANS), which can lead to numerous negative outcomes in mental and physical health (Schumacher, Ströhle, Fydrich, & Kirschbaum, 2013; Marsh, Beauchaine & Williams, 2008; Brosschot & Janssen, 1998). Research indicates that asymmetry may be particularly problematic for children as they are experiencing rapid physical and psychological development (Marsh et al., 2008; Cicchetti & Toth, 2009).

Understanding factors that contribute to behavioral and physiological asymmetry may help to identify children who are at a higher risk for experiencing long-term physical and psychological health problems. While individual and environmental factors have been investigated in relation to behavioral and physiological asymmetry, the contributions of internalizing symptoms are thus far unexamined in the literature. The present study examines depressive symptoms as a moderator of behavioral and physiological asymmetry as research indicates that depression may exert opposing influences on these domains (Morris, Kouros, Fox, Rao & Garber, 2014; Ebata & Moos, 1991; Tanaka et al., 2012; Schumacher, et al. 2013).

The Sympathetic Nervous System

The physiological stress response consists of two distinct but interrelated systems: the
locus ceruleus-norepinephrine/ sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (Chrousos & Gold, 1992). The HPA axis is a slow-acting stress response system, whereas the sympathetic branch of the ANS is a fast-acting system that is responsible for the “fight or flight” response (Rudolph, Troop-Gordon, & Granger, 2011). Activation of the sympathetic nervous system (SNS) affects a number of body systems, causing enhanced cardiovascular tone, respiratory rate, blood flow to skeletal muscles, and elevated blood glucose (Bauer, Quas, & Boyce, 2002).

Salivary biomarkers have become increasingly popular in research, as they provide an accessible, non-invasive means by which to evaluate physiological stress response systems (Nater et al., 2005). In recent years, salivary alpha-amylase (sAA) has emerged as a marker of SNS activity as its secretion is regulated by the sympathetic branch of the autonomic nervous system (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). The measurement of sAA is proposed to reflect stress-related changes in the ANS (Nater et al., 2007) as activation of the ANS results in release of norepinephrine, which elicits the secretion of alpha-amylase by the salivary glands (Veen et al., 2013). Research indicates that sAA levels increase in response to both physical and psychological stress (Nater et al., 2007). In healthy children who experience a psychosocial stressor, there is a marked increase in sAA, followed by a return to baseline levels within 10-30 minutes after removal of the stressor (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Strahler, Mueller, Rosenloecher, Kirschbaum & Rohleder, 2010).

In adults, sAA follows a diurnal profile with a decrease in the first 30 minutes after waking followed by a steady increase during the afternoon and evening (Nater et al., 2007). Children and adolescents follow a similar pattern to adults, with lower levels in the morning followed by a gradual increase in sAA levels throughout the day (Adam, Till Hoyt, & Granger,
2011; Wolf, Nicholls, & Chen, 2008). However, unlike adults, children and adolescents do not seem to experience a significant drop in sAA levels within the first hour post-awakening (Adam et al., 2011; Bright, Frick, Out, & Granger, 2014; Wolf et al., 2008).

The current research on sAA indicates that individual differences in sAA are associated with numerous mental and physical health problems. Specifically, higher sAA reactivity in response to stressors is associated with internalizing symptoms and poor cognitive and academic performance (Granger et al., 2007). Furthermore, higher sAA baseline and reactivity levels are associated with posttraumatic stress disorder, separation anxiety disorder, generalized anxiety disorder, major depressive disorder, and schizophrenia (Schumacher et al., 2013). In addition to mental health problems, higher sAA reactivity is associated with increased health problems including respiratory problems, fatigue, and frequency of illness (Granger et al., 2007). Low sAA reactivity can be equally problematic and is associated with anorexia nervosa, borderline personality disorder, aggressive behaviors, oppositional defiant disorder, and conduct disorder (Schumacher et al., 2013; Granger et al., 2007). Overall, sAA may serve as an indicator for physiological processes that affect and are affected by psychological, behavioral, and social processes (Granger et al., 2007).

**Asymmetry between Behavioral and Physiological Stress Responses**

Emotion is manifested in multiple response domains, including physiological and behavioral reactions (Newton & Contrada, 1992). When presented with stressors, healthy individuals use various sources of internal feedback, such as autonomic responses, to organize their adaptive behavioral responses (Brosschot & Janssen, 1998; Derakshan et al., 2007). However, some individuals exhibit reduced cognitive processing of threatening stimuli, which prevents autonomic responses from being adequately interpreted as threats to psychological well-
being (Brosschot & Janssen, 1998; Coifman, Bonanno, Ray & Gross, 2007). This under-utilization of physiological feedback is reflected in dissociation between emotional, behavioral, and autonomic responses to stressors (Brosschot & Janssen, 1998). When confronted with stressful situations, these individuals show emotional and behavioral signs of self-control, such as social smiles and low levels of negative affect, despite manifesting high autonomic arousal (Contrada, Czarnecki & Pan, 1997; Derakshan et al., 2007). Individuals who exhibit this type of dissociation are termed “repressors” (Brosschot & Janssen, 1998). When examining “repressors” during stressful tasks, research indicates that they have lower emotional responses to stress as compared to non-repressors, whereas their sympathetic activity is similar, or sometimes higher, than that of non-repressors (Brosschot & Janssen, 1998).

“Repressors” evidence asymmetry in the direction of high autonomic arousal accompanied by low behavioral arousal; however, the reverse situation of asymmetry also occurs. These individuals are termed “high-anxious” and exhibit increased negative affect in response to stressors that is not accompanied by commensurate increases in autonomic activity (Newton & Contrada, 1992; Derakshan et al., 2007). Rather than ignore internal states, as is the case for repressors, “high-anxious” individuals attend to their physiological feedback (Newton & Contrada, 1992). While both types of dissociation are likely to be problematic for functioning, the majority of research focuses on the repressor-type, as it seems to exert a greater impact on current and long-term functioning, which will be discussed later in more detail.

The majority of literature on dissociation between behavioral and physiological response systems has been conducted in adult samples. However, research indicates that similar dissociation emerges in children (Marsh et al., 2008). The purpose of the present study is to
expand the literature on dissociation of response systems in children, which is currently sparse and inconclusive.

**Causes of asymmetry.** The research into causes of dissociation between behavioral and physiological responses to stress is quite limited. The most frequently cited cause of behavioral and physiological asymmetry is prolonged exposure to uncontrollable environmental stress (Brosschot & Janssen, 1998; Contrada et al., 1997; Coifman et al., 2007). Efforts to master uncontrollable environments will evoke high sympathetic activity (Contrada et al., 1997). However, individuals who have a high need for self-control, are motivated to display fewer behavioral indicators of stress (Contrada et al., 1997). Thus, creating a situation of high sympathetic arousal while efforts at self-control attenuate increases in stress behaviors (Contrada et al., 1997).

While a high need for self-control may predispose individuals to develop dissociation between systems, other individual-level characteristics may also be involved. Individuals differ in their tolerance of internal distress, which may affect the attention given to physiological indicators of stress and strategies used to manage this stress (Brosschot & Janssen, 1998). Additionally, individuals differ in their desire to create particular impressions on others, with individuals who frequently use impression management strategies at a higher risk of developing dissociation between response systems (Newton & Contrada, 1992). Whereas it is relatively easy for individuals to modify their behavior to fit a desired impression, sympathetic activity is more difficult to modify or control merely due to a desire to remain physiologically unaffected (Newton & Contrada, 1992). Therefore, sympathetic activity remains high while behavioral indicators of stress remain relatively low. Lastly, individuals differ in the degree to which they use external cues to interpret internal sensations (Brosschot & Janssen, 1998). Research indicates
that individuals who direct more attention toward external cues, rather than internal sensations, are more likely to have the repressor-type of dissociation (Brosschot & Janssen, 1998). While not explicitly discussed in the literature, it seems likely that individual differences in brain structure and function may be associated with the attention given to external cues. This speculation stems from research indicating that the anterior cingulate cortex and amygdala play an important role in environmental assessment of threat and selective attention to stimuli (Hammen, Rudolph, & Abaied, 2014, Chapter 5). Therefore, individuals with alternations to these brain regions may be more likely to attend to external stimuli and thereby develop dissociation of response systems.

The existing literature primarily focuses on the separate influences of individual characteristics and environmental factors on asymmetry of response systems. However, developmental psychopathology indicates that processes are reciprocally interactive and that it is the mutual relationship between at least two components of the developmental system that influences developmental organization or disorganization (Cicchetti & Toth, 2009). Therefore, whereas individual characteristics may predispose children to develop dissociation of systems, these characteristics are likely impacted by environmental demands. Specifically, many of these characteristics may develop due to frequent encounters with situations where remaining restrained, unreactive, and giving greater attention to external cues is beneficial (Brosschot & Janssen, 1998). For example, for children growing up in violent neighborhoods, giving greater attention to environmental cues may protect them from adverse experiences. Similarly, children raised in abusive households may develop restrained and unreactive behaviors as a way to escape the notice of abusers. Thus, individual characteristics do not develop in isolation. Similarly, these characteristics may exert influences on environmental situations.

**Role of Depression in Asymmetry**
There is still much that remains unknown about the causes of behavioral and physiological dissociation. Whereas the adult literature has not explicitly examined the role of psychological disorder symptoms in regards to dissociation of systems, the research indicates that personality characteristics and anxiety may play an influential role (Brosschot & Janssen, 1998; Contrada et al., 1997). Little is known currently about the role of psychopathology in the development of behavioral-physiological dissociation in youth.

Until recently, literature has focused exclusively on adult populations. In one of the first studies to examine discrepancies in children, research indicates behavior problems may contribute to this dissociation. Specifically, boys with disruptive behavior disorders exhibit greater behavioral reactivity while simultaneously lower autonomic reactivity to emotion-eliciting stimuli (Marsh et al., 2008). This research with externalizing disorders raises the question as to the role of internalizing disorders in the dissociation of response systems in children. In adults, research has noted dissociation of emotion response systems in individuals with depression (Sloan, Strauss, Quirk, & Sajatovic, 1997). Furthermore, depression is characterized by poor emotion regulation and low emotional expressiveness (Marsh et al., 2008), both of which occur in individuals with asymmetry between behavioral and physiological indicators of stress. Finally, research indicates that depression differentially impacts physiological systems and behavioral responses to stress (Tanaka et al., 2012; Schumacher et al., 2013; Morris et al., 2014), thus lending further support for the role of depressive symptoms in dissociation of response systems.

In children, depression can begin as early as ages 2-3 and is most frequently cited in relation to adverse environments and chronic and/or acute stressors (Hankin, 2015). However, there are numerous other biological, emotional, cognitive, and interpersonal causes of early-onset
depression (Hammen, Rudolph, & Abaied, 2014, Chapter 5). Biological and prenatal conditions are thought to be some of the strongest predictors of depression in young children (Hammen et al., 2014, p. 233; Hankin, 2015). Depression during early childhood is problematic for daily functioning as well as long-term functioning. Early-onset depression often results in a reoccurrence of depressive symptoms into adolescence and adulthood (Kovacs, 1996). While reoccurrence of depressive episodes is the primary outcome of childhood depression, there is also an increased risk for other psychological disorders. Research indicates that early childhood major depressive disorder (MDD) increases the risk for anxiety disorders and ADHD in later childhood and adolescence (Hankin, 2015). Additionally, early-onset depression is more problematic for long-term functioning, as compared to later-onset depression, as it interferes with critical developmental periods in childhood (Kovacs, Obrosky, & George, 2016). Specifically, children with prepubertal-onset MDD are at an increased risk for developing bipolar 1 disorder and antisocial personality disorder as adults (Weissman et al., 1999). Additionally, childhood depression increases the risk for later development of substance use disorders (Rao & Chen, 2009). Thus, childhood depression can impact numerous areas of functioning and early intervention is key to minimizing impairment during childhood, adolescence, and adulthood.

**Depression and salivary alpha-amylase.** Researchers theorizing about the relation between sAA and depression draw heavily from the adult literature, as the studies among children and adolescents are limited. In a review article on the relation between sAA and psychological disorders, Schumacher et al (2013) found that individuals with psychological disorders show different patterns and/or levels of sAA following stressful experiences. As compared to healthy controls, depressed individuals exhibit higher levels of sAA before, during, and after stressful situations (Tanaka et al., 2012; Schumacher et al., 2013). These results are
supported by other studies, which find similarly elevated sAA levels among individuals with depressive disorders (Veith et al., 1994; Ishitobi, 2010; Vigil, Geary, Granger & Flinn, 2010, Rudolph et al., 2011). Furthermore, research indicates that prolonged exposure to stressful situations suppresses the sAA “fight-or-flight” response in healthy controls, whereas individuals with depression experience no suppression of this response (Tanaka et al., 2012). This finding suggests a preservation of physiological responsiveness in individuals with depression (Tanaka et al., 2012). While the relation between depressive symptoms and sAA seems to be consistent across studies, the directionality of the relationship remains unclear. Hill-Soderlund and colleagues (2015) suggest that high sAA levels contribute to the maintenance of depression and may predict the development of other mental health disorders. Based on the existing research, it seems likely sAA and depression are related, and that bi-directional effects may occur.

**Depression and behavioral observations.** The literature on the relationship between depressive symptoms and behavioral observations of stress indicates that observers often have greater difficulty correctly identifying depressive symptoms, as compared to other psychological symptoms, in children. Specifically, in comparison to externalizing disorders, parents and other observers tend to have more difficulties recognizing the distress caused by internalizing symptoms in children (Bein et al., 2015). Despite some children projecting less outward stress in response to internalizing symptoms, these children still experience significant internal distress (Bein et al., 2015). Difficulties in recognizing internalizing symptoms likely result because, during times of stress, depressed children can become more avoidant or disengaged rather than outwardly distressed by the situation, as may be the case for children with externalizing disorders (Morris et al., 2014; Ebata & Moos, 1991). Furthermore, externalizing symptoms may be more
distressing for parents and thereby increase awareness of symptoms and increase the likelihood of treatment-seeking (Duchovic, Gerkensmeyer, & Wu, 2009).

The above findings indicate that depression may exert opposing influences on behavioral and physiological systems during times of stress, such that sAA reactivity increases while behavioral reactivity decreases. The opposing influence of depressive symptoms suggests that it may contribute to the dissociation of response systems. Furthermore, depressive symptoms contribute to a preservation of sympathetic activity, which is associated with the repressor-type of dissociation. However, the role of depression in relation to behavioral and physiological dissociation is currently unexamined in the literature. Therefore, the present study expands the literature on dissociation of response systems in children to include an examination of the role of depressive symptoms.

Implications of Behavioral and Physiological Asymmetry

Research indicates that synchronization of behavioral, affective, and physiological emotional response systems is critical for emotional health (Marsh et al., 2008). Therefore, misalignment between these systems can have numerous implications for functioning. The greatest risk posed by desynchronization of these systems is the emergence and maintenance of physical and psychological disorders (Marsh et al., 2008). While both “repressor” and “high-anxious” types of dissociation pose a significant risk for mental and physical health, the repressor-type may exert a greater influence on both current and long-term functioning (Derakshan et al., 2007; Brosschot & Janssen, 1998). This difference seems to arise due to “repressors” avoidance of physiological distress cues (Brosschot & Janssen, 1998). This neglect of autonomic activity may result in failed cognitive or behavioral attempts to cope with stressors, which then leads to persistence, and perhaps increases, in autonomic reactivity (Brosschot &
“High-anxious” individuals experience similar autonomic arousal, although perhaps not to the same degree; however, they attend to internal feedback and enact strategies to manage the stress and thereby prevent prolonged exposure to high autonomic activity (Brosschot & Janssen, 1998).

As previously discussed, prolonged sympathetic activity is associated with psychological, physical, and social problems (Schumacher et al., 2013; Granger et al., 2007). For children, prolonged exposure to sympathetic activity during this early period of growth and development may have long-lasting consequences for functioning. Chronic sympathetic activation is associated with heart and other organ damage, insulin resistance, hypertension, atherosclerosis, obesity, altered cellular functioning, and increased disease risk (Oparil, Zaman, & Calhoun, 2003; Parati & Esler, 2012; Fisher, Young, & Fadel, 2009; Qi & Ding, 2016; Brosschot & Janssen, 1998). Developmental psychopathology indicates that alterations to brain or physiological development can trigger a cascade of growth and function changes that lead an individual to developing enduring forms of mental and physical health problems (Cicchetti & Toth, 2009). Therefore, alterations to development at this early stage due to prolonged sympathetic activity may create numerous, long-lasting problems.

In addition to impacting physical development, dissociation of behavioral and physiological response systems can also impact social functioning. This dissociation impacts emotion regulation abilities, specifically constricted emotions for “repressors” and excessive emotional reactivity for “high-anxious” individuals (Coifman et al., 2007). This emotion dysregulation may in turn impact children’s social functioning and lead to isolation and peer rejection (Kim & Cicchetti, 2010). Additionally, emotion dysregulation contributes to later
psychopathology in the form of internalizing and externalizing disorders, which have emotion
dysregulation as a core feature (Kim & Cicchetti, 2010).

Asymmetry between behavioral and physiological systems may also place children at an
increased risk for experiencing stressors. Sympathetic activity of the autonomic nervous system
serves as an alert for the presence of threats (Bauer et al., 2002). Failure to attend to these
internal cues may prevent individuals from responding appropriately to threats in their
environment and ultimately place them at risk for trauma (Brosschot & Janssen, 1998).
Therefore, “repressors” are at the greatest risk for stress and trauma due to their failure to attend
to internal cues. However, the repressor-type of dissociation may not be entirely harmful in
stressful environments. Research indicates that this style may help to prevent children from
dwelling on the negative aspects of stressful situations and to remember fewer negative events
(Coifman et al., 2007). Additionally, restraining from behavioral reactivity in volatile
environments may be protective for children (Cicchetti & Toth, 2009). Whereas the repressor-
type of dissociation may have some benefits in the short-term for individuals dealing with
chronic stress, it is not likely to be beneficial for long-term functioning due to the previously
discussed impact on physical and psychological health.

Finally, asymmetry between behavioral and physiological indicators of stress may
prevent caregivers and healthcare providers from recognizing children who are experiencing
difficulties with stress management (Derakshan et al., 2007). Among “repressors,” who project
low levels of behavioral distress, caregivers or healthcare providers may be unaware of the
internal distress that is occurring and therefore not actively seeking interventions to help children
address their physiological stress. In comparison to adults, children may be particularly
vulnerable to prolonged dissociation between systems that goes untreated as they are completely
reliant upon parents to access intervention services. Therefore, it is left up to parents to recognize a problem and pursue treatment. However, research indicates that, for certain psychological disorders, namely internalizing disorders, observers have difficulties correctly identifying problematic behaviors as indicative of disorders (Bein et al., 2015). Therefore, in the case of children with internalizing disorders, there may actually be dual factors impacting detection of problems: difficulties recognizing internalizing symptoms and repressor-type dissociation.

While this inability to detect symptoms may not ultimately prevent treatment, it may significantly delay the time before treatment occurs (Contrada et al., 1997), which is problematic because research indicates that early identification and treatment of mental health problems in children is important to prevent persistence of problems into adulthood (van de Looij-Jansen et al., 2011). Furthermore, asymmetry between behavioral and physiological systems may impact the course of treatment by delaying diagnosis, skewing symptom reports, affecting physician judgments, and negatively impacting physician-patient relationships (Derakshan et al., 2007). Additionally, parent accuracy in recognizing and reporting symptoms is particularly important, as research indicates that clinicians and healthcare providers often place more importance on parental reports than child reports, particularly with younger children (Guyatt & Juniper, 1997; Grills & Ollendick, 2003; Helm, 2011). However, parents are unlikely to be accurate in reporting in the case of “repressors”, who consistently alter their behavior to appear unaffected to stressors.

**Aims of the Present Study**

Overall, research indicates that asymmetry between behavioral and physiological responses to stressors contributes to numerous negative physical, psychological, and social outcomes that may extend beyond childhood. Additionally, this discrepancy may prevent detection and treatment of psychological disorders. Research in this area is quite limited and has
typically concentrated on adult populations. To my knowledge, this is only the second study to investigate asymmetry between behavioral and physiological responses to stressors in children. Additionally, while existing research suggests depressive symptoms may impact the relation between behavioral and physiological response systems, there have been no studies explicating examining this relationship among children.

The purpose of this study is to examine the relation between sAA reactivity and observer-reported behavioral responses to stress. Furthermore, depressive symptoms are examined as a potential moderator of this relationship. I hypothesize that depression will moderate the relationship between observed stress behaviors and sAA reactivity. Specifically, I hypothesize that individuals with lower levels of depression will exhibit greater symmetry between their observer-rated stress behaviors and sAA reactivity, reflected by a positive relationship between behaviors and physiology. Additionally, I hypothesize that individuals with higher levels of depression will exhibit greater asymmetry between stress behaviors and sAA reactivity, as evidenced by no significant relationship between behaviors and physiology. The goal of this study is to extend the research on dissociation between subjective and objective measures of distress in children. Furthermore, this study is a first step toward examining moderators of this dissociation.

Methods

Participants

As part of a larger study examining community violence exposure and responses to stress in a sample of urban at-risk youth, 51 participants between the ages of 9-12 years old were recruited from one Boys and Girls Club in New York. The youth who participated in this study were recruited from Schenectady, NY, a city with high rates of crime (In 2014, Schenectady
County had the highest rate of index crimes in the state of New York; New York State Division of Criminal Justice Services, 2011) and poverty (22.5% of residents live below the poverty line; U.S. Census Bureau, 2012) allowing us to focus on an understudied population of youth at-risk for developing mental health disorders.

The current study examined data available from 49 youth. The remaining participants were excluded from analysis due to lack of child assent and parental consent. The average age of participants included in the analysis was 10.7 years (range = 9-12, SD = 1.01) and approximately half (51.0%) were female. Ethnic and racial composition was 38.8% African-American, 34.7% biracial, 12.2% Caucasian, 4.1% Hispanic or Latino, and 10.2% mixed race or “other” ethnic background.

**Procedures**

All procedures used in this study were approved by the University at Albany Institutional Review Board (IRB). After obtaining parental consent and child assent, youth were asked to provide a baseline saliva sample via passive drool. This was followed by a 5-minute relaxation task where participants viewed a tranquil beach scene and listened to sounds of waves. Following the relaxation task, youth were asked to provide another saliva sample before beginning the serial subtraction subtest of the Trier Social Stress Task (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). While participating in this task, participant stress behaviors were observed and coded by two research assistants. Saliva samples continued to be collected at 5, 10, 20, and 30 minutes following the serial subtraction task, during which time the participants completed self-report measures. Depending on the reading and comprehension abilities of the participants, questionnaires were either read aloud by a research assistant or the child was asked to read the questions aloud to the researcher. To ensure confidentiality and prevent possible
influence on participant responses, precautions were taken to ensure that the research personnel were not aware of the responses given by the participants. Following data collection procedures, participants were debriefed and compensated for their time with a snack, drink, and five dollars.

**Measures**

**Demographics.** Participant demographics were collected through a self-report measure, which assessed for ethnicity (based on current US census categories), date of birth, sex, grade in school, medication use, cigarette use, and, for female youth, whether they had reached menarche.

**Salivary alpha-amylase/autonomic activity.** Participants provided a total of six saliva samples: one immediately following consent and assent (baseline), one prior to the TSST immediately following the relaxation task (pre-task), and four following the TSST at 5, 10, 20 and 30 minutes after the task. These time points were chosen to ensure that peak reactivity to the stressful task (serial subtraction task) would be captured. Activity of the sympathetic nervous system, as measured by sAA, occurs within 5 minutes following the task (Nater et al., 2005). To reduce the effects of changes in the diurnal pattern of sAA, all data were collected from participants between the hours of 2:00 PM-6:00 PM (Nater et al., 2007). On average, the pre-task sample was collected at 2:19 pm (SD = 33.87 min).

Saliva samples were collected via passive drool into a 2 mL cryogenic vial. This method was selected because it does not artificially inflate levels of biomarkers, which can occur when using cotton materials to stimulate saliva production (Shirtcliff, Granger, Schwartz, & Curran, 2001). Samples were frozen at −20 C until shipped to the Institute for Interdisciplinary Salivary Bioscience Research (IISBR) at Arizona State University (Salimetrics, LLC) for immunoassay. The assay measures amylolytic activity using a chromagenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose (Gordis, Granger, Susman, & Trickett, 2008). The enzymatic action of sAA
on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm (Gordis et al., 2008). The amount of sAA activity present in the sample is directly proportional to the increase (over a 2 minute period) in absorbance at 405 nm (Gordis et al., 2008). Results are computed in U/mL of sAA using the formula: 

\[
\text{Absorbance difference per minute} \times \text{total assay volume (328 ml)} \times \text{dilution factor (200)} / \text{millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)} \times \text{sample volume (.008 ml)} \times \text{light path (.97)}
\]

(Gordis et al., 2008). All sAA samples were assayed in singlet.

Since multiple saliva samples were collected, formulas outlined by Pruessner, Kirschbaum, Meinschmidt, & Hellhammer (2003) were used to calculate area under the curve (AUC) scores. Both area under the curve with respect to ground (AUC\textsubscript{G}) and increase (AUC\textsubscript{I}) scores were calculated. AUC\textsubscript{G} describes the change in sAA concentration over time and the level at which the concentrations changes, whereas AUC\textsubscript{I} describes the specific sAA response to the stressor. The formulas for AUC\textsubscript{G} and AUC\textsubscript{I} calculation can be found in Figure 1. The first saliva sample was removed from analysis in order to establish a more reliable baseline. Since this sample was collected immediately upon arrival, it is not possible to account for external factors that may have influenced the sAA levels prior to the start of the study. Researchers have found that mood prior to saliva collection can influence levels of salivary alpha-amylase (Nater et al., 2007). Therefore, the first saliva sample was removed from analysis and the post-relaxation sample was used as a baseline. Salivary alpha-amylase was log transformed to address positive skew.

**Trier Social Stress Test (TSST).** The Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) is a widely used protocol designed to induce psychological stress in a laboratory setting. The TSST consists of two separate tasks: a public speaking task and a serial
subtraction task. This protocol has been found to induce changes in SNS activity, as measured by sAA activity (Nater et al., 2005).

For the purposes of this study, only the serial subtraction task was included in the procedures. The public speaking task was not included in order to shorten the duration of the study and not unduly stress the young participants. During the serial subtraction task, youth were instructed to serially subtract the number 7 starting with 756 for four minutes. If the participants provided an incorrect number, they were asked to start again from the beginning until the four-minute time had elapsed. To further ensure that the task induced a stress response, two research assistants acted as judges, and the participants were informed that these judges were judging them on their task performance. In actuality, the judges were coding for participant stress behaviors rather than participant performance.

**Behavioral coding of stress.** Participant stress behaviors were observed and coded during administration of the TSST using a behavioral coding measure. This measure was designed to assess participants’ behavioral responses to stress. This measure assessed for a total of fourteen, a priori identified, continuous and discrete stress behaviors. Continuous events were assessed using a Likert scale where judges rated participants from 0 to 2 (0-None, 1-A little, and 2-A lot) based on how often they exhibited the stress behaviors of fidgeting, shuffling feet, shaking, touching their face, turning red, nervous laughter/smiles, and becoming quiet/whispering. Discrete events were measured through a tally count of how often the participants engaged in a specified stress behavior. These behaviors consisted of task-related talk, task-deprecating talk, self-deprecating talk, licking lips, deep sighs, unrelated conversation, and crying. Prior to data collection, all research assistants were thoroughly trained on the behavioral coding measure to ensure acceptable inter-rater reliability. During data collection, two
judges rated each participant and the average of the two ratings was calculated and included in analyses. In the study sample, this coding measure showed acceptable inter-rater reliability as measured through intraclass correlation with the continuous events at an \( ICC = .734 \) and the discrete events at an \( ICC = .724 \).

To reduce the large number of individual behaviors into meaningful groups, exploratory factor analysis (EFA) with maximum likelihood extraction and varimax rotation was performed on items from the behavioral coding measure. Prior to the EFA, two behaviors from the behavioral coding measure, unrelated conversation and crying, were removed from the analysis due to infrequent coding. Per convention, coefficients less than .32 were suppressed in the analysis (Tabachnick & Fidell, 2001). Two factors were extracted in the EFA and the final factor structure is presented in Table 1. Several stress behaviors were excluded from the final factor structure due to failure to load onto any factor in the analysis. The two-factor structure explained 45.5% of the variance in the sample. The first factor is labeled verbal stress (e.g., self-deprecating, task-deprecating, task-related talk), and the second factor is physical stress (e.g., turning red, touching face/hair, nervous laughter, shaking, and fidgeting). Both the physical and verbal stress behavior factors were log transformed to address positive skew.

**Depression.** Participant depression symptoms were measured through the Center for Epidemiologic Studies Depression Scale for Children (CESD-C; Weissman, Orvaschel, & Padian, 1980), which is a widely used child self-report measure of depression. This 20-item measure asked participants to report on how they have been feeling and acting in the past week. For each item, participants responded on a 4-point scale where not at all = 0, a little = 1, some = 2, and a lot = 3. Participant item responses were summed and a total score was calculated, with total scores ranging from 0-55 in this sample. Higher scores indicate increased depression.
severity. The CESD-C has good internal consistency in the general population, although there is variability across studies ($\alpha = .77$ to .91; Barkmann, Erhart, & Schulte-Markwort, 2008). In the study sample, the internal consistency was $\alpha = .907$, and 76% of participants had scores above the clinical cutoff score of 9, with 57% of participants in the moderately to severely depressed range. While the depression rates found in this sample are surprisingly high, this sample was collected in an area with high rates of community violence, poverty, and crime, which can increase children’s risk for mental health disorders (Leventhal & Brooks-Gunn, 2000; Fowler, Tompsett, Braciszewski, Jacques-Tiura, & Baltes, 2009). Additionally, other studies among children exposed to chronic stressors, community violence, and wartime violence have found similarly high rates, ranging from 43-63 percent (Beardslee, Versage, & Gladstone, 1998; Attanayake et al., 2009; Berthold, 2000). The CES-DC variable was square root transformed to address positive skew.

**Statistical Methods**

All statistical analyses were conducted using the SPSS 21.0 software (SPSS, Inc., Chicago, IL). All analyses include the log transformed behavioral coding factors and sAA variables and square root transformed depression variable. Transformed variables were centered on the mean in order to reduce multicollinearity and aid interpretation (Rosenthal & Rosnow, 2008).

**Regression analyses.** Regression analyses examined the main and interactive effects of observed stress behaviors and depression symptoms on salivary alpha-amylase ($\text{AUC}_G$ and $\text{AUC}_I$). Separate models were examined for both behavior factors and for both area under the curve scores, yielding four models of equations. The first two models examined sAA ($\text{AUC}_G$ and $\text{AUC}_I$) as a function of depression symptoms and observed physical stress behaviors. The second
two models examined sAA (AUC_G and AUC_I) as a function of depression symptoms and observed verbal stress behaviors. All regression analyses controlled for time of first saliva collection, sex, and age due to longstanding literature indicating differences in sAA on these factors (Nater et al., 2007). Significant interactions were probed according to procedures described by Aiken and West (1991) to facilitate interpretation.

**Results**

**Descriptive Information and Correlations Among Depression, Stress Behaviors Factors, and sAA**

Descriptive data for all raw and transformed study variables appear in Table 2. Bivariate correlations examining relations among depression, observer-rated stress behaviors, and sAA appear in Table 3. Analyses revealed that there was a significant positive correlation between the verbal stress behaviors factor and the physical stress behaviors factor. There were no other significant correlations.

**Regression Analyses Predicting sAA From Physical Stress Behaviors Factor**

Regression analysis examining main and interactive effects of observer-coded physical stress behaviors and depression on sAA (AUC_G) was not significant (Table 4). Although the overall equation was not significant, the two-way interaction between the physical stress factor and depression was significant ($B = .205, t = 2.114, p < .05$; see Table 4). Follow-up probes of this interaction according to the procedures described by Aiken and West (1991) revealed that, at depression values 1 SD below the mean, the slope of the relation between observed physical stress behaviors and sAA (AUC_G) was positive and significant ($B = .576, t = 2.529, p < .05$). At values of depression 1 SD above the mean, the relation between observed physical stress behaviors and sAA (AUC_G) was not significant ($B = -.081, t = -.440, p = .662$). Results from
slope difference tests are graphed in Figure 2. Regression analysis examining the main and interactive effects of physical stress behaviors and depression on sAA (AUCᵢ) was also examined and the results were not significant ($R^2 = .179, p = .206$)

**Regression analyses predicting salivary alpha-amylase from verbal stress behaviors factor**

Regression analysis examining main and interactive effects of observer coded verbal stress behaviors and depression on sAA (AUCᵢ) was not significant (Table 4). Additionally, the two-way interaction between verbal stress behaviors and depression was not significant ($B = .010, t = .071, p = .944$; see Table 4). Regression analysis examining the main and interactive effects of verbal stress behaviors and depression on sAA (AUCᵢ) was also examined and the results were not significant ($R^2 = .124, p = .461$).

**Discussion**

The present study examined the role of depressive symptoms in the relation between behavioral and physiological measures of stress in a sample of youth aged 9-12 years old. Results indicate a behavior-specific pattern in the moderation effect of depressive symptoms on the relations between behavioral and physiological measures of stress responses. Specifically, for physical stress behaviors, greater symmetry between behavioral and physiological stress responses was observed at lower levels of depressive symptoms. For verbal stress behaviors, the interaction with depressive symptoms did not emerge as a significant predictor of sAA levels.

For the physical stress behaviors, the regression equation was not significant, but the two-way interaction between observed behaviors and depressive symptoms emerged as a significant predictor of sAA reactivity levels and was therefore interpreted. Examination of the simple slope analysis revealed that, at lower levels of depressive symptoms, the relationship between physical stress behaviors and sAA reactivity was significant and positive. Thus, in the absence of
depressive symptoms, youth exhibit more congruence between behavioral and physiological measures of stress. This finding aligns with hypotheses and existing research, which indicates that healthy children exhibit commensurate increases in behavioral and physiological arousal in response to stressors (Gunnar, 1992; Granger et al., 2007; Strahler et al., 2010). The simple slope analysis for high levels of depressive symptoms was non-significant.

Taken together, these findings align with predictions and indicate that fewer depressive symptoms result in greater symmetry between behavioral and physiological indicators of stress. Symmetry between behavioral and physiological stress responses predicts better physical and psychological health outcomes, as compared to asymmetry in these systems (Brosschot & Janssen, 1998; Coifman et al., 2007; Derakshan et al., 2007). Additionally, these results indicate that depressive symptoms make important contributions to asymmetry in behavioral and physiological response systems. This asymmetry is in turn associated with impaired physical and psychological development in children (Oparil et al., 2003, Parati & Esler, 2012; Fisher et al., 2009; Qi & Ding, 2016; Brosschot & Janssen, 1998).

In regard to the verbal stress factor, the two-way interaction between observed behaviors and depressive symptoms did not significantly predict salivary alpha-amylase. One possible explanation for this result is that fewer verbal behaviors were observed in the study as compared to physical behaviors. As shown in Table 2, the mean number of verbal behaviors was 1.84 compared to 5.31 for physical behaviors. Furthermore, 28.6% of participants in the sample exhibited zero verbal stress behaviors during the TSST, compared to only 2% of participants for physical stress behaviors. These low rates of verbal stress behaviors may result because children with depression become reserved during times of stress (Morris et al., 2014; Ebata & Moos, 1991) and, rather than verbalize their stress, they will engage in behaviors such as turning red,
fidgeting, or shaking, which were associated with the physical stress factor in this study. Additionally, the TSST math task required participants to serially subtract numbers aloud, which may have constrained their verbal stress behaviors.

The present results should be interpreted in light of the sample composition. As previously discussed, the depression rates were considerably higher (57%) when compared to rates in the general population (1-3% in children between ages 7-12; Hammen et al., 2014, p. 232). However, these depression rates are not abnormal when compared to other child and adolescent samples with similarly high rates of violence exposure (43-63%; Beardslee et al., 1998; Attanayake et al., 2009; Berthold, 2000). In the present sample, more than half of the children reported having been punched or slapped, having had a bottle or hard object thrown at them, having witnessed gunshots, and having witnessed someone being chased by gangs. Additionally, more than a third of the sample reported having seen someone carrying a gun/weapon, being forced to do something with threat of violence, being chased by gangs, and been threatened with bodily harm. Finally, a quarter of the sample had been attacked with a bat or stick. The high rates of depressive symptoms coupled with the high rates of community violence likely impacted the results in this study. Therefore, these findings may not generalize to other samples with lower rates of depression and violence.

There are several limitations to consider when interpreting these results. Saliva samples may have been impacted by individual-level factors that were outside of the experimenter’s control, including time of day and time since awakening. We collected all saliva samples between the hours of 2:00-6:00PM; however, some variability still remained across participants. Additionally, dietary or lifestyle factors may have affected the sAA levels in this sample. Prior research indicates that caffeine, high amounts of carbohydrates, certain medications, and exercise
can increase sAA activity (Nater & Rohleder, 2009). While participants were instructed to refrain from eating or drinking during the period between the end of school and beginning of data collection, it is possible that some participants did not adhere to these instructions. Another limitation is the small sample size and the reliance on self-report questionnaires for several key variables.

**Clinical Implications**

Despite the above limitations, this study has notable strengths. First, this study incorporated multiple methods of reporting (i.e. self-report, behavioral coding, and physiological biomarkers), rather than relying solely on self-report data. Additionally, the present study contributes to an area of research that is relatively unexamined. The results of this study extend the findings of Marsh et al (2008) to indicate that, in addition to externalizing symptoms, internalizing symptoms also significantly impact the symmetry between response systems. To my knowledge, this is the first study to find evidence of depressive symptoms moderating the symmetry between behavioral and physiological stress responses. While the negative effects of childhood depression are well understood, the present study indicates that asymmetry of behavioral and physiological stress response systems may present a new area of functioning that is impaired by childhood depression. Furthermore, the results indicate that asymmetry between behavioral and physiological systems is a domain that needs to be addressed during interventions to prevent damage to bodily systems and psychological wellbeing. Understanding the relation between behavioral and physiological stress responses as well as the moderators of this relationship will help to identify children at risk for long-term impairments in physical and psychological functioning. Finally, research in this area highlights the importance of using
multiple methods of reporting in assessments, as behavioral observations may not always be indicative of physiological responses.

Future work should continue to examine factors that contribute to behavioral and physiological asymmetry in children. Specifically, longitudinal research should examine children starting at an earlier stage of development to determine the onset of behavioral and physiological asymmetry. Additionally, future research should consider including parasympathetic nervous system activity to examine its relation with stress behaviors and psychological disorder symptoms. Finally, interventions that promote alignment between behavioral and physiological stress responses should be a focus of research and clinical practice.
References


doi:10.1016/j.biopsycho.2007.12.004
### Table 1

*Exploratory Factor Analysis Structure of the Observational Behavior Coding*

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Discrete Behaviors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Licking lips</td>
<td>0.062</td>
<td>0.017</td>
</tr>
<tr>
<td>Deep sigh</td>
<td>0.206</td>
<td>0.206</td>
</tr>
<tr>
<td>Self-deprecating talk</td>
<td>0.986</td>
<td>0.166</td>
</tr>
<tr>
<td>Task-deprecating talk</td>
<td>0.817</td>
<td>0.128</td>
</tr>
<tr>
<td>Task-related talk</td>
<td>0.360</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>Continuous Behaviors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shifting weight</td>
<td>0.034</td>
<td>0.263</td>
</tr>
<tr>
<td>Becomes quiet/whispering tone</td>
<td>-0.204</td>
<td>0.071</td>
</tr>
<tr>
<td>Turning red/blushing</td>
<td>-0.177</td>
<td>0.677</td>
</tr>
<tr>
<td>Touching face, hair, etc.</td>
<td>0.255</td>
<td>0.700</td>
</tr>
<tr>
<td>Shaking</td>
<td>-0.095</td>
<td>0.471</td>
</tr>
<tr>
<td>Nervous laughter/smiling</td>
<td>0.050</td>
<td>0.547</td>
</tr>
<tr>
<td>Fidgeting</td>
<td>0.282</td>
<td>0.395</td>
</tr>
</tbody>
</table>

*Notes.* Factor 1 is verbal stress behaviors. Factor 2 is physical stress behaviors. Bolded items are those included in the factor.
Table 2

*Means and Standard Deviations of Study Variables*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-Transformed</th>
<th>Transformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal Stress Behaviors</td>
<td>1.84(2.25)</td>
<td>.80(0.69)</td>
</tr>
<tr>
<td>Physical Stress Behaviors</td>
<td>5.31(5.01)</td>
<td>1.56(0.77)</td>
</tr>
<tr>
<td>CESD</td>
<td>19.82(13.76)</td>
<td>4.28(1.60)</td>
</tr>
<tr>
<td>Alpha-Amylase (AUC₉)</td>
<td>4954.37(3207.04)</td>
<td>8.30(0.67)</td>
</tr>
<tr>
<td>Alpha-Amylase (AUCᵢ)</td>
<td>373.72(1359.81)</td>
<td>8.33(0.36)</td>
</tr>
</tbody>
</table>

*Notes.* Transformed means and SDs are those before centering on zero. CESD = Depression measure.
Table 3

Correlations of Study Variables

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PSB</td>
<td>-</td>
<td>.456*</td>
<td>.082</td>
<td>.199</td>
<td>.268</td>
</tr>
<tr>
<td>2. VSB</td>
<td>-</td>
<td>-</td>
<td>-.084</td>
<td>-.093</td>
<td>-.223</td>
</tr>
<tr>
<td>3. CESD</td>
<td>-</td>
<td></td>
<td>.101</td>
<td></td>
<td>.212</td>
</tr>
<tr>
<td>4. sAA (AUC&lt;sub&gt;G&lt;/sub&gt;)</td>
<td>-</td>
<td></td>
<td></td>
<td>.166</td>
<td></td>
</tr>
<tr>
<td>5. sAA (AUC&lt;sub&gt;I&lt;/sub&gt;)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes. PSB= Physical stress behaviors factor; VSB= Verbal stress behaviors factor; CESD= Depression measure; sAA=Salivary alpha-amylase. *p<.01.
Table 4

*Regression Analysis of Salivary Alpha-Amylase (AUC$_G$) on Depression and Stress Variables*

<table>
<thead>
<tr>
<th></th>
<th>$\beta^a$</th>
<th>$t$</th>
<th>$F^b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Equation</td>
<td>1.15</td>
<td></td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Physical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical</td>
<td>0.28</td>
<td>1.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CESD</td>
<td>0.13</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical x CESD</td>
<td>0.32</td>
<td>2.11*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal</td>
<td>0.12</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal</td>
<td>-0.08</td>
<td>-0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CESD</td>
<td>0.09</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal x CESD</td>
<td>0.01</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Notes.* Covaried by age, sex, and saliva collection time. Physical = Physical stress behaviors factor; Verbal = Verbal stress behaviors factor.

*p<.05.

$^a$ Standardized $\beta$; $^b$ For $F$-tests, df=6.47.
\[
AUC_G = \frac{(m_2 + m_1) \times t_1}{2} + \frac{(m_3 + m_2) \times t_2}{2} + \frac{(m_4 + m_3) \times t_3}{2} + \frac{(m_5 + m_4) \times t_4}{2} + \frac{(m_6 + m_5) \times t_5}{2}
\]

Note. \( m \) is a measurement at time, \( t \).

\[
AUC_I = AUC_G - m_1 \sum_{i=1}^{n-1} t_i
\]

Figure 1. Formulas for calculating \( AUC_G \) and \( AUC_I \). \( m \) is a measurement, \( t \) is time between measurements, and \( n \) is the total number of measurements. Adapted from “Two formulas for the computation of area under the curve represent measures of total hormone concentration versus time-dependent change” by J. Pruessner, C. Kirschbaum, G. Meinlschmid, & D. Hellhammer, 2003, *Psychoneuroendocrinology*, 28, p. 916-931.
Figure 2. Probing two-way interaction (physical stress x depression).
PSB= Physical stress behaviors factor; SD= Standard deviation; sAA=Salivary alpha-amylase.