EXPOSURE AND PHYSIOLOGICAL REACTIVITY TO DAILY STRESSORS: THE ROLE OF PERCEIVED STRESS, PERCEIVED STRESS REACTIVITY, AGE, AND COGNITIVE ABILITY

A Thesis
Presented to
The Academic Faculty

by

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In Partial Fulfillment
of the Requirements for the Degree
Master of Science in
Psychology

Georgia Institute of Technology
May 2021

EXPOSURE AND PHYSIOLOGICAL REACTIVITY TO DAILY STRESSORS: THE ROLE OF PERCEIVED STRESS, PERCEIVED STRESS REACTIVITY, AGE, AND COGNITIVE ABILITY

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Date Approved: January 25, 2021

ACKNOWLEDGEMENTS

I received a great deal of support throughout this project. I would first like to thank my advisor, Dr. Christopher Hertzog, who provided me with the opportunity to carry out this secondary analysis. His expertise was invaluable for helping me generate my research questions and I greatly appreciate his commitment to working with me during my first exposure to multilevel modeling and analyzing biological markers of stress. I would also like to gratefully acknowledge the support I received from my parents, John and Lallie Hughes, and my partner, Jamal Anyalebechi. Not only did these individuals provide me the encouragement needed to complete this project amidst the pandemic, but provided me with much-needed distractions along the way. Finally, I would like to thank the late Dr. Fredda Blanchard-Fields, whose work and ingenuity made this project possible.

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LIST OF SYMBOLS AND ABBREVIATIONS

HPA axis	Hypothalamic-pituitary-adrenal axis
SNS	Sympathetic nervous system
EMA	Ecological momentary assessment

SUMMARY

Identifying individual differences that buffer or exacerbate physiological reactivity to stressors can have important implications for health and well-being. This study focused on the roles perceived life stress, perceived stress reactivity, age, and cognitive ability have on within-person and between-person associations between naturally occurring stressors and cortisol and alpha-amylase activity. Using ecological momentary assessment for 10 consecutive days, 156 individuals ages 20-77 years old (M = 51.45, SD = 18.30) reported stressor exposures and provided seven saliva samples each day from which cortisol and alpha-amylase were assayed. Multilevel modeling was used to examine the role of daily stressors as well as each individual difference variable on the cortisol and alpha-amylase awakening response, diurnal slope, and area under the curve (i.e., total daily output). Results showed that the frequency of daily stressors had a significant positive correlation with perceived stress reactivity and cognitive ability. Within-persons, days with stressor exposures were associated with higher total daily cortisol output and steeper diurnal alpha-amylase slopes. A significant cross-level interaction showed individuals high in perceived stress reactivity who experienced days with more stressor exposure had steeper daily alpha-amylase awakening responses. Between-persons, people who experienced more stressors per day tended to have flatter diurnal alpha-amylase slopes. Individuals higher in perceived stress who experienced more stressors on average had steeper diurnal cortisol slopes. Although there were no age differences in the frequency of reported stressors, older people who experienced more stressors on average had steeper alpha-amylase awakening responses compared to younger people. All effects remained significant after controlling on neuroticism. Findings indicated that naturally occurring stressors predict within-person fluctuations as well as individual differences in cortisol and alpha-amylase activity. The Perceived Stress Reactivity Scale may predict within-person variability in alpha-amylase reactivity to stress, whereas the Perceived Stress Scale may be more closely associated with long-term changes in cortisol activity. Given these findings, higher perceived stress reactivity and perceived stress may be vulnerability factors associated with stressor exposure and reactivity, and age and cognitive ability may be resilience factors.

CHAPTER 1. INTRODUCTION

Experiencing stress in everyday life is an inevitable aspect of living. Stressors are disruptive events or hassles that occur during day-to-day living, such as having an argument with a spouse or being behind on an approaching work deadline. Manifestations of stress can be observed in physiological stress systems, which become activated when the body is challenged. Individual differences could explain why some people react more strongly to stressful situations than other people. Due to the potential negative consequences associated with elevated (and blunted) physiological activity on indices of health (Adam et al., 2017; McEwen & Seeman, 1999), it is important to understand how physiological manifestations of stress unfold in everyday life and to identify conditions that play a role in regulating the impact of daily stressors on stressor reactivity.

Using a sample of adults across the lifespan, this study explored the roles of perceived life stress, perceived stress reactivity, age, and cognitive ability on the relationship between daily stressor exposures and physiological activity. The outcome variables included cortisol and alpha-amylase, two biomarkers associated with stress that can be found in saliva. Using ecological momentary assessment, daily stressor exposures and saliva samples were collected over the course of 10 consecutive days, permitting between-person as well as within-person observations between stressor exposures and physiological reactivity. Multilevel modeling was used to examine the associations between daily stressors, the individual difference variables, and the diurnal cortisol and alpha-amylase patterns.

1.1 The Stress Process

One framework that connects stressor exposure and reactivity is the stress process (Pearlin, 1999b). The stress process includes three main components: stressors, outcomes, and moderators (Pearlin, 1999b). These three components help to explain why two people exposed to the same stressor may not necessarily be affected by the stressor to the same extent. Each component of the stress process is discussed in more detail in the following sections.

1.1.1 Stressors

Daily stressors are minor disruptive events and hassles that arise during day-to-day living. Compared to major life events, such as the death of a loved one, daily disruptions and hassles occur with a greater frequency and can cumulatively have similar or potentially even more adverse consequences on health and well-being (Almeida, 2005; Almeida et al., 2002). In the current stress literature, minor daily hassles are often contrasted with chronic stressors, which are ongoing, prolonged stressful situations such as caregiving for a cognitively impaired loved one (Piazza et al., 2010).

For an event to be considered stressful, the individual must appraise the event as stressful (Lazarus & Folkman, 1984). Daily stressors are situation-specific and can include a wide range of events that vary on a number of different conditions. Situational features of stressors, such as the features of self-threat and negative valence, predict our perception of stressors (Lebois et al., 2016). Specific features of past stressful events can help individuals categorize new events as stressful (Lebois et al., 2016).

The extent to which an individual reacts to a stressful event can depend on the type of stressor encountered and the everyday life domain in which the stressor emerges and impacts (e.g., interpersonal tensions, work-related stressors, and network stressors; Almeida et al., 2002). For example, Neupert et al. (2007) showed that individual differences such as age and control beliefs influenced stressor reactivity specifically to interpersonal, network, and work-related stressors. Being able to anticipate stressors before they occur, including the temporal timeframe and the context of daily life in which the stressor will impact, can also influence reactivity. Anticipating an upcoming stressor can allow a person to adopt specific types of coping strategies not otherwise available for unanticipated stressors (Neupert et al., 2019). For example, when disruptive events are expected, individuals can try to prevent the events before they occur or try to prepare themselves for the negative consequences associated with the upcoming events (Neupert et al., 2018). Finally, stressors can also differ in their level of subjective controllability. Uncontrollable events are considered unmodifiable and/or unavoidable, where efforts cannot be made to lessen the negative consequences of the events (Dickerson & Kemeny, 2004). Perceiving at least some level of influence or control over stressful situations tends to be associated with less adverse psychological and physiological responses (Breier et al., 1987; Dickerson & Kemeny, 2004; van Eck et al., 1998). The context-specific nature of stressors play an important role on stressor exposure and reactivity and should be considered when examining stress in the context of daily life.

1.1.1.1 Assessing Stressor Exposure

Exposure to daily stressors can be measured using self-report questionnaires and repeated assessment of experiences as they unfold in everyday life. For many years, research studies have relied on self-report measures of stress and self-report measures of physical health more generally (Piazza et al., 2010). Self-report questionnaires typically measure traits of individuals by asking them to reflect on their experiences over a specified period (e.g., the past week or month) and report how they generally feel or behave. Questionnaires focused specifically on stress commonly assess dispositional, or trait-like aspects of individuals' perceptions of stress, such as how much stress they experience on average. Results from such questionnaires are often treated as time-invariant, where stress-related experiences are assumed to be relatively stable within people and across time.

Advantages of using self-report questionnaires include their ease of administration in both research and clinical settings; most questionnaires can be completed by a participant in a short amount of time. Although these types of measures can be relatively easy to collect, self-report questionnaires can be vulnerable to bias and retrospective memory reconstruction (Shiffman et al., 2008; Stone et al., 1998). To report one's typical behavior on a questionnaire, individuals oftentimes have to reflect across days or even weeks and aggregate their experiences into single ratings. Doing so often requires the use of heuristics, which can bias reports of frequencies of past events (Stone et al., 1998). The Perceived Stress Scale (Cohen et al., 1983) is one type of widely-used self-report questionnaire that measures how often people felt or thought a certain way in regards to stressors. The questionnaire asks respondents to reflect over the past month and rate the frequency in which they experienced different stressors. Although questionnaires have added important information to the body of knowledge on stress, there are other approaches to measuring exposure to stressors that may be more ecologically valid.

Another approach to assess stressor exposure is to repeatedly assess individuals' experiences across time. This type of repeated assessment is called ecological momentary assessment (EMA) and it allows the collection of current psychological states and behaviors as they unfold in real time within the natural environment (Shiffman et al., 2008). One approach of EMA, called randomly-prompted EMA sampling, can be carried out using mobile technology such as a smartphone. This type of sampling prompts individuals at random intervals throughout the day to answer survey questions on a momentary basis, such as how they currently feel and whether they experienced anything disruptive or stressful since the last EMA survey. Randomly-prompted EMA may more accurately collect state-like characteristics compared to self-report questionnaires because individuals are asked to report their experiences multiple times across short time intervals, such as every three hours, for a predetermined number of days (Shiffman et al., 2008). Compared to self-report questionnaires, repeated assessment of current experiences throughout the day reduces retrospective recall bias and the need for heuristics (Shiffman et al., 2008). One additional advantage of repeated assessment is that it allows researchers to not only examine a within-person process as it unfolds over time, but also allows researchers to aggregate assessments to examine the role of individual differences. Self-report questionnaires and ecological momentary assessment are important tools for understanding individuals' perceptions and experiences with stressors encountered in everyday life. However, encountering stressors is only one component of the stress process.

1.2 Outcomes

Mental health and well-being are common outcomes in stress research (Pearlin, 1999a). Responding to stressors, or stressor reactivity, is the change in well-being associated with experiencing a stressor, linking stressor exposure and well-being over time (Almeida, McGonagle, et al., 2009). Manifestations of stress can take many forms, including psychological changes and physiological changes in response to stressors.

Compared to psychological responses to daily stressors, less is known about the relationship between naturally occurring stressors and physiological responses. Examining psychological as well as physiological reactions to stress within the same individual can provide a more holistic understanding of mental and physical well-being as well as the factors that influence it (Almeida, Piazza, et al., 2020).

1.2.1 Psychological Reactivity to Stressors

A large body of research has focused on psychological manifestations of stress. Evidence suggests that naturally occurring stressors are associated with increases in distress (Neupert et al., 2007), negative affect (Bolger et al., 1989; Scott et al., 2013; Stawski et al., 2008), and even memory failures (Neupert, Almeida, et al., 2006). In addition, the severity of a stressor may be related to negative mood such that more severe stressors are related to greater levels of negative affect (Scott et al., 2013).

Multiple methods can be used to measure psychological reactions to stressor exposures. Self-report questionnaires that focus on individuals' subjective perceptions of their typical responses to stressors is one way of measuring stressor reactivity. For example, the relatively new Perceived Stress Reactivity Scale (Schulz et al., 2005) asks individuals to rate the extent to which they typically respond to different types of stressors. Additional methods for assessing psychological reactivity to stressors can include using EMA techniques to repeatedly ask individuals to report their current mood, emotions, or levels of distress.

Although measuring psychological manifestations of stress in everyday life contexts has produced valuable information regarding the link between naturally occurring stressors and stressor reactivity, certain questions cannot be answered with subjective ratings of experiences. Specifically, stress questionnaires and momentary reports of mood, emotions, and distress do not directly measure stressor reactivity in terms of underlying biological systems sensitive to stress, which may have important implications for health outcomes (McEwen & Seeman, 1999; Lupien et al., 1998; Schlotz, 2019). Exposure to stressors in daily life may not only affect individuals' psychological states, but influence biological activity, independent of stress-related increases in negative affect (Stawski, Cichy, et al., 2013).

1.2.2 Physiological Reactivity to Stressors

In addition to psychological responses to stress, stressor reactivity may be reflected in changes in physiological activity. When confronted with a challenge, the body undergoes allostasis, the process by which the body responds to stressors by adjusting to internal and external environmental demands to maintain stability. In the short-term, allostatic responses are adaptive as they allow individuals to react, adjust, and recover from challenges. Allostatic load refers to the wear and tear the body undergoes after repeated efforts to maintain stability. The accumulation of unpleasant life experiences and demands placed on the body can lead to physiological dysregulation and ultimately increase one's susceptibility to disease (McEwen & Seeman, 1999). Biological indictors of stress, or biomarkers, allow researchers to connect behavioral, environmental, and social factors when examining health and well-being. Normative as well as pathogenic physiological processes, particularly in response to stimuli such as daily stressors, can be observed using biomarkers to understand the biological signatures of acute and chronic stress in daily life (Engeland et al., 2019).

Two biological systems involved with engaging the body's stress response are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). When an individual perceives a threat or challenge, the HPA axis and the SNS activate via endocrine and neural pathways (Engeland et al., 2019). Activity in these systems can be measured in components of saliva, providing an easy to collect, non-invasive method of data collection that can be conducted in field studies to assess underlying physiological reactions to daily stressors (Almeida, Piazza, et al., 2020). One widely examined biomarker related to stress is salivary cortisol, a hormone associated with HPA axis activity. Alpha-amylase, a digestive enzyme used as a proxy of SNS activity, is another stress-related biomarker but has received comparatively less research attention than cortisol.

1.2.2.1 Stress and the Hypothalamic-Pituitary-Adrenal Axis

The HPA axis is activated during times when the system is exposed to acute stress, leading to an increase in a glucocorticoid called cortisol. Cortisol mobilizes energy and influences the metabolism in response to acute stress (Almeida, Piazza, et al., 2020; Oster et al., 2017). When individuals perceive a stressor, neurons of the hypothalamus trigger the release of corticotrophin-releasing hormone, which travels to the pituitary gland and stimulates the release of adrenocorticotropin hormone (ACTH). In the adrenal glands, ACTH plays a role in the release of glucocorticoids, including cortisol (Sapolsky et al., 2000). After glucocorticoids have been released and the system is ready to recover following the stressor exposure, the HPA axis restores itself in a negative feedback loop and levels of cortisol decrease.

Throughout the day and night, cortisol is secreted in a pulsatile manner where the HPA axis alternates between activation and inhibition (Lightman et al., 2008; Windle et al., 1998). The pulses in cortisol that occur within the 24-hour cycle form the basis of the typical diurnal cortisol rhythm (Lightman, 2008; Young et al., 2004). In healthy individuals, cortisol levels start high at awakening and increase approximately 30-45 minutes after awakening (Fries et al., 2009). The magnitude of the increase in cortisol post-awakening is called the cortisol awakening response. After the sharp increase in cortisol levels following morning awakening, healthy individuals show a steep decrease in cortisol levels throughout the rest of the day. The magnitude of the decrease in cortisol levels from morning to evening is called the diurnal cortisol slope. Although cortisol levels follow a distinct diurnal profile, elevations in cortisol levels can be examined on top of its diurnal profile when an individual is exposed to a stressor (Almeida, 2005; Nater et al., 2006). An increase in cortisol associated with an acute stressor occurs approximately 20 minutes following the initial exposure to the stressor (Granger et al., 2007). These spikes in cortisol levels corresponding to acute stress can lead to deviations from one's typical diurnal pattern, potentially influencing the steepness of the awakening response and diurnal slope (Miller et al., 2007; Stawski, Cichy, et al., 2013). Changes in the magnitude of the awakening response and diurnal slope due to spikes in cortisol from daily stressors can also lead to changes in total daily cortisol output, or area under the curve with respect to ground (Stawski, Cichy, et al., 2013). Understanding how naturally occurring stressors influence diurnal cortisol profiles and area under the curve can provide further insight into the role diurnal profiles have in the stress process. For example, a steeper cortisol awakening response may be indicative of the system preparing itself for a particularly stressful day (Adam et al., 2006; Fries et al., 2009).

Independent of stressors, confounding factors can influence cortisol levels by either activating the HPA axis or by impacting saliva production. Confounding factors can include age, gender, pregnancy, smoking status, medication, consuming coffee and alcohol, and ingestion of certain foods (Kudielka et al., 2009). Although results have been mixed (see Epel et al., 2007), potentially due to some studies using a limited number of saliva samples and/or exposing participants to different types of stressors, older age is often associated with greater HPA axis activity (Almeida et al., 2011; Almela et al., 2011). Analyzing the same data used in the current study, Nater et al. (2013) showed that older age was associated with greater total cortisol output, an attenuated diurnal cortisol slope, and more pronounced morning rises. Even though older adults generally have higher cortisol levels across the day compared to younger adults (Almeida et al., 2011), research studies have demonstrated that older adults still experience a significant increase in cortisol levels above and beyond their typical diurnal rhythm when they are exposed to stressors (Stawski, Cichy, et al., 2013). Older age may also be related to greater cortisol reactivity and a slower rate of recovery following acute stressors, although increased HPA axis activity is not inevitable with age (Epel et al., 2007; Otte et al., 2005). Kudielka et al. (2009) recommended that research studies examining cortisol reactivity to stressors consider including these potentially confounding factors as exclusionary criteria, or statistically controlling for the confounders by adding them as covariates into statistical models.

1.2.2.2 Stress and the Sympathetic Nervous System

The relationship between naturally occurring stressors and the diurnal cortisol rhythm has received more research attention than the relationship between stressors and the diurnal alpha-amylase rhythm. Alpha-amylase, an oral cavity enzyme that primarily aids in the digestion of carbohydrates, is used as a surrogate marker of SNS activation. Upon activation, the SNS stimulates the release of the catecholamines epinephrine and norepinephrine (Epel et al., 2007). Although epinephrine and norepinephrine can be found in saliva, logistical constraints limit researchers' ability to collect these catecholamines in field studies (e.g., there are complicated processing requirements and catecholamines can take an extended length of time to transfer from blood to saliva). Because epinephrine and norepinephrine are difficult to collect in field studies, researchers have used salivary alpha-amylase as an alternative indicator of SNS activity (Rohleder et al., 2004). The rationale for using alpha-amylase as a proxy of SNS activity is that when the SNS is activated and epinephrine and norepinephrine are released, the catecholamines stimulate and alter activity in salivary glands, ultimately influencing concentrations of alpha-amylase (see Nater & Rohleder, 2009). Although research findings are mixed, stress-related increases in alpha-amylase are associated with increases in epinephrine and norepinephrine (Ditzen et al., 2014; Rohleder et al., 2004; Thoma et al., 2012).

Diurnal alpha-amylase profiles mirror the diurnal profiles of cortisol. In healthy individuals, alpha-amylase starts moderately low at awakening, decreases in the first 30 minutes after awakening, and rises throughout the afternoon (Nater et al., 2007). On top of its diurnal rhythm, sharp increases in alpha-amylase levels associated with acute stress can be observed (Nater et al., 2006; Nater et al., 2005). Under acute stress, alpha-amylase levels rapidly increase, peaking approximately five minutes following a stressor exposure, and return to baseline approximately 20 minutes following the stressor exposure (Granger et al., 2007). Although relatively few studies have examined the effect of naturally occurring stressors on diurnal alpha-amylase profiles, exposure to daily stressors can lead to deviations in the diurnal alpha-amylase rhythm (Liu et al., 2017).

Compared to cortisol, alpha-amylase may be more resistant to confounding factors that impact HPA axis activity. Although findings are mixed, Nater et al. (2007) suggested the diurnal alpha-amylase profile is not influenced by body mass index (BMI), sex, exercise, smoking status, or food and drink consumption. Although few studies have investigated age differences in alpha-amylase reactivity to stressors, evidence suggests that older age is associated with greater reactivity in catecholamines and a slower rate of recovery following a stressor (Epel et al., 2007). In addition, previous research has shown that older age is associated with greater total alpha-amylase output (Almela et al., 2011), a less pronounced increase across the day (Nater et al., 2013; Nater et al., 2007), and no age-related differences in the awakening response (Nater et al., 2013).

1.2.2.3 Contrasting the HPA Axis and Sympathetic Nervous System.

Although cortisol and alpha-amylase follow distinct diurnal rhythms and increase in response to stressful events, there are observable differences in HPA axis and SNS reactivity to stressors. Within-person fluctuations in cortisol levels may not be associated with within-person fluctuations in alpha-amylase levels across the day (Nater et al., 2007). In addition, the increase in cortisol associated with encountering a stressor may not be correlated with the increase in alpha-amylase associated with encountering the same stressor (Nater et al., 2005). However, elevated cardiovascular responses to stress associated with activation in the SNS has been shown to predict elevated responses in the HPA axis, potentially indicating some level of coordination between the two stress systems (Poppelaars et al., 2019).

The diurnal rhythm of alpha-amylase might be more stable across time and conditions compared to the diurnal rhythm of cortisol (Out et al., 2013), potentially indicating that stressor-related spikes in alpha-amylase levels have a different (or weaker) impact on its diurnal rhythm compared to the impact stressor-related increases in cortisol levels have on its diurnal profile.

Despite being activated by the same stressor, the HPA axis and SNS have temporal differences in their response patterns. When exposed to a stressor, the SNS provides an immediate response, demonstrated by the rapid increase and recovery in alpha-amylase levels. The HPA axis may provide a longer-term response to stress, observed in the slower increase of cortisol levels following a stressor (Granger et al., 2007). These temporal differences in activation may differentially impact fluctuations in the diurnal rhythms of cortisol and alpha-amylase, indexed at a daily-level rather than a momentary-level. There may be evidence that SNS activation precedes HPA axis activation by first initiating inflammatory mechanisms, later downregulated by cortisol (Engeland et al., 2019). This sequence could indicate that the increase in SNS activity is the system preparing for a challenge, followed by down regulation coordinated by the HPA axis (Engeland et al., 2019).

The HPA axis and SNS may also differ in their sensitivity to the nature of different types of stressors. Activation in the SNS may occur at a lower threshold compared to the

HPA axis. Some types of stressors, particularly mild stressors, may activate the SNS but are not strong (or severe) enough to activate the HPA axis (Engeland et al., 2019; Savla et al., 2013). For example, Hunter et al. (2018) showed that the stress of not having one's smartphone device during a time of social exclusion influenced levels of alpha-amylase but not levels of cortisol, highlighting the potential sensitivity of alpha-amylase as an indicator of mild stress relative to cortisol. Savla et al. (2013) examined cortisol and alpha-amylase activity in spousal care partners of individuals with mild cognitive impairment (MCI). Uncontrollable problem behaviors exhibited by individuals with MCI elicited elevated cortisol levels in the spousal caregivers but did not impact alpha-amylase levels. Marital problems between the spouses, which could be more emotionally charged compared to problem behaviors, prompted elevations in alpha-amylase levels but did not impact cortisol levels. Similarly, events that are perceived as uncontrollable, potentially leading to greater levels of distress, are often associated with elevated HPA axis as well as SNS activity, whereas stressful events that are perceived as controllable may be more closely related to elevated SNS activity but not HPA axis activity (Frankenhaeuser, 1982; Savla et al., 2013). More research is needed to better understand the specific types of unpleasant situations that differentially influence HPA axis and SNS activity.

Despite their differences, the HPA axis and SNS are sensitive to exposure to daily stressors, producing biomarkers observable in saliva. Because the two systems may play different roles in the stress response, it is of interest to examine how these systems behave in response to the same naturally occurring stressful events. Physiological responses observed in one system could have important implications for the other system. Both between-person as well as within-person analyses will be used in this study to better understand predictors of cortisol and alpha-amylase activity.

1.2.3 Stress Reactivity as a Within-Person Process

Because some individuals are more susceptible to encountering and reacting to daily stressors than other individuals, yet some days are more stressful than others, variability in physiological reactivity to stressors can be examined between-persons as well as within-persons. Research results on stressor exposure and reactivity can differ considerably depending on the approach one takes (Stawski, Cichy, et al., 2013).

Early studies on stressor exposure and reactivity focused on stable, trait-based characteristics of individuals' experiences. Approaches compared people based on their average, or typical, experiences. This focus on examining aggregated measures of stressor exposure and reactivity between-persons allows researchers to understand the role of individual differences in the stress process. For example, Stawski, Cichy, et al. (2013) found that a higher overall frequency of stressor exposures was associated with higher levels of cortisol 30 minutes post-awakening and a steeper diurnal slope. That is, individuals who generally experienced more daily hassles had higher peak morning cortisol levels and steeper slopes across the day. This type of statistical approach does not allow researchers to understand how stress unfolds within the same individual.

Stressor reactivity is differentially impacted by state and trait characteristics (Hellhammer et al., 2007). Although between-person analyses on stress-related experiences have added to our understanding of the stress process, it is well established that reactivity to stressors fluctuates across time and situations (Tennen et al., 2000).

Almeida (2005) suggested research designs must take into account that daily stress is a process occurring within people. Studies relying exclusively on aggregated measures of stressor reactivity cannot consider within-person variability or the situation-specific nature of stressors. Within-person examinations of the stress process allow researchers to not only examine individual differences associated with stressor exposure and reactivity, but also the circumstances in which individuals are exposed and react to stressors.

The notion of stress as a within-person process is supported by the significant amount of day-to-day variability in cortisol levels rather than consistent differences between people (Almeida, Piazza, et al., 2009; Ross et al., 2014). In fact, it is recommended that researchers collect cortisol samples for at least six days to calculate stable estimates (Hellhammer et al., 2007). Minor daily hassles have been found to affect the diurnal cortisol rhythm (Stawski, Cichy, et al., 2013; van Eck et al., 1996). Stawski, Cichy, et al. (2013) examined the intraindividual relationship of daily stressor exposures and diurnal cortisol patterns and showed that compared to days without exposure to stressors, days with stressors were significantly related to greater levels of cortisol 30 minutes post-awakening, greater levels of cortisol before lunch, and elevated total cortisol output. The same study found that between-persons, individuals who reported more stressor exposure tended to have elevated cortisol levels 30 minutes post-awakening, a steeper cortisol awakening response, and a steeper diurnal slope. Findings differed based on whether the associations were examined within-persons versus between-persons, highlighting the importance of considering stressor reactivity as a fluctuating, within-person process (Stawski, Cichy, et al., 2013).

Although alpha-amylase is sensitive to exposure to daily hassles on a momentary basis, the daily rhythm of salivary alpha-amylase, particularly an aggregated measure of total daily output, may be relatively stable across time and conditions (Out et al., 2013). In a longitudinal measurement burst design, Out et al. (2013) showed that 65.2% and 61.2% of the variance in daily mean alpha-amylase and area under the curve, respectively, were attributable to between-person differences. In addition to the relatively stable pattern of alpha-amylase levels across days, Out et al. (2013) demonstrated that only a small amount of variance in the diurnal alpha-amylase rhythm was due to differences between measurement bursts. Few empirical studies have examined the intraindividual relationship between daily stressors and diurnal alpha-amylase profiles. Studies that have examined the relationship show mixed results (e.g., Liu et al., 2017; Nater et al., 2007), possibly due to differences in data collection methods and the sample of participants. Studies that have examined the connection between daily stressors and diurnal alpha-amylase measures focused on chronically stressed groups. For example, Liu et al. (2017) found that the diurnal rhythm of alpha-amylase had significant within-person and between-person associations with care-related stressor exposures in a group of family caregivers of individuals with dementia. Nater et al. (2007) did not find a significant intraindividual relationship between alpha-amylase levels and momentary (i.e., hourly) reports of stress levels but suggested as a limitation that the sample was not particularly stressed during the data collection period. The lack of interindividual and intraindividual findings on the relationship between naturally occurring stressors and daily alpha-amylase parameters could be due to the relatively fast reacting and recovery of alpha-amylase in response to stressful situations (Granger et al., 2007). The diurnal rhythm of alpha-amylase may be robust against within-day, momentary spikes associated with acute stress, even though alpha-amylase is highly sensitive to stress on a moment-to-moment basis. Because there may be fundamental differences in the way diurnal cortisol patterns fluctuate across situations compared to the way alpha-amylase patterns fluctuate (i.e., day-level measures of cortisol potentially fluctuate more across time while day-level measures of alpha-amylase remain relatively stable), taking a between-person and within-person approach to examine the relationship of these biomarkers with daily stressors can provide additional insight on stress-cortisol and stress-alpha-amylase linkages.

Examining cortisol and alpha-amylase as outcomes of the stress process will allow a side-by-side comparison of HPA axis and SNS activity in response to daily stressors. Using a multi-system approach can advance our understanding of the stress response and can also be a particularly useful method when identifying individual differences that impact stressor reactivity (Bauer et al., 2002; Pearlin, 1999b). Individual differences can play an important role in the link between stressor exposure and reactivity; identifying characteristics of people who may be more vulnerable to stressor exposures as well as more vulnerable to elevated reactivity to stressors will not only improve our understanding of the stress process, but also provide potential targets for intervention.

1.3 Moderators

Although the body strives to achieve stability when faced with stressors (McEwen, 2016), individual differences can play a role in the interpretation of potentially stressful situations as well as the extent to which a person reacts to stressors (Pearlin, 1999b). Personal characteristics and resources can affect psychological and physiological outcomes when exposed to challenges. These differences in outcomes can be partially attributed to individuals' ability to cope with and manage daily hassles (Pearlin, 1999a).

Individual differences can be viewed as vulnerability and resilience factors in the daily stress process. Vulnerability factors are characteristics that make individuals more susceptible to adverse consequences of stressors. Resilience factors are characteristics that help protect individuals from the effects of stressors (Diehl et al., 2012). In terms of physiological responses to stressors, vulnerability factors could be associated with exacerbated increases in cortisol and alpha-amylase levels when stressors occur, or increased variability in physiological activity over time. Resilience factors, on the other hand, could be associated with buffered elevations in cortisol and alpha-amylase levels when stressors occur, and potentially less variability in physiological activity over time. However, it should be noted that allostatic overload can be related to abnormal physiological activity, including inadequate responses to stressors that lack the initial reactivity as well as prolonged responses to stressors that lack recovery after activation (McEwen, 1998). The current study uses a healthy lifespan sample to examine the exacerbating or buffering effects of perceived life stress, perceived stress reactivity, age, and cognitive ability on physiological reactivity to stress.

1.3.1 Perceived Stress

Perceived stress is the extent to which a person experiences their life demands as unpredictable, uncontrollable, or overloaded (Cohen et al., 1983). Higher levels of perceived stress may be associated with greater levels of trait anxiety, depression, anger, negative affect, psychosomatic complaints, and exposure to daily stressors (van Eck &

Nicolson, 1994). Perceived life stress is often measured by the Perceived Stress Scale (Cohen et al., 1983), a short self-report questionnaire that asks respondents to report the frequency in which the experiences in their lives are perceived as stressful. Consequently, perceived stress is often positively related to the frequency of encountering everyday stressor exposures in both younger and older adults (Stawski et al., 2008; van Eck et al., 1998).

Higher levels of perceived stress may be associated with elevated HPA axis activity. On average, workers in a high perceived stress group had higher aggregated cortisol levels during workdays compared to workers in a low perceived stress group (van Eck & Nicolson, 1994). Within-person observations on the same sample examined by van Eck and Nicolson (1994) showed that cortisol levels were higher on days with stressors, but stress-related cortisol reactivity was not exacerbated in individuals high in perceived stress. State negative affect, however, was significantly related to elevated cortisol (van Eck et al., 1996; van Eck & Nicolson, 1994).

Perceived life stress may not be a reliable predictor of momentary alpha-amylase levels, although a measure of chronic stress significantly predicted momentary alpha-amylase levels (Nater et al., 2007). Chronic stress experienced when caregiving for a loved one with dementia was associated with an attenuated diurnal alpha-amylase profile (Liu et al., 2017). Within-persons, days with more caregiving-related stressors were associated with blunted rises between 30 minutes post-awakening and before lunch and a steeper rise between late afternoon and before bed (Liu et al., 2017). In a study focused on office workers, individuals who reported high levels of distress were found to have lower alpha-amylase levels at awakening and higher levels during the afternoon and at bedtime (Marchand et al., 2016).

Evidence suggests that higher levels of perceived stress are related to experiencing more stressors in daily life and may be associated with deviations in diurnal cortisol levels, but potentially not associated with deviations in diurnal alpha-amylase levels. The current study focuses on a healthy adult sample to understand the role of perceived stress on physiological processes in daily life.

1.3.2 Perceived Stress Reactivity

Perceived stress reactivity is the extent to which an individual typically reports responding to stressors with immediate, intense, and/or long-lasting reactions (Schulz et al., 2005). Perceived stress reactivity can be measured using the Perceived Stress Reactivity Scale (Schulz et al., 2005), which asks respondents to report the extent to which they typically respond to different types of everyday life stressors. The Perceived Stress Reactivity Scale is moderately correlated with the Perceived Stress Scale (Morgan et al., 2014), indicating that individuals who perceive higher levels of chronic stress may also perceive themselves as reacting more strongly to stressors.

Findings have been mixed regarding the relationship between perceived stress reactivity and HPA axis activity. Schlotz et al. (2011) showed that higher perceived stress reactivity was associated with steeper increases in cortisol following the Trier Social Stress Test (Kirschbaum et al., 1993), demonstrating perceived stress reactivity may predict physiological reactivity associated with acute stressors. Diurnal cortisol profiles, however, did not significantly differ between individuals with higher levels of perceived stress reactivity compared to individuals with lower levels of perceived reactivity (Limm et al., 2010).

Regarding the relationship between perceived stress reactivity and alpha-amylase levels, subjective reactivity significantly predicted momentary (i.e., hourly) alpha-amylase levels (Nater et al., 2007). Few studies have examined the role of subjective reactivity on diurnal alpha-amylase patterns. The current study aims to better understand the relationship between subjective reports of stress reactivity and diurnal cortisol and alpha-amylase patterns within the context of everyday life.

A large body of research has focused on understanding the role perceived stress and perceived stress reactivity (although to a lesser extent) have on physiological arousal when stressors are introduced in the laboratory (e.g., Nater et al., 2006). Laboratory settings offer a controlled environment where reactivity and recovery of the HPA axis and SNS can be observed across individuals high and low in subjective levels of stress. There is emerging evidence, however, that these individual differences observed in the laboratory are also related to stress responses within the context of daily life (Nater et al., 2007; Schlotz et al., 2011; Scott et al., 2013; Stawski et al., 2008). More work is needed to understand the specific impact subjective experiences have on day-to-day HPA axis and SNS activity.

1.3.3 Age

Age may have a role on one’s frequency of encounters with stressors as well as stressor reactivity. Older adults typically report fewer exposures to daily stressors compared to younger and middle-aged adults (Stawski et al., 2008). Older adults are oftentimes retired and no longer encountering work-related stressors. Older individuals may also be motivated to avoid unpleasant situations in daily life (Carstensen et al., 1999). In addition to age differences in the frequency of stressor exposures, there may also be age differences in the extent to which people react physiologically to stressors. Some studies have shown that, compared to younger adults, older adults have greater levels of physiological activation when exposed to stressors, potentially because older adults take longer to return to baseline levels following a stressor (Gotthardt et al., 1995; Neupert, Miller, et al., 2006; Otte et al., 2005). However, findings on age-related changes in cortisol reactivity have been mixed (Kudielka et al., 2000; Nicolson et al., 1997). Regarding alpha-amylase, older age may be associated with reduced reactivity to acute stressors (Strahler et al., 2010), but not all studies have observed such age differences in alpha-amylase levels (Almela et al., 2011). These mixed findings suggest that there may be times or situations where older adults are less reactive to stressors, and other times or situations where older adults are more reactive to stressors.

Instead of asking whether age is exclusively a vulnerability or resilience factor in the context of daily stress, Diehl et al. (2012) recommended researchers instead ask, "*when and under what conditions* is age associated with *greater vulnerability* to daily stress and *when and under what conditions* is age associated with *greater resilience* to daily stress." (p. 13). There may be specific situations where older adults are more reactive to stressors compared to younger adults (Mroczek & Almeida, 2004), situations where people across the lifespan react similarly to stressors (Stawski et al., 2008), and situations where the life experiences that come with being older better equip a person to manage and cope with unpleasant events (Blanchard-Fields, 2007; Uchino et al., 2006).

The Strength and Vulnerability Integration model (SAVI; Charles, 2010) provides a framework to explain why researchers might observe (and not observe) age-related changes in stressor exposure and physiological reactivity across the lifespan. The SAVI model considers both age-related strengths and vulnerabilities regarding emotion regulation and maintaining well-being. Older age can be associated with greater use and success with attentional strategies, appraisals, and behaviors to regulate emotional experiences in daily life. When older adults can successfully use these strategies to deescalate or avoid a negative or unpleasant situation, age-related strengths in well-being emerge and can buffer physiological arousal related to the event.

The SAVI model considers the temporal aspects of emotion regulation. Age-related benefits in regulation may be small immediately before and after a stressful event. Given time, however, the benefits of age-related strengths in emotion regulation become evident. Time may allow older adults to engage in regulatory strategies more effectively compared to younger adults. For example, Scott et al. (2017) found no age differences in negative affect associated with stressor exposures within the first 0-10 minutes following stressor exposure, but older age was associated with buffered stressor-related increases in negative affect between 10 minutes to 2.5 hours following the exposure. When daily stressors occur, these temporal differences in age-related strengths could have important implications on HPA axis and SNS reactivity. Given the potential length of time needed for age-related strengths to emerge following stressor exposures, these strengths may not be observable in the fast reacting and recovery of the SNS but may be more easily observable in the slower reacting and recovery of the HPA axis.

The SAVI model also suggests that age-related vulnerabilities, such as having fewer social supports, offset older adults' strengths in regulation when exposed to stressors. In situations where stressors cannot be deescalated or avoided using attentional strategies, appraisals, and/or self-regulatory behaviors, the age advantage associated with regulation is reduced or eliminated. Subjective states reported by older adults will be similar to or more adverse than the subjective states reported by younger adults, which could also impact physiological states. The unavoidable or uncontrollable nature of some life events, such as naturally occurring stressors, can reveal age-related vulnerabilities in regulation, making it more difficult for older adults to regulate their physiological arousal and potentially prolong their reactions to stressors.

Although encountering stressful situations is an inevitable part of life, acute stress as well as chronic, prolonged exposure to stressors in daily life can have negative impacts on health. Physiological adjustments are made each time the stress response is activated, which, over time, can lead to adverse health outcomes by causing cumulative wear and tear of the system (McEwen, 1998; Piazza et al., 2010). This cumulative wear and tear can be observed in greater as well as blunted physiological reactions to stress. Prolonged elevated physiological arousal is associated with an increased risk of disease (McEwen, 1998), hippocampal atrophy, and memory deficits (Lupien et al., 1998). Although these negative health outcomes are not inevitable with age, it is critical to understand the role age might have on the relationship between daily stressor exposures and physiological arousal.

1.3.4 Cognitive Ability

The resources people have may influence the extent to which they can avoid or cope with naturally occurring stressors. The Selection, Optimization, and Compensation with Emotion Regulation framework (SOC-ER; Urry & Gross, 2010) seeks to explain when and why some individuals more successfully regulate their responses to stressors than others. People select regulation strategies based on their available resources, including both internal resources (e.g., capabilities) and external resources (e.g., environmental affordances) (Urry & Gross, 2010). The SOC-ER framework supports the notion that available resources can vary between people as well as within people from one situation to the next, and success with self-regulation varies accordingly. One resource that could play an important role in the extent to which a person reacts to stressors is cognitive ability.

Cognitive ability, including crystallized and fluid ability, and education are associated with the frequency in which people are exposed to stressors and react to stressors. Crystallized cognitive ability reflects knowledge and skills learned through education and experiences (Cattell, 1963), and learned life experiences could help people manage and cope with daily stressors (Blanchard-Fields, 2007). Fluid cognitive ability is the capacity to solve problems and flexibly process new information (Cattell, 1963), which could also be a particularly useful tool when confronted with unpleasant life events.

Many research studies have focused on the role cognitive ability and education have on psychological reactivity to stressors. Individuals with higher levels of fluid and crystallized cognitive ability tend to report smaller stress-related increases in negative mood compared to individuals with lower levels of ability (Hyun et al., 2018; Stawski et al., 2010; Stawski, Mogle, et al., 2013) and people with lower levels of education may experience greater levels of psychological distress and physical symptoms compared to people with higher levels of education (Almeida et al., 2005; Grzywacz et al., 2004). Individuals with greater cognitive ability and more education also tend to report more stressful events in daily life, potentially indicating that these individuals self-select into more challenging and demanding lifestyles (Grzywacz et al., 2004; Seeman & Crimmins, 2001; Stawski et al., 2010; Stawski, Mogle, et al., 2013).

Compared to examining the benefits cognitive ability might have on regulating psychological reactivity to stressors, relatively few empirical studies have examined the role of cognitive ability on reactivity to stressors indexed by changes in cortisol and alpha-amylase levels. Although the study did not examine the role of cognitive ability on physiological reactivity to stress, healthier diurnal cortisol rhythms, particularly higher morning levels, lower levels in the evening, and steeper diurnal slopes are associated with higher levels of cognitive function (Stawski et al., 2011). Individuals with better cognitive ability may have adaptive diurnal profiles, and this evidence suggests higher levels of physiological activation may not always be a poor indicator of health.

Consistent with what might be expected given the buffering effect cognitive ability has on psychological reactivity to stressors, Wright et al. (2005) demonstrated that older adults who had poorer cognitive performance showed greater cortisol reactivity to a laboratory stressor. Wolf et al. (2001) observed the same association in a sample of men. Findings on this relationship, however, have been mixed. For example, after inducing a stressor in the laboratory, individuals with poorer cognitive performance showed lower levels of cortisol reactivity and area under the curve (Ginty et al., 2012). Attenuated stressor reactivity associated with lower levels of cognitive ability may partially explain the relationship between blunted stressor reactivity and adverse health outcomes (Ginty et al., 2012), but more research is needed to understand the association between cognitive ability and physiological reactivity to stress.

One explanation for the association between higher levels of cognitive ability and reduced reactivity to stressors is the potential role cognitive ability has on successful emotion regulation. Labouvie-Vief (2003) suggested that negative emotions are more cognitively demanding compared to positive emotions. Cognitive ability could provide the resources needed to allocate towards processing the negative emotions associated with experiencing stressful events (Labouvie-Vief, 2003). Specific types of emotion regulation strategies may draw more heavily on executive functioning than other types of strategies, which could contribute to one's success with regulating their emotions (Shiota & Levenson, 2009). For example, Opitz et al. (2014) showed that individuals with higher levels of fluid cognitive ability (and not age or crystallized ability) tended to have more success with using reappraisal to reduce their emotional reactivity to stressors. This study aims to examine whether the observed benefits of cognitive ability on emotion regulation may also be observed in buffered physiological arousal on days when unpleasant events occur.

Because emotion regulation is considered resource demanding and has been shown to negatively impact cognitive performance (Richards, 2004), a common assumption is that the cognitive declines associated with older age negatively impact older adults' ability to effectively engage in emotion regulation. However, Blanchard-Fields and colleagues argued that the declines in fluid ability associated with older age do not necessarily translate into impaired problem solving and poor emotion regulation. In fact, emotion regulation may be less costly for older adults compared to younger adults such that emotion regulation may be more automatic and efficient in older age (Blanchard-Fields, 2009). With a lifetime of experiences, older adults may have a wider array of emotion regulation strategies to use and may also be more effective at adaptively selecting strategies when they encounter situations that could negatively impact their goals (Blanchard-Fields, 2007). It could even be the case that age differentially influences the type of resources used to successfully manage problems in everyday life (Chen et al., 2017). Crystallized knowledge could help older adults continue to effectively solve problems in everyday life, whereas fluid cognitive ability may be a more important resource for everyday problem solving in younger adults (Chen et al., 2017). These findings suggest that, contrary to what is commonly expected, older people approach daily hassles and challenges with emotionally mature functioning, despite age-related cognitive declines (Blanchard-Fields, 2009).

Although there are conditions in which available cognitive resources may not necessarily imply better self-regulation, cognitive ability may be particularly useful for coping with unpleasant situations (Gottfredson & Deary, 2004). Higher levels of cognitive ability may act as a buffer against indices of poor health and well-being by helping individuals more successfully navigate the challenges and hassles faced in everyday life. It is important to understand when reduced reactivity to stressors is adaptive versus maladaptive and which conditions play a buffering versus exacerbating role on stressor reactivity.

1.3.5 Neuroticism

Neuroticism is a facet of personality associated with emotional instability and the tendency to experience distress. The personality dimension is associated with irritability, depression, anxiety, and low self-esteem (Costa & McCrae, 1987). Individuals high in neuroticism are prone to encountering stressors and reacting more strongly to stressors compared to individuals low in neuroticism (Bolger & Zuckerman, 1995). Not only do people high in neuroticism tend to report elevated levels of negative affect and distress in response to stressors, but they also potentially use less-adaptive coping strategies when stressors occur (Gunthert et al., 1999; Sliwinski et al., 2009). Analyzing the same data set as the current study, Morgan et al. (2014) showed a positive relationship between perceived stress, perceived stress reactivity, and neuroticism, though neuroticism was meaningfully different than stressor reactivity.

Although neuroticism may be associated with elevated psychological responses to stress, individuals high in neuroticism may show blunted physiological stressor reactivity (Bibbey et al., 2013). Individuals high in neuroticism showed diminished responses to acute stress, potentially indicating that this negative facet of personality is associated with maladaptive physiological responses to stressors (Bibbey et al., 2013). Neuroticism was included as a covariate in this study to show that any significant relationship between physiological activity and perceived stress and perceived stress reactivity was independent of trait neuroticism.

1.4 Present Study

This study utilized multilevel modeling to examine the between-person and within-person relationships between daily stressor exposures, perceived stress, perceived stress reactivity, age, cognitive ability, and diurnal profiles of cortisol and alpha-amylase. The research questions included the following:

1.a. Within-persons, are days with stressor exposure associated with greater diurnal cortisol and alpha-amylase measures compared to days without stressor exposure?

1.b. Between-persons, is there a relationship between a greater average frequency of days with stressor exposures and the average diurnal cortisol and alpha-amylase measures?

Based on previous research, it was hypothesized that within-persons, stressor days would be associated with a steeper cortisol and alpha-amylase awakening response, a flatter diurnal cortisol slope, a steeper alpha-amylase slope, and greater cortisol and alpha-amylase area under the curve. Between-persons, a greater average frequency of stressor days will be associated with a steeper cortisol and alpha-amylase awakening response, a steeper diurnal cortisol and alpha-amylase slope, and greater cortisol and alpha-amylase area under the curve.

2. Are perceived stress, perceived stress reactivity, age, and cognitive ability significantly correlated with the frequency of days with stressors reported across study days?

It was predicted that the frequency of days with stressors would positively correlate with perceived stress, perceived stress reactivity, and cognitive ability. That is, individuals with higher levels of perceived stress, perceived stress reactivity, and cognitive ability would report more days with stressors. It was also predicted that the frequency of stressor days would negatively correlate with age, such that older adults would report fewer stressors than younger adults.

3.a. Within-persons, does perceived stress, perceived stress reactivity, age, and cognitive ability moderate the relationship between days with stressors and the diurnal cortisol and alpha-amylase measures? That is, do the diurnal cortisol and alpha-amylase measures of people with higher perceived stress, perceived stress reactivity, age, and cognitive ability fluctuate more on days with stressors compared to people with lower perceived stress, perceived stress reactivity, age, and cognitive ability?

3.b. Between-persons, does the relationship between the average frequency of stressor days and the average diurnal cortisol and alpha-amylase measures depend on perceived stress, perceived stress reactivity, age, and cognitive ability?

Within-persons, it was predicted that perceived stress, perceived stress reactivity, and age would act as vulnerability factors on days when stressors occurred. On stressor days, individuals who reported higher levels of perceived stress would show greater HPA axis activation (i.e., a steeper awakening response, flatter diurnal slope, and greater total daily cortisol output) compared to individuals who reported lower levels of perceived stress. Perceived stress was not expected to be a significant moderator of the relationship between daily stressors and the diurnal alpha-amylase measures. Regarding perceived stress reactivity, on stressor days, individuals who reported higher levels of perceived stress reactivity would show greater SNS activation (i.e., a flatter awakening response, steeper diurnal slope, and greater total daily alpha-amylase output) compared to individuals who reported lower levels of perceived stress reactivity. Perceived stress reactivity was not expected to be a significant moderator of the relationship between daily stressors and the diurnal cortisol measures. Consistent with the SAVI model, it was expected that older adults would have greater HPA axis and SNS activation on days with stressors compared to younger adults. Finally, it was predicted that cognitive ability would act as a resilience factor on days when stressors occurred. On stressor days, individuals with higher cognitive ability would show buffered HPA axis and SNS activation (i.e., steeper cortisol slopes, flatter alpha-amylase slopes, and lower total cortisol and alpha-amylase output) compared to individuals with lower cognitive ability.

Between-persons, it was predicted that the association between the average frequency of days with stressors and the cortisol measures would depend on perceived stress, such that individuals high in perceived stress who experienced more stressors would show greater HPA axis activation. The relationship between the average frequency of stressors and the alpha-amylase measures would depend on perceived stress reactivity, such that a greater frequency of stressor days would be related to greater SNS activation for individuals who perceive higher levels of stress reactivity. Finally, it was hypothesized that the association between the average frequency of stressor days and the cortisol and alpha-amylase measures would also depend on age and cognitive ability. Compared to younger adults, older individuals would report fewer stressors but show greater HPA axis and SNS activation. Compared to people with lower cognitive ability, people with higher cognitive ability would be exposed to a greater frequency of stressors but show reduced HPA axis and SNS activation. In addition to the hypothesis-driven analyses, supplemental analyses will be discussed.

CHAPTER 2. METHOD

Archival data focused on aging and everyday problem-solving, goals, and emotions were examined in this secondary analysis (research funded by the National Institute on Aging at the National Institutes of Health to Fredda Blanchard-Fields, PhD, grant number NIA R01AG015019). Data from the primary study were collected between spring 2008 and fall 2010 at the Georgia Institute of Technology in Atlanta, Georgia. The study was approved by the Georgia Institute of Technology Institutional Review Board.

2.1 Participants

Participants were originally recruited from a database of individuals who had previously participated in research or had expressed interest in participating in research at the Georgia Institute of Technology. Additional younger adults and middle-aged adults were recruited by mailing postcards to a purchased list of names from an advertising agency.

Individuals were excluded from participating in the study if they did not speak English, did not have at least a high school education, were pregnant or breastfeeding, had a BMI greater than 35, indicated recreational drug use, indicated taking depression or anxiety medication, had a history of alcohol abuse, or reported a diagnosis of post-traumatic stress disorder, bipolar disorder, psychosis, eating disorder, dementia (e.g., Parkinson’s or Alzheimer’s Disease), hormone-producing cancers, or conditions related to the endocrine system (i.e., Cushing’s disease or Addison’s disease). Additional exclusion criteria included experiencing a nontypical week (i.e., death in the family or surgery) or having a schedule that would interfere with the intense nature of data collection (i.e., shift work). Full-time students (n = 18) were excluded from analyses, but part-time students (n = 9) were included. This subset of full-time students consisted primarily of Georgia

Institute of Technology students and was not representative of the general younger adult population in terms of cognitive ability.

2.2 Measures

2.2.1 Perceived Stress

The Perceived Stress Scale (Cohen et al., 1983) is a self-report questionnaire that measures one's current perceived stress. An adapted version of the questionnaire was used with 10 items to be answered by reflecting over the past month. An example item includes, "In the last month, how often have you been upset because of something that happened unexpectedly?" All items are rated on a 5-point scale ranging from 1 (*never*) to 5 (*very often*). Four items were reverse coded so that higher scores indicate higher levels of perceived stress. Three participants were missing the full measure. A sum score was calculated for each participant. Cronbach's alpha for the current study was .88.

2.2.2 Perceived Stress Reactivity

The Perceived Stress Reactivity Scale (Schulz et al., 2005) is a self-report questionnaire that measures the disposition of responding to stressors with immediate, intense, and/or long-lasting reactions. The questionnaire includes 29 items to be rated on a unique 3-point scale tailored to each item. An example item includes, "When I have many tasks and duties to fulfill…" and the respondent is given the option of indicating: "In general, I stay calm", "I usually get impatient", or "I often get irritable." Each rating was coded on a scale ranging from 1 (*lowest reactivity*) to 3 (*highest reactivity*). Fifteen items were reverse coded so that higher scores indicate higher levels of stress reactivity. Four participants were missing the full measure. An overall reactivity sum score was calculated for each participant. Cronbach's alpha for the current study was .89.

2.2.3 Cognitive Ability

A cognitive ability composite score using the mean of z-scores was created using five measures of cognitive ability: digit symbols, letter sets, number series, size judgement span, and category fluency. Higher composite scores indicate higher levels of cognitive ability. None of the participants were missing data on the following measures of cognitive ability.

2.2.3.1 Digit Symbols

Digit Symbols (Wechsler, 1997) is a measure of perceptual speed. The task has a key consisting of the numbers 1 through 9 with each number paired with a unique symbol. A series of the symbols are in random order and repeated below the key. Individuals are to write the correct number corresponding to each symbol for as many symbols as they can in two minutes. The digit symbols score is the total number of correct items.

2.2.3.2 Letter Sets

Letter Sets (Ekstrom et al., 1976) is a measure of fluid intelligence. Each item has five sets of letters with four letters in each set. Four of the sets are alike in some way while one set differs from the others. Individuals are to indicate which set of letters does not belong in each item for as many items as they can in seven minutes. The letter sets total score is the number of correct items.

2.2.3.3 Number Series

Number Series (Salthouse & Prill, 1987) is a measure of inductive reasoning. Five items were included in the task. Each item includes a series of numbers that either increase or decrease. Each set consists of a pattern and at the end of each set of numbers, individuals are to indicate what the next number in the series should be. The total number series score is the number of correct items.

2.2.3.4 Size Judgment Span

Size Judgement Span (Cherry & Park, 1993) is a measure of working memory. Individuals are read aloud a list of words and are to remember the words while they wait for a tone to sound. Once individuals hear the tone, they must write the words they heard in order of size (i.e., smallest object to largest object). For example, if the words were tiger, cell phone, and pineapple, the correct order would be cell phone, pineapple, tiger. With each trial, the lists of words become progressively longer. The total size judgement span score is the number of correctly ordered lists.

2.2.3.5 Category Fluency

Category Fluency (Drachman & Leavitt, 1972) is a measure of executive function. Individuals are given a category and asked to name as many examples that they can think of from that category in one minute. Five categories were used in this study, including fruits, flowers, girls' names, vegetables, and animals. The total category fluency score is the number of appropriately non-repeated responses given for each category.

2.2.4 Neuroticism

The Big Five Inventory (John et al., 1991) is a self-report questionnaire that assesses five dimensions of personality, including openness, conscientiousness, extraversion, agreeableness, and neuroticism. The questionnaire includes 44 items to be answered on a 5-point scale ranging from 1 (*Strongly Disagree*) to 5 (*Strongly Agree*). For purposes of this study, only neuroticism was evaluated, which assesses individuals' emotional stability. For example, respondents are asked to rate, "I see myself as someone who gets nervous easily" on the 5-point scale. Three participants were missing the full measure. Higher scores reflect greater levels of neuroticism. This personality variable was included as a covariate. Cronbach's alpha for the current study was .82.

2.2.5 Daily Stressor Exposure

Stressful events were reported on Palm Pilots using ecological momentary assessment. Five times per day for 10 consecutive days, participants indicated whether they experienced a stressful event by answering either "Yes" or "No" to the question, "Did you experience a problem/disruptive event [since the last beep]?". Survey prompts occurred at random times each day, approximately every three hours between 9:00 AM and 9:00 PM. Each participant could have reported up to 50 stressful events during the 10-day period.

A binary coding scheme was used to dichotomize non-stressor days versus stressor days. Days without any reported stressors were coded 0 and days with at least one reported stressor were coded 1. This binary variable was aggregated at the day-level to correspond to the diurnal cortisol and alpha-amylase measures (described below). In addition, the frequency of stressors reported per day was calculated by summing the number of times each participant indicated "yes" to experiencing a disruptive event each day on the Palm Pilots. A minimum of 0 stressors and a maximum of 5 stressors could be reported each day.

2.2.6 Salivary Cortisol and Alpha-Amylase

During the same 10-day period that stressful events were recorded on Palm Pilots, participants used Starstedt Salivettes to provide saliva samples. To give each saliva sample, participants were instructed to chew on cotton rolls for one minute (see Nater et al., 2013 for more information). Seven saliva samples were provided each day of the 10-day data collection period: once upon awakening, another 30-minutes post-awakening, and five additional saliva samples provided concurrently to the five daily EMA surveys. The saliva samples were assayed for free cortisol (nmol/L), alpha-amylase (U/ml), and dehydroepiandrosterone-sulfate (DHEA-S; ng/mol). DHEA-S was not analyzed in this study. The cortisol samples were analyzed using commercial chemiluminescence immunoassay (IBL in Hamburg, Germany) and the alpha-amylase samples were analyzed using a commercially available substrate reagent (EPS Sys; Roche Diagnostics). Each participant could have provided up to 70 saliva samples during the 10-day period, producing 70 possible values of cortisol and alpha-amylase per person.

2.3 Procedure

Participants were mailed a packet of paper-and-pencil self-report questionnaires prior to their first in-person laboratory visit. This packet included demographic questions, the Perceived Stress Scale (Cohen et al., 1983), Perceived Stress Reactivity Scale (Schulz et al., 2005), and the Big Five Inventory (John et al., 1991). Participants completed the questionnaires in their own time and brought the completed packet to their first session. Digit Symbols and Letter Sets were completed during participants' first laboratory visit. During this initial session, participants received instructions for the study and participated in one to three hours of training on how to use the Palm Pilot Tungsten T2 (Palm Inc., 2003).

The day following the in-person training session, participants began the ecological momentary assessment period where they reported their stressful experiences. At five randomly selected times per day for 10 consecutive days, participants were asked to complete surveys on Tungsten T2 handheld computers (Palm Inc., 2003). The beeps occurred within 15 minutes of 9:00 AM, 12:00 PM, 3:00 PM, 6:00 PM, and 9:00 PM each day. Each survey took approximately five minutes to complete. Research assistants followed up with each participant within 24 hours of the initial Palm Pilot training session to ensure that the participant understood the protocol. Participants who completed at least 80% of the surveys in the first three days of data collection were asked to continue completing surveys for the remainder of the data collection period. Individuals who did not complete at least 80% of the surveys in the first three days of the data collection period were asked to exit the study and were compensated $30.

In addition to completing surveys on the Palm Pilots during the 10-day data collection period, participants were also asked to provide saliva samples on Starstedt Salivettes seven times per day: immediately upon awakening while still lying in bed, 30 minutes post-awakening, and five additional times during each day. After the two morning samples, the remaining five daily saliva samples were provided concurrently with the EMA surveys on the Palm Pilots. The saliva samples yielded cortisol, alpha-amylase & DHEA-s readings.

Following the 10-day assessment period, participants returned to the laboratory. Category Fluency, Number Series, and Size Judgment Span were administered at this time. In addition, participants delivered their saliva samples and returned the Palm Pilots during this session. Participants were compensated $10 if they only completed the mailout packet of questionnaires and the baseline session. Individuals who did not complete at least 80% of the EMA surveys and corresponding salivettes within the first three days of the data collection period were asked to exit the study and were compensated $30. Participants were compensated $100 for completing the full study protocol.

2.3.1 Covariates

Confounding factors can affect salivary cortisol and alpha-amylase levels. Previous analyses on the data set in the current study demonstrated that sex, BMI, self-reported average daily cigarettes, and self-reported average daily coffees did not significantly influence the outcome variables (Nater et al., 2013). Even though these confounding factors did not influence the outcome variables in previous analyses, the current study still controlled for these variables in the models. Body mass index (using the formula BMI = [weight in pounds * 703] / [height in inches]^2), sex, age, daily cigarettes, and daily coffees were included as covariates in all models. Wakeup time (i.e., Sample Time 1) was included as a covariate for the models predicating the cortisol and alpha-amylase awakening responses (Skoluda et al., 2016; Stalder et al., 2016). For the daily covariates (i.e., wakeup time, daily coffees, daily cigarettes), person means and person-centered variables were included in the models to account for between- and within-person variability, respectively (Hoffman & Stawski, 2009). Finally, neuroticism was included as a covariate for its known relationship with stressor exposure and reactivity.

2.3.2 Indices of Cortisol and Alpha-Amylase Activity

Raw cortisol and alpha-amylase values that were three standard deviations above the mean of the respective assessment time were removed (Wrosch et al., 2007). For example, the raw cortisol values associated with Sample Time 1 (i.e., the first assessment of the day) were compared to the mean of cortisol at Sample Time 1. In addition, cortisol and alpha-amylase values that were missing time reports were removed before analysis, as missing time reports could lead to unreliable slope and area under the curve calculations.

The seven within-day cortisol and alpha-amylase observations were aggregated to produce day-level variables: the cortisol and alpha-amylase awakening response, the cortisol and alpha-amylase diurnal slopes based on waking and peak, and cortisol and alpha-amylase area under the curve with respect to ground (i.e., total daily output). These values were calculated for each of the 10 days of data collection as well as aggregated across the 10 days to create person-level variables. Due to the non-normal, positively skewed distribution of the biomarker data, the raw cortisol and alpha-amylase values were log transformed before calculating the awakening response and diurnal slopes. Area under the curve was calculated using raw cortisol and alpha-amylase values (Stawski, Cichy, et al., 2013).

Natural log transformed cortisol and alpha-amylase values were used to calculate the awakening response. The awakening response was calculated by modeling cortisol and alpha-amylase values as a function of wakening levels and time since waking (Almeida, Piazza, et al., 2009). The following equation was used to calculate the awakening response: *(ln Sample 2 – ln Sample 1) / (Sample Time 2 – Sample Time 1)*.

The diurnal slope from waking was calculated as change per hour using log transformed cortisol and alpha-amylase values. The diurnal wake-evening slope was calculated by subtracting the first sample post-awakening from the final, evening sample while taking into account time since wakening using the following equation: *(ln Sample 7 – ln Sample 1) / (Sample Time 7 – Sample Time 1)*.

The diurnal slope from peak was calculated as change per hour using log transformed cortisol and alpha-amylase values. The diurnal peak-evening slope was calculated by subtracting the second sample at 30-minute post-awakening from the final, evening sample while taking into account time since peaking level using the following equation: *(ln Sample 7 – ln Sample 2) / (Sample Time 7 – Sample Time 2)*.

Raw cortisol and alpha-amylase values were used to calculate area under the curve with respect to ground using the trapezoid formula from Pruessner et al. (2003). Area under the curve was not calculated if a morning sample (i.e., Sample Time 1) was missing or if there were more than three missing or invalid samples.

2.4 Statistical Analysis

Multilevel modeling (Raudenbush & Bryk, 2002) using maximum likelihood estimation in SAS PROC MIXED software (SAS Institute, 2008) was used to examine the hierarchically nested data. This statistical approach accounts for the nesting of days (Level 1) within people (Level 2) and permits observations of between-person and within-person variability. The variables at Level 1 vary across time (days) and the variables at Level 2 represent characteristics of the individuals in the sample. Models were conducted separately for each dependent variable, which included the cortisol and alpha-amylase awakening response, diurnal slopes, and area under the curve. Coefficients included error terms; intercepts and slopes were modeled as randomly varying, but non-significant random slopes were not retained.

Days with stressors as well as the daily covariates were entered as day-level (Level 1) predictors. Between-person (person mean) as well as within-person (person mean centered) variables of all Level 1 predictors were included in the models. Person-level (Level 2) predictors (body mass index, neuroticism, age, perceived stress, perceived stress reactivity, and cognitive ability) were grand mean centered. The statistical approach included all available data in the analyses. The mean was substituted for missing item-level data.

Regarding the additional analyses focused on the total number of stressors reported each day, the Level 1 variable was person mean centered and entered into the models in place of the dichotomous stressor day variable. The person mean of the frequency of stressors reported across the 10-day period was also included in the models in place of the person mean dichotomous stressor day variable to account for the fact that people differ in their average frequency of stressor exposures each day.

Before adding predictors to the models, variance estimates from unconditional models with only the dependent variable and random intercept were run to examine the proportion of variance associated with daily fluctuations (Level 1 variance; σ^2) versus variance associated with individual differences (Level 2 variance; τ^2). Intraclass correlation coefficients using the Level 1 and Level 2 variance estimates were calculated to examine the distribution of variance between-persons versus within-persons using the following formula: $\rho_I = \tau^2 / (\tau^2 + \sigma^2)$. An example of an unconditional model is below. Separate equations were used to account for the different diurnal cortisol and alpha amylase measures (denoted BIOMARKER). In the equations, days (i.e., timepoints) are represented by *t* (Level 1) and individuals are represented by *i* (Level 2).

Level 1: $\text{BIOMARKER}_{ti} = \beta_{0ti} + r_{ti}$

Level 2: $\beta_{0i} = \gamma_{00} + u_{0i}$

Using a forward stepping approach, covariates and predictors were added one at a time to each model. Squared predictors and interactions were entered at the second step. Each interaction was entered independently to examine whether the interaction term was significant and whether it improved model fit. Significant interactions were retained, and non-significant interactions were dropped from the models. If predictors remained significant after controlling for all other predictors in the models, then the final models included all predictors. Significant interactions were decomposed by computing simple slopes at the mean and ± 1 SD of the predictor variables. In addition to examining the moderating role perceived stress, perceived stress reactivity, age, and cognitive ability had on the relationship between daily stressors and the physiological measures, supplemental interactions were tested using the same criteria listed above. For example, it was examined whether the relationship between perceived stress and the biomarkers depended on age. A critical value of .05 was used for all statistical tests.

To understand the amount of within-person and between-person variance in the dependent variables explained by the predictors and to compare the variance estimates between the unconditional models and the final models, pseudo R^2 values were calculated for each model and a likelihood ratio test was run. The following equation was used to estimate the proportion of within-person variance explained by each model: $R_1^2 = 1 - [(\sigma^2 \text{ final} + \tau^2 \text{ final}) / (\sigma^2 \text{ unconditional} + \tau^2 \text{ unconditional})]$, where "$\sigma^2$ final" and "τ^2 final" are the Level 1 and Level 2 variance estimates for the final model, and "σ^2 unconditional" and

"τ^2 unconditional" are the Level 1 and Level 2 variance estimates for the unconditional model (Snijders & Bosker, 2011). Regarding the proportion of between-person variance explained by each model, the following equation was used: $R_2^2 = 1 - [(\sigma^2 \text{ final} / n + \tau^2 \text{ final}) / (\sigma^2 \text{ unconditional} / n + \tau^2 \text{ unconditional})]$, where "n" is number of days nested within individuals (Snijders & Bosker, 2011).

The following model is an example of the multilevel equations that will be estimated. This example allows a cross-level interaction with stressor days and the moderating individual difference variable perceived stress reactivity (PSRS).

(1) Level 1: $\text{BIOMARKER}_{ti} = \beta_{0ti} + \beta_{1ti}(\text{Stressor}) + \beta_{2ti}(\text{Wake Time}) + \beta_{3ti}(\text{Coffees}) + \beta_{4ti}(\text{Cigarettes}) + r_{ti}$

(2) Level 2: $\beta_{0i} = \gamma_{00} + \gamma_{01}(\text{Person Mean Stressor}) + \gamma_{02}(\text{Person Mean Wake Time}) + \gamma_{03}(\text{Person Mean Coffees}) + \gamma_{04}(\text{Person Mean Cigarettes}) + \gamma_{05}(\text{BMI}) + \gamma_{06}(\text{Sex}) + \gamma_{07}(\text{Age}) + \gamma_{08}(\text{Neuroticism}) + \gamma_{09}(\text{PSRS}) + \gamma_{010}(\text{PSS}) + \gamma_{011}(\text{Cognitive Ability}) + u_{0i}$

(3) $\beta_{1i} = \gamma_{10} + \gamma_{11}(\text{PSRS}) + u_{1i}$

$\beta_{2i} = \gamma_{20}$

$\beta_{3i} = \gamma_{30}$

$\beta_{4i} = \gamma_{40}$

In Equation 1 at Level 1, the intercept β_{0ti} is the expected physiological level for person *i* on days without stressor exposures (i.e., Stressor = 0). Note that the dichotomous "Stressor" variable was left uncentered so that the interpretation of the intercept could contrast stressor days with non-stressor days. Stressor reactivity, or the expected change in the physiological levels related to days with stressor exposures (Stressor = 1), is represented by the slope β_{1ti}. Day-level covariates (Wake Time, Coffees, Cigarettes) were included in Level 1. The error term r_{it} at Level 1 represents person *i*'s variability around their own average, or their unique effect.

The intercept and slope become outcome variables at Level 2 (see Equation 2). The covariates (Person Mean Wake Time, Person Mean Coffees, Person Mean Cigarettes, BMI, Sex [males = 0, females = 1], Age, Neuroticism), including an adjustment for individual differences in stressor exposures by including each person's mean number of stressor days across study days (Person Mean Stressor), were added at Level 2. Daily stressor exposure can vary across days and across people and therefore the person mean of stressor days was added as a covariate at Level 2 to adjust for the fact that some individuals have more stressor days than other individuals (Hoffman & Stawski, 2009). This same logic was applied to all Level 1 covariates (Wake Time, Coffees, Cigarettes). The intercept γ_{00} represents the average level of physiological arousal on stressor-free days when all variables are at their means. The slope β_{0i} examines the between-person effect of the individual difference variables (e.g., PSRS) on the average level of the diurnal physiological measures. In other words, γ_{01} through γ_{011} represent the main effects of stressor days, the covariates, and the individual difference variables on the biomarker data. Between-person variability from the average level of the diurnal psychological profiles is represented by u_{0i}.

In Equation 3, β_{1i} tests for differences in the moderating variables (e.g., PSRS) in the intraindividual relationship between days with stressors and diurnal physiological arousal. In this equation, γ_{10} depicts the mean change in the biomarker data on days with and without stressors while all other variables are at their means. γ_{11} depicts the cross-level interaction between stressor days at Level 1 and the person-level variables (e.g., PSRS) at Level 2. The random slope for the stressor day variable is represented by u_{1i}.

CHAPTER 3. RESULTS

3.1 Sample Characteristics

The final sample included 156 participants (50.6% female) ranging from young adulthood to older age (*M* = 51.45, *SD* = 18.30, range = 20-77). Seven (12.28%) of the middle-aged adults and 52 (89.66%) of the older adults were retired. There were no age differences in self-reported health [$F(2,151) = 0.37$, $p = .69$]. The sample was representative of the Atlanta, Georgia metropolitan area regarding gender, socioeconomic status, and racial and ethnic diversity (Scott et al., 2013). Sample characteristics are in Table 1.

Table 1 – Sample Characteristics

Variable	*N* (%)	*M* (*SD*)
Sex		
Female	79 (50.6)	
Male	77 (49.4)	
Age [a] (20-77 years)		51.45 (18.30)
Young (20-34 years)	41 (26.3)	26.58 (3.09)
Middle (35-64 years)	57 (36.5)	49.43 (7.36)
Old (65+ years)	58 (37.2)	71.03 (3.46)
Hispanic/Latino	3 (2.0)	
Non-Hispanic/Latino	150 (98.0)	
Race		
African American	28 (17.9)	
Asian	3 (1.9)	
Caucasian	117 (75.0)	
More than one race	6 (3.8)	
Other	2 (1.3)	
Education		
Less than 12 years	1 (0.7)	
High school diploma	8 (5.3)	
Technical/vocational/trade school	11 (7.2)	
College freshman	4 (2.6)	
College sophomore	14 (9.2)	
College junior	10 (6.6)	
Bachelor's degree	64 (42.1)	
Master's degree	32 (21.1)	
JD, MD, or PhD	8 (5.3)	

Note. [a] Age was treated as a continuous variable in analyses but depicted as a categorical variable in Table 1 to display the age distribution.

3.2 Stress Surveys and Saliva Samples

Of the 7,800 EMA surveys (156 persons X 5 surveys X 10 days), 7,321 were complete (479 surveys were missing, or 6.14%). Participants reported a total of 997 stressful events across 7,321 valid EMA surveys during the 10-day study period (13.62% of the momentary reports). The EMA surveys were aggregated across days to produce a dichotomous variable of stressor days and stressor-free days. At the daily-level, participants experienced 686 stressor days out of 1,560 study days (156 persons X 10 days), indicating an aggregate daily stressor prevalence rate of 43.97%.

Regarding the frequency of stressors reported per day, zero stressors were reported on 872 days (55.97% of days), one stressor was reported on 451 days (28.95% of days) and two or more stressors were reported on 235 days (15.08% of days). The range of the number of stressors reported per day was 0 to 5 with a mean of 0.64 (*SD* = 0.47) stressors per day across people.

Of the 10,920 cortisol and alpha-amylase samples (156 persons X 7 saliva samples X 10 days), 9,954 valid cortisol and 9,884 valid alpha-amylase values were aggregated to calculate the awakening responses, diurnal slopes, and total daily output measures. Of the 1,560 study days, this study included 1,424 cortisol awakening responses, 1,376 wake-evening cortisol slopes, 1,328 peak-evening cortisol slopes, 1,484 cortisol area under the curve values, 1,360 alpha-amylase awakening responses, 1,335 wake-evening alpha-amylase slopes, 1,296 peak-evening alpha-amylase slopes, and 1,444 alpha-amylase area under the curve values. Figure 1 and Figure 2 show the average diurnal cortisol and alpha-amylase profiles, respectively, across people and study days.

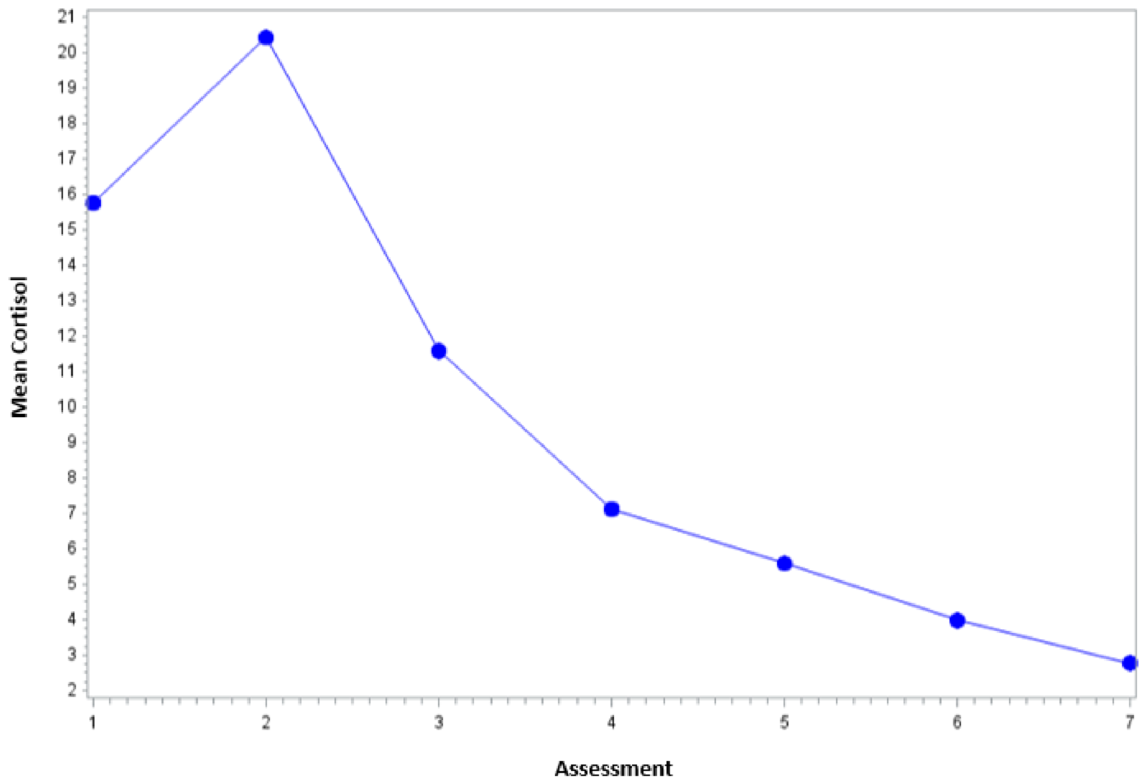

Figure 1 – Diurnal Cortisol Rhythm

Note. Raw cortisol values were averaged across people and study days at each of the seven daily assessments.

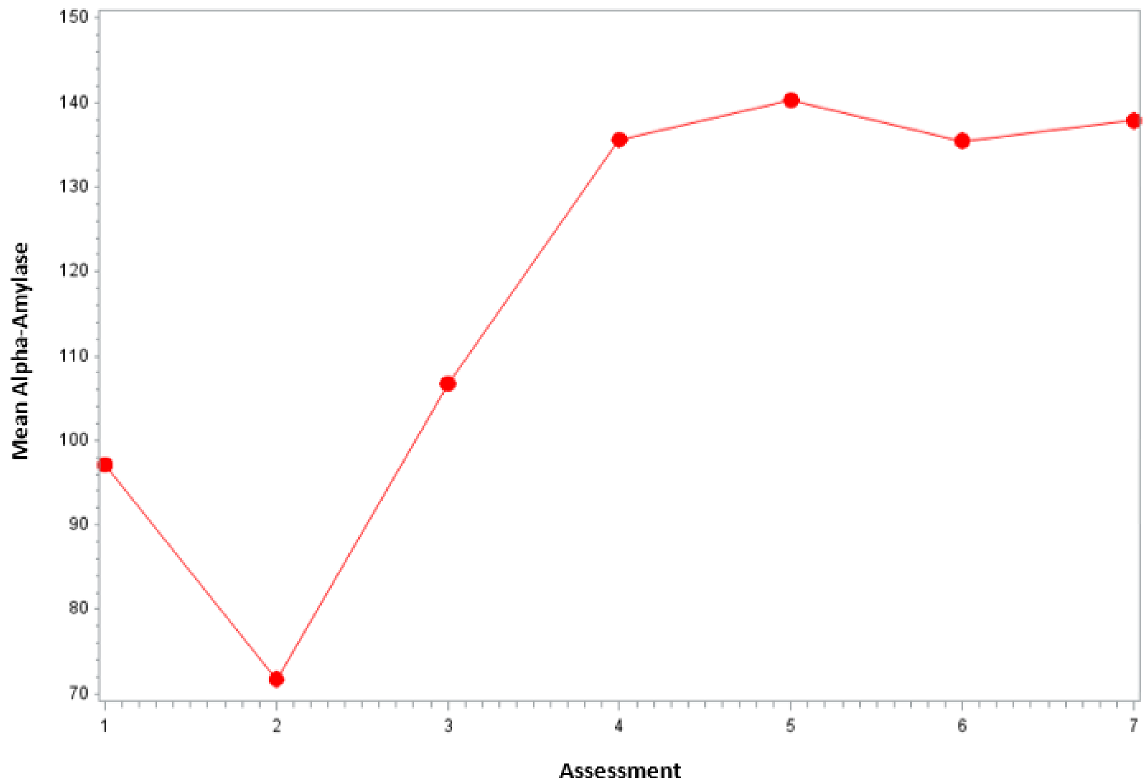

Figure 2 – Diurnal Alpha-Amylase Rhythm

Note. Raw alpha-amylase values were averaged across people and study days at each of the seven daily assessments.

3.3 Zero-Order Correlations

Descriptive statistics and between-person correlations among the primary study variables are in Table 2. There were several noteworthy correlations among the cortisol and alpha-amylase measures. The cortisol awakening response was negatively correlated with the cortisol peak-evening slope and the cortisol wake-evening slope was positively correlated with cortisol area under the curve. The alpha-amylase awakening response had a significant positive relationship with the alpha-amylase wake-evening slope and a significant negative correlation with the alpha-amylase peak-evening slope.

Table 2 – Means, Standard Deviations, and Between-Person Correlations Among Study Variables

Variable	*M*	*SD*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Daily Stressors [a,b]	0.44	0.25	-														
2. Total Stressors Per Day [c]	0.64	0.47	.91**	-													
3. Age	51.45	18.30	-.08	-.08	-												
4. Neuroticism	2.24	0.70	.08	.09	-.25**	-											
5. Perceived Stress	22.65	5.70	.08	.08	-.20*	.56**	-										
6. Perceived Stress Reactivity	48.64	8.03	.20*	.23**	-.20*	.64**	.50**	-									
7. Cognitive Ability	0.00	0.73	.24**	.24**	-.67**	.21**	.09	.21*	-								
8. Cortisol Awakening Response	0.59	0.72	-.02	-.08	.13	-.13	-.15	-.05	-.03	-							
9. Cortisol Wake-Evening Slope	-0.13	0.04	-.04	-.04	.24**	.01	-.12	.05	-.18*	.16	-						
10. Cortisol Peak-Evening Slope	-0.15	0.04	-.08	-.08	.16*	.01	-.08	.03	-.11	-.28**	.84**	-					
11. Cortisol AUC	109.65	26.12	-.03	.02	.17*	-.01	-.04	-.06	-.12	.06	.23**	.16	-				
12. sAA Awakening Response	-0.58	1.22	-.06	-.06	.05	.20*	.10	.10	-.08	-.22**	-.02	.09	-.02	-			
13. sAA Wake-Evening Slope	0.04	0.04	-.02	-.03	-.20*	.08	.09	.19*	.12	-.02	-.02	.00	.04	.48**	-		
14. sAA Peak-Evening Slope	0.06	0.04	-.07	-.09	-.29**	-.13	-.02	.07	.21**	.14	-.02	-.07	.04	-.37**	.59**	-	
15. sAA AUC	1,793.03	899.15	-.14	-.15	.17*	-.10	-.08	-.21**	-.15	.05	-.02	-.05	-.02	.04	-.14	-.14	-

Note. Cortisol values are measured in nmol/l. Alpha-amylase values are measured in U/ml. sAA = Alpha-amylase. AUC = Area Under the Curve.
[a] Daily Stressors coded 0 = stressor-free day and 1 = stressor day. [b] Mean value reflects the average number of days with a stressor across study days. [c] Mean value reflects the sum of stressors reported each day averaged across study days.
* $p \leq .05$. ** $p \leq .01$.

Regarding significant correlations across the two stress systems, the cortisol awakening response was negatively correlated with the alpha-amylase awakening response. None of the other cortisol measures were significantly correlated with the alpha-amylase measures.

The frequency of stressor days across people was not significantly correlated with any of the diurnal cortisol or alpha-amylase measures. The frequency of stressors days was also not significantly correlated with age, neuroticism, or perceived stress, as measured by the Perceived Stress Scale. The frequency of stressor days had a significant positive correlation with the Perceived Stress Reactivity total score and with cognitive ability. People with higher perceived stress reactivity and people with better cognitive ability reported stressor days more frequently.

3.4 Unconditional Models

Fully unconditional models were estimated to partition the variance of cortisol and alpha-amylase into within-person and between-person sources (see Table 3). In general, a greater proportion of variance in the diurnal cortisol and alpha-amylase measures was found within-people compared to between-people except for alpha-amylase area under the curve, where 77.59% of the variance was between-persons. Results from the fully unconditional models indicated that there was sufficient variability in the outcome variables for further analyses using multilevel modeling.

Table 3 – Fully Unconditional Multilevel Models Predicting Diurnal Physiological Measures: Proportion of Variance Within-Persons Versus Between-Persons

Biomarker	Variance Within-Persons	Variance Between-Persons
Cortisol		
Awakening Response	90.09%	9.91%
Wake-Evening Slope	63.10%	36.90%
Peak-Evening Slope	60.88%	39.12%
Area Under the Curve	52.07%	47.93%
Alpha-Amylase		
Awakening Response	71.86%	28.14%
Wake-Evening Slope	63.98%	36.02%
Peak-Evening Slope	59.65%	40.36%
Area Under the Curve	22.41%	77.59%

Note. Intraclass correlation coefficients were calculated using the formula: $\rho_I = \tau^2 / (\tau^2 + \sigma^2)$.

3.5 Diurnal Cortisol Measures

Models predicting the cortisol awakening response, wake-evening slope, peak-evening slope, and area under the curve were estimated twice. The initial models included the binary stressor day versus stressor-free day variable, and the second models replaced the binary stressor day variable with the variable that accounted for the total number of stressors reported each day.

3.5.1 Daily Stressors

Regarding the models that included the binary variable of stressor days versus stressor-free days, estimates, standard errors, and the proportion of variance accounted for by each model are reported in Table 4.

With respect to the cortisol awakening response, later daily wakeup times predicted flatter daily awakening responses (γ_{20} = -0.147, t = -3.00, p < .01). Sex was a significant between-person predictor of the average awakening response, such that females had steeper morning slopes (γ_{06} = 0.224, t = 1.97, $p \leq .05$). The model explained 1.1% of the within-person variance and 9.4% of the between-person variance in the cortisol awakening response.

With respect to the cortisol wake-evening slope, more daily cigarettes predicted steeper daily wake-evening slopes (γ_{04} = -0.010, t = -2.33, $p < .05$). There was trend towards statistical significance of daily stressors predicting flatter daily wake-evening slopes (γ_{10} = 0.005, t = 1.85, p = .06). Between-persons, there was a trend towards significance regarding higher levels of perceived stress predicting steeper average wake-evening slopes (γ_{010} = -0.001, t = -1.78, p = .08) and older age predicting flatter average slopes (γ_{07} = 0.000, t = 1.75, p = .08). The model explained 3.7% of the within-person variance and 9.2% of the between-person variance in the cortisol wake-evening slope.

With respect to the cortisol peak-evening slope, there were no significant within-person or between-person predictors. Although the relationship was not reported in Table 4, there was a trend towards significance with perceived stress moderating the relationship between the average frequency of stressor days and the average peak-evening slope (γ_{012} = -0.004, t = -1.74, p = .08). Individuals who experienced more stressor days on average and had higher levels of perceived stress had steeper cortisol peak-evening slopes. The model explained 2.5% of the within-person variance and 4.5% of the between-person variance in the cortisol peak-evening slope.

With respect to cortisol area under the curve, total daily cortisol output was higher on days with stressor exposures compared to stressor-free days (γ_{10} = 4.157, t = 2.64, $p <$ .01). Between-persons, higher BMI predicted lower total daily cortisol output (γ_{05} = -2.019,

t = -3.58, $p < .01$) and older age predicted higher total daily cortisol output (γ_{07} = 0.329, t = 2.07, $p < .05$). Perceived stress trended towards statistical significance as a moderator of the relationship between the average frequency of stressor days and average cortisol area under the curve (γ_{012} = -2.865, t = -1.93, p = .06). Individuals who experienced more stressor days and had higher levels of perceived stress typically had lower cortisol area under the curve compared to individuals lower in perceived stress. The model explained 5.4% of the within-person variance and 10.3% of the between-person variance in cortisol area under the curve.

Table 4 – Two-Level Models with Daily Stressors Predicting the Diurnal Cortisol Measures using Maximum Likelihood Estimation (*N* = 156)

	Awakening Response		Wake-Evening Slope		Peak-Evening Slope		Area Under the Curve	
Fixed Effects	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Intercept	0.181	0.511	-0.130**	0.008	-0.145**	0.009	114.780**	5.458
Daily Wakeup Time WP	-0.147**	0.049						
Average Wakeup Time BP	0.036	0.068						
Daily Coffees WP	-0.043	0.052	0.001	0.002	0.003	0.002	1.615†	0.888
Average Coffees BP	0.044	0.058	0.000	0.003	-0.002	0.003	-0.717	2.153
Daily Cigarettes WP	0.122	0.135	-0.010*	0.004	-0.008†	0.004	0.010	2.354
Average Cigarettes BP	-0.016	0.066	0.000	0.004	0.002	0.004	0.905	2.429
Daily Stressors WP	-0.056	0.092	0.005†	0.003	0.002	0.003	4.157**	1.574
Average Daily Stressors BP	0.052	0.251	-0.009	0.013	-0.018	0.014	-8.668	8.885
Body Mass Index	0.016	0.015	0.000	0.001	0.000	0.001	-2.019**	0.564
Sex	0.224*	0.113	0.007	0.006	0.000	0.007	-4.954	4.224
Age	0.005	0.004	0.000†	0.000	0.000	0.000	0.329*	0.159
Neuroticism	-0.096	0.108	0.006	0.006	0.003	0.006	2.466	4.021
Perceived Stress Scale	-0.014	0.012	-0.001†	0.001	-0.001	0.001	0.017	0.445
Stress Reactivity Scale	0.006	0.009	0.001	0.001	0.001	0.001	-0.235	0.344
Cognitive Ability	0.091	0.107	-0.005	0.006	0.000	0.006	0.171	4.003
Random Effects								
Residual	2.175**	.087	0.001**	0.000	0.002**	0.000	660.840**	25.971
Intercept Level 1	0.193**	.051	0.002**	0.000	0.001**	0.000	537.920**	69.780
Within-Person Pseudo R^2	0.011		0.037		0.025		0.054	
Between-Person Pseudo R^2	0.094		0.092		0.045		0.103	

Note. Within-person and between-person variables were included for all daily (Level 1) variables. WP = Within-Persons (Person Mean Centered). BP = Between-Persons (Person Mean). All individual difference variables were grand-mean centered. Daily Stressors coded 0 = stressor-free day, 1 = stressor day. Sex coded male = 0, female = 1.

** $p \leq .01$. * $p \leq .05$. † $p \leq .08$.

3.5.2 Total Stressors Per Day

Regarding the models that included the total number of stressors per day variable (rather than the binary variable of stressor days versus stress-free days), estimates, standard errors, and the proportion of variance accounted for by each model are in Table 5.

With respect to the cortisol awakening response, later daily wakeup times predicted flatter daily awakening responses (γ_{20} = -0.148, t = -2.99, p < .01). Women had steeper awakening responses (γ_{06} = 0.230, t = 2.04, p < .05) than men. The model explained 1.2% of the within-person variance and 9.9% of the between-person variance in the cortisol awakening response.

With respect to the cortisol wake-evening slope, more daily cigarettes predicted steeper daily slopes (γ_{40} = -0.010, t = -2.31, p < .05). There were no significant between-person effects predicting the wake-evening cortisol slope. Older age trended towards significance predicting a flatter slope (γ_{07} = 0.000, t = 1.78, p = .08). There was also a trend towards significance with perceived stress moderating the relationship between the average number of stressors per day and the wake-evening slope (γ_{012} = -0.002, t = -1.90, p = .06). People who experienced more stressors on average and had higher perceived stress tended to have steeper wake-evening slopes compared to people lower in perceived stress. The model explained 4.5% of the within-person variance and 11.2% of the between-person variance in the cortisol wake-evening slope.

With respect to the cortisol peak-evening slope, there were no significant within-person or between-person predictors. There was a significant Level 2 interaction between the average number of stressors per day and individual differences in perceived stress (γ_{012}

= -0.003, t = -1.96, $p \leq .05$), such that individuals who tended to experience more stressors per day and had higher levels of perceived stress tended to have steeper peak-evening slopes compared to individuals lower in perceived stress (see Figure 3). The model explained 3.6% of the within-person variance and 6.9% of the between-person variance in the cortisol peak-evening slope.

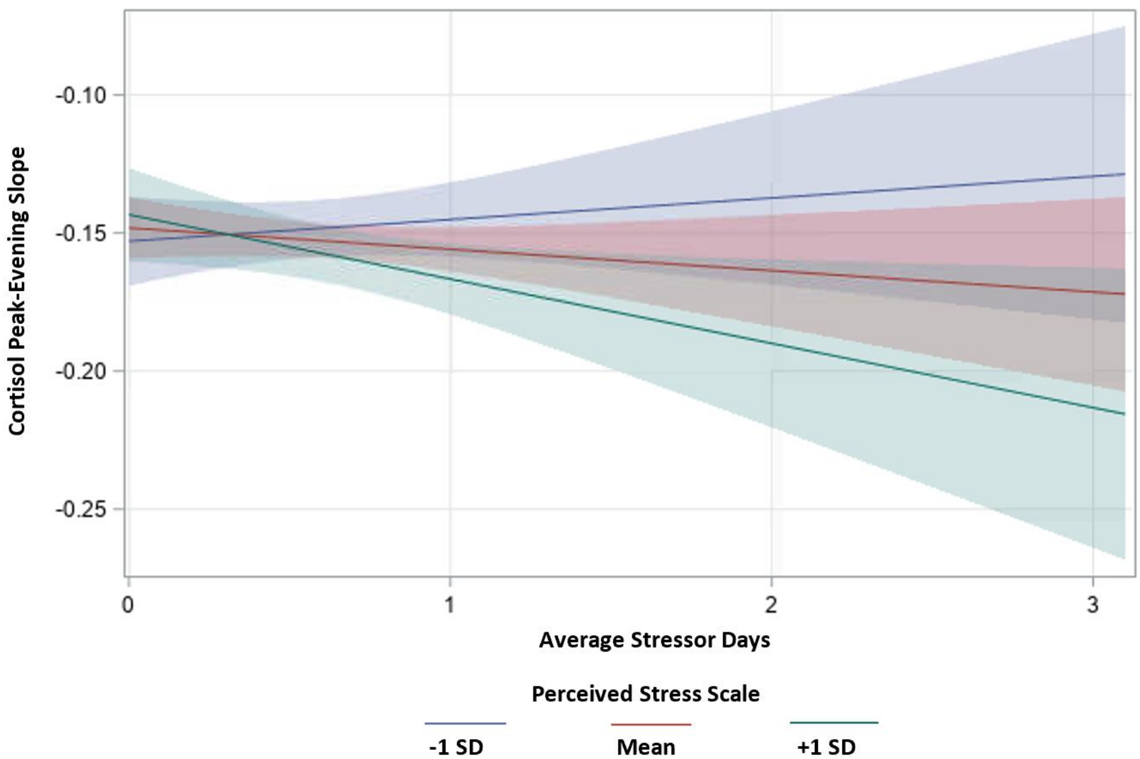

Figure 3 – The Relationship Between the Average Number of Stressors Reported Per Day and the Average Cortisol Peak-Evening Slope Depended on Perceived Stress

With respect to cortisol area under the curve, daily stressors predicted greater total daily cortisol output (γ_{10} = 2.399, t = 2.58, $p \leq .01$). Between-persons, higher BMI predicted lower average total cortisol output (γ_{05} = -1.987, t = -3.50, $p < .01$) and older age predicted higher average total cortisol output (γ_{07} = 0.313, t = 1.97, $p \leq .05$). The model explained 5.3% of the within-person variance and 10.1% of the between-person variance in cortisol area under the curve.

Table 5 – Two-Level Models with Total Daily Stressors Predicting the Diurnal Cortisol Measures using Maximum Likelihood Estimation (*N* = 156)

	Awakening Response		**Wake-Evening Slope**		**Peak-Evening Slope**		**Area Under the Curve**	
Fixed Effects	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Intercept	0.289	0.502	-0.130**	0.007	-0.146**	0.008	112.650**	4.926
Daily Wakeup Time WP	-0.148**	0.049						
Average Wakeup Time BP	0.031	0.068						
Daily Coffees WP	-0.043	0.052	0.001	0.002	0.003	0.002	1.628†	0.888
Average Coffees BP	0.037	0.058	0.001	0.002	-0.002	0.003	-0.574	2.151
Daily Cigarettes WP	0.121	0.135	-0.010*	0.004	-0.008	0.004	0.091	2.354
Average Cigarettes BP	-0.017	0.066	0.000	0.004	0.001	0.004	0.898	2.432
Total Daily Stressors WP	-0.031	0.055	0.002	0.002	0.001	0.002	2.399**	0.930
Average Total Daily Stressors BP	-0.111	0.123	-0.002	0.007	-0.008	0.007	0.203	4.586
Body Mass Index	0.014	0.015	0.000	0.001	0.000	0.001	-1.987**	0.568
Sex	0.230*	0.112	0.007	0.006	0.000	0.007	-5.268	4.203
Age	0.005	0.004	0.000†	0.000	0.000	0.000	0.313*	0.159
Neuroticism	-0.102	0.108	0.007	0.006	0.005	0.006	2.612	4.028
Perceived Stress Scale	-0.014	0.012	-0.000	0.001	0.001	0.001	0.006	0.445
Stress Reactivity Scale	0.007	0.009	0.000	0.001	0.000	0.001	-0.263	0.346
Cognitive Ability	0.114	0.107	-0.005	0.006	-0.001	0.006	-0.423	3.994
Average Total Daily Stressors BP x Perceived Stress			-0.002†	0.001	-0.003*	0.001		
Random Effects								
Residual	2.175**	0.088	0.002**	0.000	0.002**	0.000	660.94**	25.975
Intercept Level 1	0.191**	0.051	0.001*	0.000	0.001**	0.000	539.44**	69.955
Within-Person Pseudo R^2	0.012		0.045		0.036		0.053	
Between-Person Pseudo R^2	0.099		0.112		0.069		0.101	

Note. Within-person and between-person variables were included for all daily (Level 1) variables. WP = Within-Persons (Person Mean Centered). BP = Between-Persons (Person Mean). All individual difference variables were grand-mean centered. Daily Stressors coded 0 = stressor-free day, 1 = stressor day. Sex coded male = 0, female = 1.

** $p \leq .01$. * $p \leq .05$. † $p \leq .08$.

3.6 Diurnal Alpha-Amylase Measures

Models predicting the alpha-amylase awakening response, wake-evening slope, peak-evening slope, and area under the curve were estimated twice. The initial models included the binary variable of stressor days versus stressor-free days, and the second models replaced the binary stressor day variable with the variable that accounted for the total number of stressors reported each day.

3.6.1 Daily Stressors

Regarding models that included the binary variable of stressor days versus stressor-free days, estimates, standard errors, and the proportion of variance accounted for by each model are in Table 6.

With respect to the alpha-amylase awakening response, there were no significant within-person predictors. Older age predicted a steeper awakening response (γ_{07} = -0.091, t = -2.16, p < .05) and higher neuroticism predicted a flatter alpha-amylase awakening response (γ_{08} = 0.395, t = 2.18, p < .05). There was a trend towards significance with the average frequency of daily stressors predicting flatter average awakening responses (γ_{01} = 2.072 t = 1.86, p = .07). There was a quadratic effect of age on the awakening response (γ_{012} = 0.001, t = 2.90, p < .01), such that middle-aged adults had the steepest morning declines compared to younger and older adults, whose awakening responses were flatter (see Figure 4). There was a significant Level 2 interaction between average daily stressors and individual differences in age (γ_{013} = -0.040, t = -2.01, p < .05), such that a greater frequency of days with stressors were related to a steeper alpha-amylase awakening response for older adults and a flatter awakening response for younger adults (see Figure 5). The model explained 6.2% of the within-person variance and 18.4% of the between-person variance in the alpha-amylase awakening response.

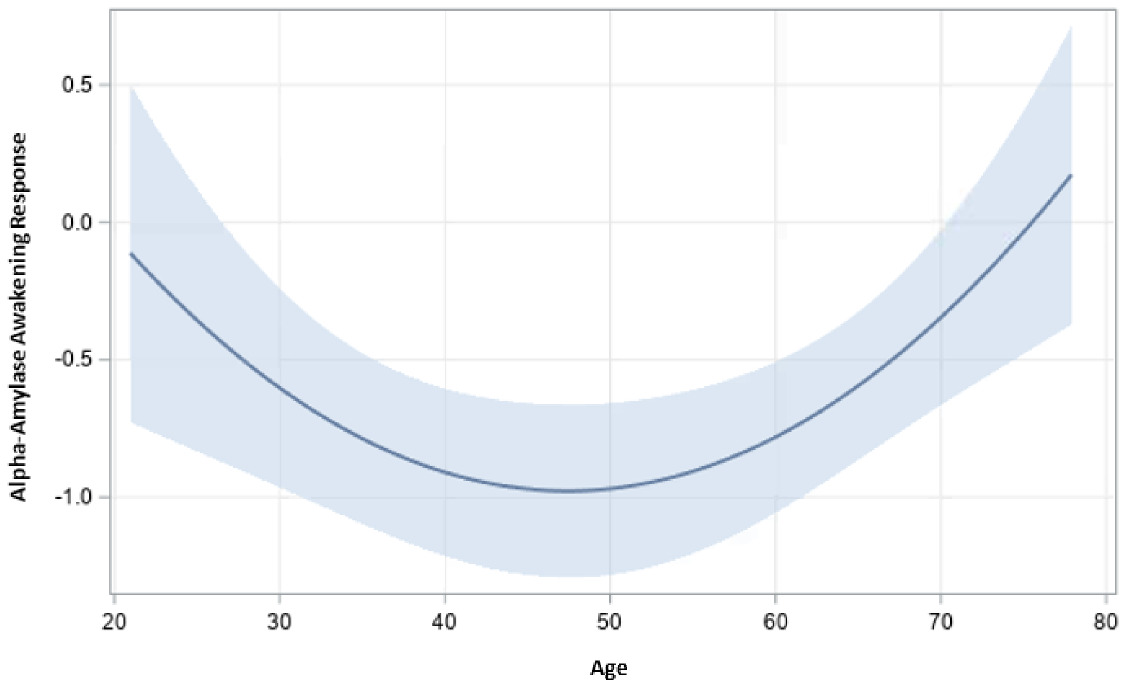

Figure 4 – Quadratic Age Effect Predicting the Alpha-Amylase Awakening Response

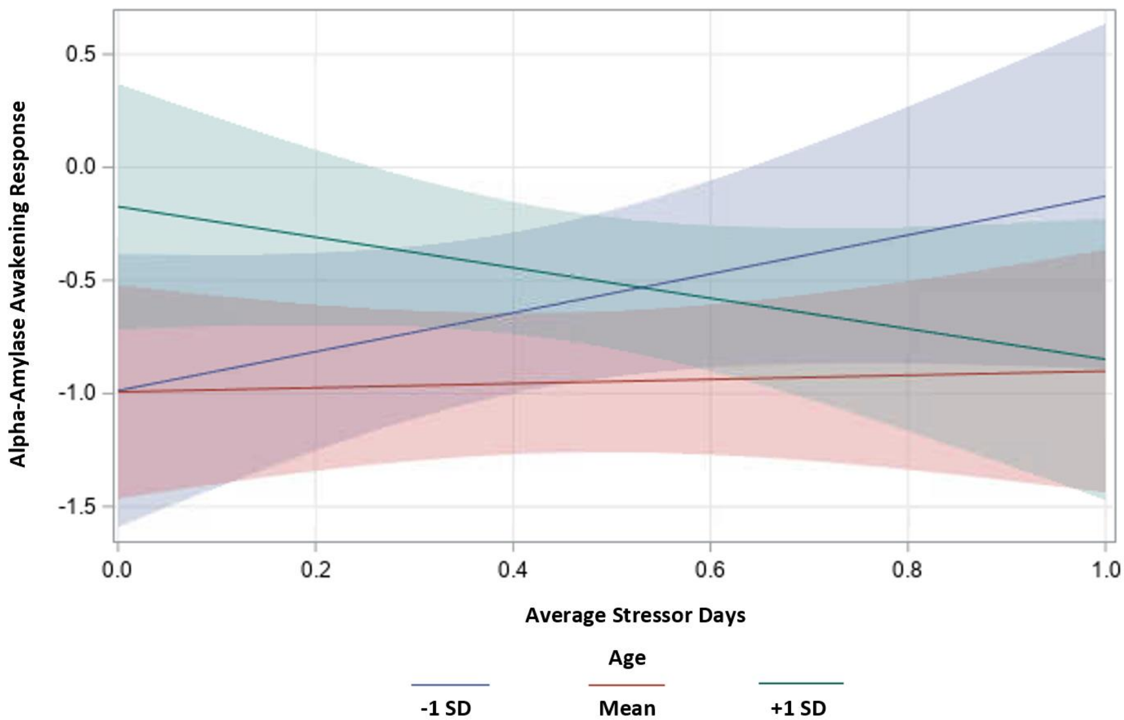

Figure 5 – The Relationship Between the Average Frequency of Daily Stressors and the Average Alpha-Amylase Awakening Response Depended on Age

With respect to the alpha-amylase wake-evening slope, there was a trend towards statistical significance with daily stressors predicting steeper daily slopes (γ_{10} = 0.006, t = 1.82, p = .07). Higher perceived stress reactivity predicted a steeper slope (γ_{09} = 0.001, t = 2.13, p < .05) and there was a trend towards significance of older age predicting a flatter slope (γ_{07} = 0.000, t = -1.81, p = .07). The model explained 5.4% of the within-person variance and 11.1% of the between-person variance in the alpha-amylase wake-evening slope.

With respect to the alpha-amylase peak-evening slope, more daily cigarettes predicted steeper daily slopes (γ_{40} = 0.010, t = 2.02, p < .05). Between-persons, a greater average frequency of daily stressors predicted a flatter average slope (γ_{01} = -0.028, t = -1.95, $p \leq$ .05). In addition, older age (γ_{07} = -0.001, t = -3.06, p < .01) and higher neuroticism

(γ_{08} = -0.023, t = -3.58, p < .01) predicted a flatter average slope, and higher perceived stress reactivity predicted a steeper average slope (γ_{09} = 0.001, t = 2.71, p < .01). A significant Level 2 interaction was detected between individual differences in cognitive ability and the sample average alpha-amylase peak-evening slope, moderated by perceived stress reactivity (γ_{012} = -.001, t = -2.17, p < .05). Persons who were high in perceived stress reactivity but low in cognitive ability tended to have steeper alpha-amylase peak-evening slopes compared to persons low in perceived stress reactivity (see Figure 6). The model explained 11% of the within-person variance and 22% of the between-person variance in the alpha-amylase peak-evening slope.

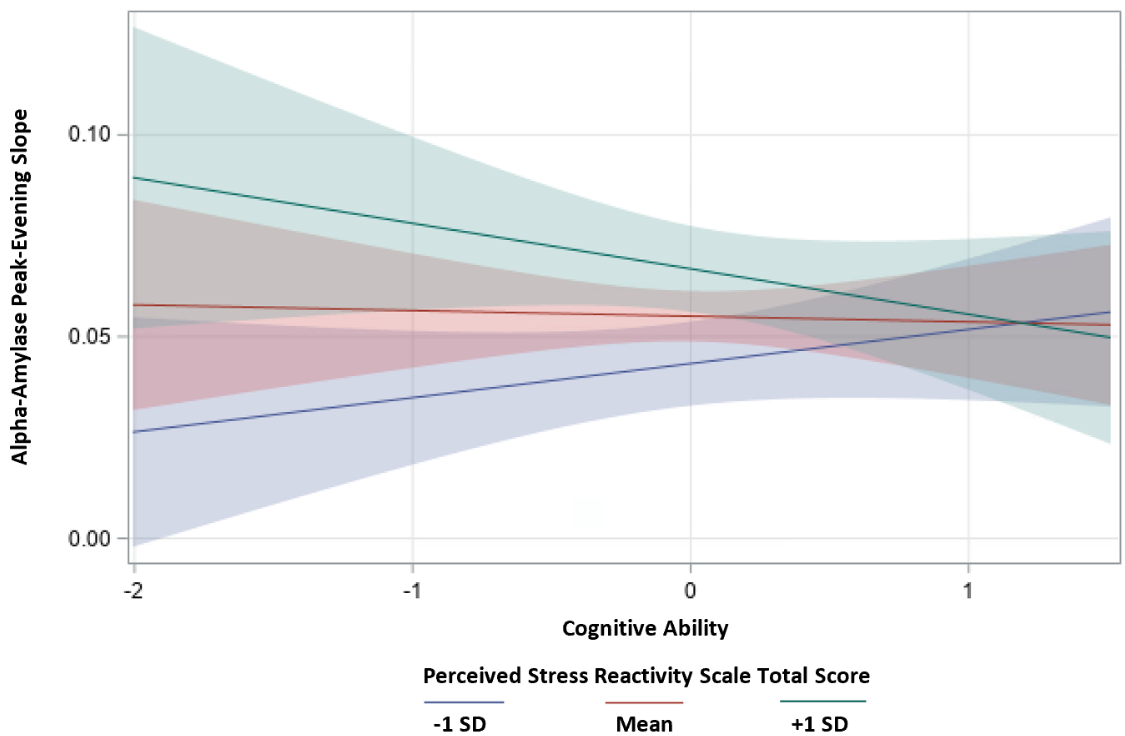

Figure 6 – The Relationship Between Cognitive Ability and the Alpha-Amylase Peak-Evening Slope Depended on Perceived Stress Reactivity

With respect to alpha-amylase area under the curve, more daily coffees predicted higher total daily alpha-amylase output (γ_{30} = 46.089, t = 2.77, $p < .01$). Between-persons, higher perceived stress reactivity predicted lower average total alpha-amylase output (γ_{09} = -23.791, t = -2.02, $p < .05$). The model explained 8.7% of the within-person variance and 10.6% of the between-person variance in alpha-amylase area under the curve.

Table 6 – Two-Level Models with Daily Stressors Predicting the Diurnal Alpha-Amylase Measures using Maximum Likelihood Estimation (*N* = 156)

	Awakening Response		**Wake-Evening Slope**		**Peak-Evening Slope**		**Area Under the Curve**	
Fixed Effects	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Intercept	0.239	1.487	0.050**	0.009	0.062**	0.009	1,853.330**	186.860
Daily Wakeup Time WP	0.092	0.059						
Average Wakeup Time BP	0.108	0.114						
Daily Coffees WP	0.081	0.062	-0.001	0.002	-0.002	0.002	46.089**	16.667
Average Coffees BP	-0.125	0.096	-0.004	0.003	0.001	0.003	-11.732	73.784
Daily Cigarettes WP	-0.174	0.159	0.007	0.005	0.010*	0.005	20.524	43.181
Average Cigarettes BP	0.046	0.112	-0.003	0.004	-0.004	0.004	65.332	83.340
Daily Stressors WP	0.045	0.108	0.006†	0.003	0.004	0.003	15.802	29.169
Average Daily Stressors BP	2.072†	1.114	-0.013	0.014	-0.028*	0.014	-279.960	300.640
Body Mass Index	-0.003	0.025	0.000	0.001	0.000	0.001	37.457†	19.315
Sex	-0.347†	0.186	-0.009	0.007	0.008	0.007	153.580	144.620
Age	-0.091*	0.042	0.000†	0.000	-0.001**	0.000	4.233	5.447
Neuroticism	0.395*	0.181	-0.005	0.006	-0.023**	0.006	111.450	137.850
Perceived Stress Scale	-0.012	0.021	0.000	0.001	0.000	0.001	-0.336	15.233
Stress Reactivity Scale	-0.005	0.015	0.001*	0.001	0.001**	0.001	-23.791*	11.804
Cognitive Ability	-0.073	0.176	-0.001	0.006	-0.001	0.006	-43.973	137.100
Age x Age	0.001**	0.000						
Average Daily Stressors BP x Age	-0.040*	0.020						
Cognitive Ability x Perceived Stress Reactivity					-0.001*	0.001		
Random Effects								
Residual	2.816**	0.116	0.002**	0.000	0.002**	0.000	220,857**	8,809.260
Intercept Level 1	0.842**	0.137	0.001**	0.000	0.001**	0.000	688,592**	82,041
Within-Person Pseudo R^2	0.062		0.054		0.110		0.087	
Between-Person Pseudo R^2	0.184		0.111		0.220		0.106	

Note. Within-person and between-person variables were included for all daily (Level 1) variables. WP = Within-Persons (Person Mean Centered). BP = Between-Persons (Person Mean). All individual difference variables were grand-mean centered. Sex coded male = 0, female = 1.
** $p \leq .01$. * $p \leq .05$. † $p \leq .08$.

3.6.2 Total Stressors Per Day

Regarding models that included the total number of stressors per day instead of the binary stressor day variable, estimates, standard errors, and the proportion of variance accounted for by each model are in Table 7.

With respect to the alpha-amylase awakening response, there were no significant within-person predictors. Older age (γ_{07} = 0.023, t = 2.38, $p < .05$) and higher neuroticism (γ_{08} = 0.396, t = 2.19, $p < .05$) predicted flatter alpha-amylase awakening responses. Similar to the model controlling for daily stressors, there was also a quadratic effect of age on the awakening response (γ_{012} = 0.001, t = 2.77, $p < .01$). A significant Level 2 interaction was found between average daily stressors and age (γ_{013} = -0.022, t = -2.01, $p \leq .05$), such that individuals who tended to experience more stressors and were older had steeper awakening responses compared to younger individuals. Finally, there was a significant cross-level interaction between daily fluctuations in the number of stressor exposures reported and individual differences in perceived stress reactivity (γ_{011} = -0.020, t = -2.61, $p < .01$). Individuals with higher levels of perceived stress reactivity had steeper alpha-amylase awakening responses on days with more stressor exposures, whereas individuals with lower levels of perceived stress reactivity had flatter alpha-amylase awakening responses on days with more stressor exposures (see Figure 7). The model explained 6.5% of the within-person variance and 18.1% of the between-person variance in the alpha-amylase awakening response.

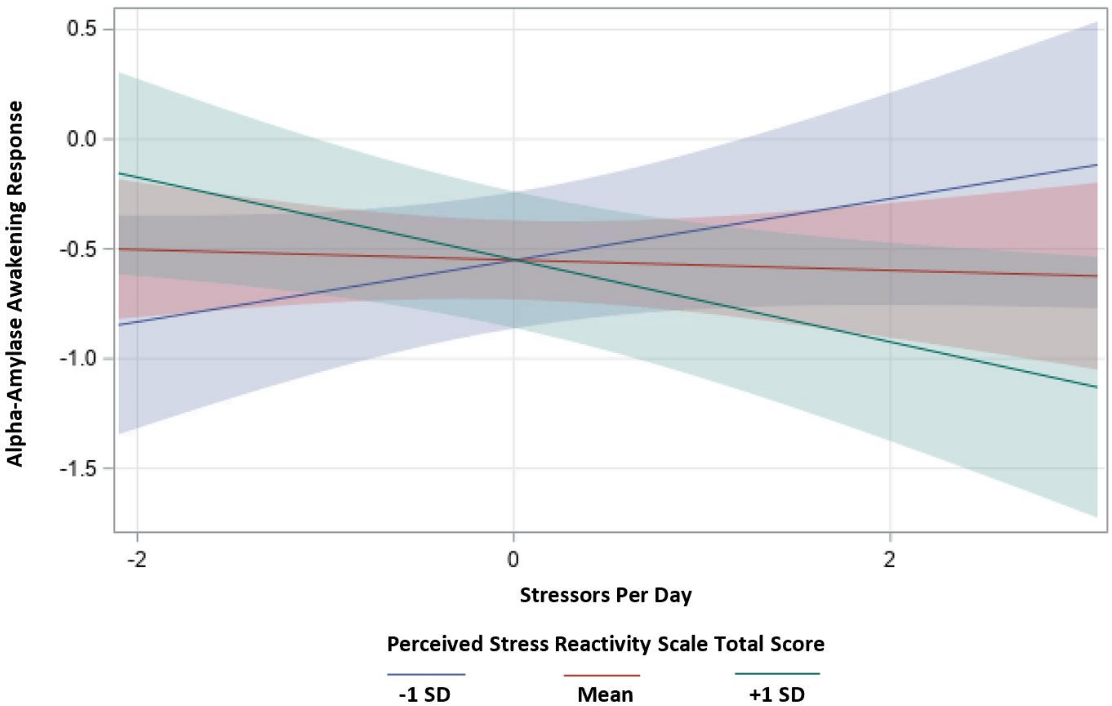

Figure 7 – Cross-Level Interaction Between the Number of Stressors Reported Per Day and Perceived Stress Reactivity Predicting the Alpha-Amylase Awakening Response

With respect to the alpha-amylase wake-evening slope, there were no significant within-person predictors. Higher perceived stress reactivity predicted a steeper average slope (γ_{09} = 0.001, t = 2.23, p < .05) and there was a trend towards older age predicting a flatter slope (γ_{07} = 0.000, t = -1.75, p =.08). The model explained 5.5% of the within-person variance and 11.4% of the between-person variance in the alpha-amylase wake-evening slope.

With respect to the alpha-amylase peak-evening slope, more daily cigarettes (γ_{40} = 0.010, t = 2.02, p < .05) and days with more stressors than usual (γ_{10} = .004, t = 2.39, p <

.05) predicted steeper daily slopes. A greater average number of stressors reported per day (γ_{01} = -0.014, t = -1.97, $p \leq .05$), older age (γ_{07} = -0.001, t = -3.05, $p < .01$), and higher neuroticism (γ_{08} = -0.023, t = -3.61, $p < .01$) predicted flatter slopes, and higher perceived stress reactivity predicted steeper slopes (γ_{09} = 0.002, t = 2.82, $p < .01$). Similar to the model that controlled for the frequency of daily stressors, a significant Level 2 interaction was detected between individual differences in cognitive ability and the sample average alpha-amylase peak-evening slope, moderated by perceived stress reactivity (γ_{012} = -0.001, t = -2.11, $p < .05$). The model explained 11.3% of the within-person variance and 22.3% of the between-person variance in the alpha-amylase peak-evening slope.

With respect to alpha-amylase area under the curve, more daily coffees predicted higher total daily alpha-amylase output (γ_{30} = 46.160, t = 2.77, $p < .01$). Between-persons, higher perceived stress reactivity predicted lower average total alpha-amylase output (γ_{09} = -23.339, t = -1.97, $p \leq .05$). The model explained 8.7% of the within-person variance and 10.6% of the between-person variance in alpha-amylase area under the curve.

Table 7 – Two-Level Models with Total Daily Stressors Predicting the Diurnal Alpha-Amylase Measures using Maximum Likelihood Estimation (*N* = 156)

	Awakening Response		Wake-Evening Slope		Peak-Evening Slope		Area Under the Curve	
Fixed Effects	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***	**Estimate**	***SE***
Intercept	-1.340	0.832	0.052**	0.008	0.062**	0.008	1,832.650**	168.390
Daily Wakeup Time WP	0.080	0.059						
Average Wakeup Time BP	0.109	0.114						
Daily Coffees WP	0.076	0.062	-0.001	0.002	-0.002	0.002	46.160**	16.667
Average Coffees BP	-0.129	0.096	-0.004	0.003	0.001	0.003	10.814	73.598
Daily Cigarettes WP	-0.181	0.158	0.007	0.005	0.010*	0.005	20.701	43.172
Average Cigarettes BP	0.042	0.112	-0.003	0.004	-0.005	0.004	62.221	83.342
Total Daily Stressors WP	-0.025	0.064	0.003	0.002	0.004*	0.002	11.628	17.128
Average Total Daily Stressors BP	-0.059	0.203	-0.007	0.007	-0.014*	0.007	-142.940	156.570
Body Mass Index	-0.004	0.025	-0.001	0.001	0.000	0.001	36.556†	19.422
Sex	-0.338†	0.186	-0.009	0.007	0.007	0.007	145.270	143.710
Age	0.023*	0.010	0.000	0.000	-0.001**	0.000	4.188	5.432
Neuroticism	0.396*	0.181	-0.005	0.006	-0.023**	0.006	110.400	137.860
Perceived Stress Scale	0.012	0.021	0.000	0.001	0.000	0.001	-0.563	15.221
Stress Reactivity Scale	-0.005	0.016	0.001*	0.001	0.002**	0.001	-23.339*	11.871
Cognitive Ability	-0.074	0.175	-0.001	0.006	-0.001	0.006	-44.641	136.550
Age x Age	0.001**	0.000						
Total Daily Stressors WP x Perceived Stress Reactivity	-0.020**	0.008						
Average Total Daily Stressors BP x Age	-0.022*	0.011						
Cognitive Ability x Perceived Stress Reactivity					-0.001*	0.001		
Random Effects								
Residual	2.798**	0.115	0.002**	0.000	0.002**	0.000	220,832**	8,808.270
Intercept Level 1	0.848**	0.138	0.001**	0.000	0.001**	0.000	688,222**	82,000
Within-Person Pseudo R^2	0.065		0.055		0.113		0.087	
Between-Person Pseudo R^2	0.181		0.114		0.223		0.106	

Note. Within-person and between-person variables were included for all daily (Level 1) variables. WP = Within-Persons (Person Mean Centered). BP = Between-Persons (Person Mean). All individual difference variables were grand-mean centered. Sex coded male = 0, female = 1.
** $p \leq .01$. * $p \leq .05$. † $p \leq .08$.

CHAPTER 4. DISCUSSION

This study had three primary goals: (1) to examine the between-person and within-person relationship between naturally occurring stressors and diurnal cortisol and alpha-amylase measures, (2) to examine the correlations between the frequency of reported stressors and the individual difference variables of perceived stress, perceived stress reactivity, age, and cognitive ability, and (3) to identify individual difference variables that influence the intraindividual and interindividual relationship between the frequency of daily stressor exposures and diurnal cortisol and alpha-amylase measures.

The current study had several notable findings. Different results emerged depending on whether the relationship between daily stressor exposures and physiological reactivity were observed between-persons versus within-persons over time. Regarding the first goal of the study, there was a significant within-person association between daily stressors and the diurnal cortisol and alpha-amylase measures. Days with stressor exposures were associated with greater cortisol area under the curve and days with more stressors than usual were associated with steeper daily alpha-amylase peak-evening slopes. Between-persons, there was a significant association between a greater average frequency of stressors per day and flatter average alpha-amylase peak-evening slopes. Regarding the second goal, the frequency of days with stressor exposures had a significant positive correlation with perceived stress reactivity and cognitive ability but was not significantly correlated with perceived stress or age. Regarding the third goal of the study, individuals who were high in perceived stress and experienced more stressors on average tended to have steeper diurnal cortisol slopes, particularly when compared to individuals low in perceived stress. Compared to younger adults, older adults who experienced a greater frequency of stressors had steeper alpha-amylase awakening responses. Finally, a cross-level interaction emerged that showed individuals high in perceived stress reactivity who experienced more stressors in a day tended to have steeper alpha-amylase awakening responses, particularly when compared to individuals low in perceived stress reactivity.

This study found a stressor day prevalence rate of 44% across the overall sample. This prevalence rate falls within the typical range of reported stressors (42% to 76%) from similar stress studies, as indicated by a recent coordinated analysis conducted by Zawadzki et al. (2019).

4.1 Daily Stressors and Physiological Activity

Significant between-person and within-person associations were found between naturally occurring stressors and the diurnal cortisol and alpha-amylase measures. Inconsistent with the hypotheses that days with stressor exposures would influence total daily output for both biomarkers, stressor days were associated with higher cortisol area under the curve and were not associated with daily fluctuations in alpha-amylase area under the curve. Area under the curve is an aggregated measure of the total output of cortisol and alpha-amylase across the day. In this study, the measure considered all seven salivary assessments throughout each study day. For within-day stress-related spikes in cortisol and alpha-amylase to affect the aggregated measure of total daily output, the stress-related elevations in physiological levels would need to overlap (at least partially) with the saliva sample assessment times before the levels returned to baseline.

It could be the case that, in response to stress, the slow-acting HPA axis has a greater influence on the measure of total daily output compared to the fast-acting SNS. The relatively large amount of within-person variance in total daily cortisol output may indicate that the aggregated day-level variable is sensitive to situational factors that lead to fluctuations in cortisol throughout the day, such as exposures to daily stressors (among other factors). Cortisol levels slowly increase and slowly return to baseline following acute stress, and this slow rate of reactivity and recovery likely lasts long enough to overlap with the saliva sample assessment times. The intraindividual finding of higher total daily cortisol output on days with stressors replicates findings from Stawski, Cichy, et al. (2013) and provides additional support that cortisol area under the curve is a sensitive day-level indicator for examining the stress-cortisol link in the context of everyday life.

Although alpha-amylase is a sensitive measure of stressor reactivity (Nater et al., 2006), exposure to daily stressors did not predict higher daily alpha-amylase output. There could be two reasons to explain why daily stressors did not predict higher alpha-amylase under the curve. First, alpha-amylase under the curve may be relatively more stable and resistant across time and situations. In fact, only one to three days of saliva sampling may be necessary to obtain reliable estimates of alpha-amylase area under the curve (Out et al., 2013). Stress-related fluctuations in alpha-amylase levels may not be dense enough throughout the day to influence total daily alpha-amylase output, especially considering the infrequency of experiencing multiple stressors per day (15% of days had two or more stressors exposures). In other words, stress-related elevations in alpha-amylase levels may be overlooked in the aggregated total daily output measure.

Second, the nature of the diurnal alpha-amylase rhythm may make it difficult to detect the effect of daily stressors on area under the curve. Steeper alpha-amylase awakening responses, which may be associated with greater SNS activation in the morning and stress anticipation (to be discussed later in the Discussion), could attenuate the area under the curve. Whereas a steeper cortisol awakening response can lead to greater area under the curve, a steeper alpha-amylase awakening response can reduce the area under the curve because the morning slope is negative. Total daily alpha-amylase output may not be a sensitive enough indicator to capture the within-day, time-dependent nature of the daily stress-alpha-amylase link, and a steeper alpha-amylase awakening response could attenuate total daily output. More research is needed to understand the meaning of alpha-amylase area under the curve in healthy populations.

The number of stressors reported per day was associated with the alpha-amylase peak-evening slope both within-persons and between-persons. Within-persons, days with more stressors than usual were associated with steeper alpha-amylase increases across the day. This finding was expected, as days with more stressors than usual were related to days with greater SNS activation. However, this was a surprising finding given the non-significant intraindividual relationship between daily stressors and total daily alpha-amylase output. The alpha-amylase awakening response may also explain this intraindividual finding between daily stressors and the diurnal alpha-amylase slope. Because one aspect of the awakening response is considered in the peak-evening slope calculation (i.e., the difference between Sample Time 2 and Sample Time 7), the magnitude of the decrease in alpha-amylase levels in the morning could influence the diurnal alpha-amylase peak-evening slope. To influence the steepness of the diurnal slope, days with multiple stressors would need to impact either peaking alpha-amylase levels in the morning (i.e., Sample Time 2), bedtime levels (i.e., Sample Time 7), or alpha-amylase levels at both time points. It could be the case that days with more stressors than usual impact the peaking levels in the morning (i.e., lower alpha-amylase values at Sample Time 2), which may lead to steeper slopes across the day. However, days with more stressors than usual could also be associated with greater alpha-amylase concentrations later in the evening as well, particularly if a stressor occurred later in the evening. As mentioned earlier, the alpha-amylase awakening response may be influenced by anticipatory stress. Anticipating a greater number of stressful events in a day could lead to greater SNS activation in the morning, which would lead to a lower value at Sample Time 2. Although this interpretation regarding the alpha-amylase awakening response is speculative, it could explain why days with more stressors than usual were related to daily fluctuations in the diurnal alpha-amylase slope but not significantly related to total daily output.

Between-persons, people who typically encountered more stressors per day had flatter average alpha-amylase peak-evening slopes. A flatter diurnal slope in people who typically experienced more stressors per day is consistent with the notion of allostatic overload, where chronic stress is oftentimes associated with blunted physiological reactivity and attenuated diurnal profiles (Liu et al., 2017; McEwen, 1998). The different pattern of results that emerged based on whether the association between stressor exposures and the diurnal alpha-amylase slope was observed between-persons versus within-persons highlights the importance of not assuming between-person relationships exist at the within-person level of analysis (Borsboom et al., 2003).

The between-person and within-person associations between stressor exposures and total daily cortisol output and the diurnal alpha-amylase slope remained significant after controlling for the other individual difference variables. Encounters with naturally occurring stressors can impact both daily cortisol and alpha-amylase activity in a healthy sample of adults, above and beyond perceived stress, perceived stress reactivity, neuroticism, age, and cognitive ability.

4.2 Perceptions of Stress and Physiological Activity

As expected, the frequency of stressor days had a significant positive correlation with a relatively new measure of stressor reactivity, the Perceived Stress Reactivity Scale (Schulz et al., 2005). Individuals who perceived themselves as highly reactive to stressors tended to experience a greater frequency of stressor days. Contrary to the hypotheses, two other measures associated with stress – neuroticism and the commonly administered Perceived Stress Scale (Cohen et al., 1983) – did not significantly correlate with the frequency of stressor days, even though neuroticism and the Perceived Stress Scale were moderately correlated with each other and with the Perceived Stress Reactivity Scale. These non-significant correlations were inconsistent with previous research that shows individuals who are high in neuroticism and perceived stress tend to report more stressors (Bolger & Zuckerman, 1995; Stawski et al., 2008). In this adult lifespan sample, the Perceived Stress Reactivity Scale was a better predictor of the frequency of exposures to naturally occurring stressors compared to neuroticism and the Perceived Stress Scale.

Due to previous research findings showing a relationship between perceived stress and salivary cortisol levels but not alpha-amylase levels (Nater et al., 2007; van Eck &

Nicolson, 1994), the Perceived Stress Scale was expected to be associated with activity in the HPA axis but unrelated to activity in the SNS. Findings were somewhat consistent with the hypotheses. Compared to individuals low in perceived stress, individuals high in perceived stress who experienced a greater average frequency of stressor days tended to have steeper diurnal cortisol slopes and potentially lower cortisol area under the curve. Because people high in perceived stress did not necessarily experience a greater frequency of stressor days, the people who were high in perceived stress and experienced a higher frequency of stressors may be in a unique group in this sample. It was hypothesized that the interaction between perceived stress and daily stressors would predict a flatter diurnal cortisol slope rather than a steeper slope. Blunted reactivity observed in people who experience chronic stress could be due to allostatic overload, where individuals show inadequate physiological responses to stressors (Liu et al., 2017; Liu et al., 2018; McEwen, 1998). For example, high baseline stress levels are related to blunted stressor reactivity (Poppelaars et al., 2019). The Perceived Stress Scale may be capturing the aspects of one's life that are contributing to chronic stress and blunted physiological activity. This idea is supported by the findings that perceived stress was related to reduced HPA axis activation across the average day for individuals who tended to experience a greater frequency of stressors, but perceived stress did not significantly predict greater HPA axis activation on days with stressors. Additional research is needed to better understand the interindividual relationship between perceived stress and long-term changes in physiological arousal.

Consistent with the hypotheses, the Perceived Stress Reactivity Scale was not associated with any of the cortisol measures. The Perceived Stress Reactivity Scale predicted steeper diurnal alpha-amylase slopes and lower alpha-amylase area under the curve. Individuals who perceive themselves as highly reactive to stressors may have greater SNS activation across the day, even after controlling on daily exposures to naturally occurring stressors and individual differences in neuroticism and perceived stress. More research is needed to understand why higher levels of perceived stress reactivity were related to lower alpha-amylase area under the curve values. Because perceived stress reactivity was correlated with the frequency of daily stressors as well as deviations in the diurnal alpha-amylase measures, the Perceived Stress Reactivity Scale may better capture the specific types of stress-related experiences to which the people in this sample were exposed.

As expected, days with more stressors were differentially associated with daily alpha-amylase awakening responses depending on one's perceived stress reactivity. Individuals who considered themselves highly reactive to stressors tended to have steeper alpha-amylase declines after awakening on days they experienced more stressors than usual. In the context of the HPA axis, steeper awakening responses are believed to be indicative of healthier HPA axis function (Adam & Kumari, 2009) with the adaptive purpose of providing individuals with the energy needed to meet the demands of the upcoming day (Adam et al., 2006; Fries et al., 2009). If the alpha-amylase awakening response serves a similar purpose of preparing the system for the upcoming day, one could argue that individuals with higher levels of perceived stress reactivity, who generally encounter a greater frequency of stressors, have steeper awakening responses on days they anticipate having more encounters with stressors than usual.

In supplemental analyses, a significant cross-level interaction also emerged between individual differences in the Anticipatory Stress subscale of the Perceived Stress

Reactivity Scale and days with more stressor exposures than usual. People with greater perceived anticipatory stress reactivity had steeper alpha-amylase awakening responses on days with more stressor exposures than usual. Although it is too early to speculate the physiological function of the alpha-amylase awakening response because the function remains unknown (Skoluda et al., 2016), these findings could show a connection between the alpha-amylase awakening response and anticipatory stress for the upcoming day. However, it should be noted that a flatter alpha-amylase awakening response is indicative of higher levels of sustained alpha-amylase in the morning, which has been associated with days with more frequent negative interpersonal interactions (Birditt et al., 2018). As a result, this anticipatory stress-related interpretation should be considered with caution. Future research should continue to examine the relationship between anticipatory stress and the alpha-amylase awakening response as well as examine the influence the Perceived Stress Reactivity subscales (i.e., Anticipatory Stress, Recovery, Work Overload, Social Conflict, Social Evaluation, and Failure) may have on physiological markers when stressors occur.

It was hypothesized that the Perceived Stress Scale and the Perceived Stress Reactivity Scale would be associated with greater activation in the HPA axis and SNS, respectively, on days when stressors occur. Different findings emerged between the two scales. The Perceived Stress Scale may have predicted inadequate, or blunted, stressor reactivity in the HPA axis specifically for individuals who tended to experience a higher frequency of stressors. The Perceived Stress Reactivity Scale was associated with greater SNS activation in general for individuals who considered themselves more reactive to stressors and steeper alpha-amylase awakening responses specifically on days with stressors. Although there is substantial convergent validity between the Perceived Stress Reactivity Scale and the Perceived Stress Scale (Morgan et al., 2014), there may be meaningful differences between the two scales as predictors of physiological activity.

The two scales differ in the way they ask individuals to reflect on their past stress-related experiences, which may explain why the Perceived Stress Reactivity Scale predicted daily fluctuations in alpha-amylase activity whereas the Perceived Stress Scale predicted individual differences in cortisol activity. The Perceived Stress Reactivity Scale focuses on the extent to which people have reacted to specific stressors they may have encountered in the past, such as arguments with other people or when tasks and duties pile up and become difficult to cope with. The scale focuses on reactivity to specific domains of stress that are relevant to everyday life experiences, particularly for people who have social relationships and are employed. The scale's focus on personally relevant domains of stressor reactivity may facilitate individuals' estimates of their own behavior. As a result, the Perceived Stress Reactivity Scale total score may be a predictor of within-person fluctuations in stressor reactivity.

The Perceived Stress Scale asks relatively more general questions about the frequency in which people have had stress-related experiences in the past month, such as how often the person was upset because of something that happened unexpectedly, or how often the person could not cope with all the things they had to do. Because the scale focuses on the frequency of stressor exposures rather than on the extent to which a person reacts to stressor exposures, the Perceived Stress Scale may not capture short-term variability in stressor reactivity. Instead, the measure of global stress may be a predictor more long-term changes in physiological activity, potentially due to the negative consequences of chronic stress (Miller et al., 2007).

Although the two questionnaires measure different aspects of the stress process, the Perceived Stress Scale has received substantially more research attention compared to the relatively new Perceived Stress Reactivity Scale. The Perceived Stress Scale has been shown to have a role on the within-person relationship between negative affective responses to encounters with stressors (Scott et al., 2013; Stawski et al., 2008) as well as individual differences in cortisol activity (van Eck et al., 1996). Findings from this study, however, provide support for the Perceived Stress Reactivity Scale as a valid measure of within-person physiological reactivity to naturally occurring stressors. This is a particularly important finding as stressor reactivity is a within-person process and, to the author's knowledge, no research studies to date have shown that the Perceived Stress Scale moderates the intraindividual relationship between daily stressor exposure and physiological reactivity. More research is needed to understand when and under what circumstances the Perceived Stress Reactivity Scale is a better predictor of within-person physiological reactivity to stress compared to the Perceived Stress Scale, and when the Perceived Stress Scale is a better predictor of individual differences in physiological activity.

The sensitivity of the HPA axis compared to the SNS to different types of stressors could explain why the Perceived Stress Scale was only significantly related to the cortisol measures and the Perceived Stress Reactivity Scale was only related to the alpha-amylase measures. In response to a stressful event, the SNS may activate at a lower threshold compared to the HPA axis. Although the authors were focused on stress in a sample of caregivers for individuals with MCI, Savla et al. (2013) showed that emotionally charged stressors were associated with elevated alpha-amylase levels but not cortisol levels. In addition, stressors appraised as controllable may be associated with elevations in SNS activity and not HPA axis activity, whereas events appraised as uncontrollable may activate both systems (Frankenhaeuser, 1982). It could be the case that the items on the Perceived Stress Scale focus on the types of situations or circumstances that more commonly influence cortisol reactivity (e.g., uncontrollable situations), whereas the items on the Perceived Stress Reactivity Scale focus on the types of situations that influence alpha-amylase reactivity (e.g., unpleasant interpersonal interactions or controllable situations). Future research studies should continue to examine the influence specific types of stressors have on HPA axis and SNS activity within the same individuals.

Although neuroticism was included in the analyses as a covariate, interesting findings related to the personality facet emerged. Individual differences in neuroticism had the opposite effect on the diurnal alpha-amylase measures compared to the effect of the Perceived Stress Reactivity Scale on the diurnal measures. Individuals higher in neuroticism tended to have a flatter alpha-amylase awakening response and a flatter diurnal alpha-amylase slope, indicating less pronounced SNS activation in the morning and across the day. These findings are consistent with previous research that showed individuals higher in neuroticism tended to have blunted SNS (and HPA axis) reactivity to stressor exposure (Bibbey et al., 2013; Poppelaars et al., 2019). Higher levels of neuroticism are typically related to elevated perceptions of stress when unpleasant events occur, and the tendency to experience elevated arousal each time the system is exposed to stress could amount to something similar to chronic stress. In other words, neuroticism may be related to blunted alpha-amylase activity in a similar way chronic stress is related to blunted physiological activity, where individuals who tend to experience more distress have attenuated physiological responses to stress due to repeated wear and tear on the system (McEwen, 1998; Nater et al., 2007; Poppelaars et al., 2019). Contrasted with the personality trait of neuroticism, the Perceived Stress Reactivity Scale may be capturing the within-person fluctuations of physiological arousal. Although the Neuroticism dimension of the Big Five Inventory and the Perceived Stress Reactivity Scale are related (Morgan et al., 2014), findings suggest the two measures capture different aspects of the stress experience. Additional work is needed that focuses specifically on the relationship between neuroticism and blunted alpha-amylase activity during the day, as most research studies have focused on the relationship between neuroticism and cardiovascular responses to stress, which is a different component of the sympathetic nervous system (e.g., Bibbey et al., 2013).

4.3 Age and Physiological Activity

Inconsistent with some adult development and aging theories, and inconsistent with the hypotheses, age did not have a significant negative correlation with the frequency of reported stressors. Previous studies have shown a linear decrease in stressor exposure with older age (e.g., Stawski et al., 2008), potentially due to age-related changes in stress appraisals and/or a decrease in social role participation associated with older age, such as retirement. The lack of age differences in the frequency of stressor days could be due to this specific study’s sample of participants. The non-significant correlation between age and the frequency of reported stressor days, and the potential lack of age differences in physiological reactivity to stressors, could be due (at least in part) to the specific participants in this study being relatively healthier and more engaged in employment duties and/or extracurricular activities than typical lifespan samples, especially this sample of older adults. There were no age differences in self-rated health and nearly all younger and middle-aged adults were employed (92.7% and 82.5%, respectively). Although many of the older adults were retired, most of them (n = 44, 75.9%) had previously participated in psychological research studies, potentially indicating the older adult sample was at least moderately engaged in activities outside the home.

Stress-related experiences and well-being in daily life are evolving over time, which could also partially explain why age differences in the frequency of stressor exposure were not found in this study. Although some research findings show decreases in well-being among individuals in late life (e.g., Charles et al., 2001), a large body of research has adopted the assumptions associated with the lifespan developmental theory of Socioemotional Selectivity Theory, which suggests there is a linear increase in well-being with older age because people experience a shift towards prioritizing emotional goals when their perceived time left to live becomes limited (Carstensen et al., 1999). Almeida, Charles, et al. (2020) suggested that this linear trend in well-being may need to be reevaluated because perceptions of well-being are influenced by economic prosperity, societal structure, and other fluid factors relevant to everyday life such as current events. As a result, stress-related experiences and well-being may not be the same across all cohorts over time; what may be true for one cohort may not be true for later cohorts. For example, Almeida, Charles, et al. (2020) showed that adults in the 2010s reported more stress and lower levels of well-being compared to same-aged adults in the 1990s. Older adults in the 1990s showed the lowest levels of stress and highest levels of well-being (consistent with the assumptions of Socioemotional Selectivity Theory), but this pattern was no longer observable in same-aged adults in the 2010s. It appears that people are becoming more stressed over time, particularly middle-aged adults, who, in the 2010s reported more stressor exposures and higher levels of negative affect than the younger and older age groups (Almeida, Charles, et al., 2020). Although findings from the current study challenge theories such as Socioemotional Selectivity Theory, additional work is needed to examine how and why age-related trends in well-being may be changing.

As expected, older age predicted higher cortisol area under the curve and a flatter diurnal alpha-amylase slope. These age-related differences in HPA axis and SNS activity are consistent with previous research and could be due to age-related changes associated with a lifetime of stress (Nater et al., 2013; Nater et al., 2007; Piazza et al., 2010). Age also had a significant quadratic effect on the alpha-amylase awakening response, such that younger and older individuals tended to have flatter awakening responses and middle-aged individuals tended to have steeper awakening responses. Considering earlier speculations regarding the potential role the alpha-amylase awakening response plays on preparing the individual for the upcoming day, midlife may be a period in the life course that is met with comparatively more stressful daily challenges and hassles (Almeida, Charles, et al., 2020). The steeper morning responses may reflect these experiences specific to being middle-aged.

Compared to younger individuals, older individuals who generally experienced more stressors per day tended to have steeper alpha-amylase awakening responses. Although it was hypothesized that older individuals would have elevated HPA axis and SNS reactivity to stressor exposures, greater SNS activation in the morning for older people who experienced more daily stressors was a surprising finding. Typically, steeper awakening responses are associated with better health and well-being. Flatter alpha-amylase awakening responses, on the other hand, have been associated with days with negative social interactions as well as individual differences in greater perceived stress and over-commitment (Birditt et al., 2018; Eddy et al., 2018; Katz et al., 2016). It could be the case that older adults who are actively engaged in work or volunteer opportunities, and therefore experiencing more daily stressors, have adaptive awakening responses in the SNS, but more research is needed to understand this relationship.

Although additional analyses are needed to understand the strength of the relationship, the lack of a significant cross-level interaction between daily fluctuations in stressor exposures and age could suggest there are no age differences in physiological reactivity on days when stressors occur. Even though previous research supports the finding of no age differences in emotional reactivity to stressors (e.g., Stawski et al., 2008), the lack of a significant cross-level interaction challenges the hypotheses as well as the Strength and Vulnerability Integration model. The SAVI model suggests older individuals have strengths with emotion regulation, such as appraising stressful situations less negatively than younger adults, but also show vulnerabilities in regulation when stressors cannot be deescalated or avoided (Charles, 2010). These vulnerabilities could be due to having fewer social supports in older age and can result in older adults being equally as reactive or more reactive to stress than younger adults. Given the findings in this study, it could be the case that the SAVI model is a better fitting model of stress exposure and reactivity for older individuals who are retired, not as healthy, and/or have fewer social supports and other engagements outside the home. Compared to the older participants in this study who were primarily retired but still actively engaged in activities, retired individuals who typically do not have as many commitments, and potentially have smaller social networks, may be more vulnerable to daily stressors. Although older people who are not as socially engaged in activities may experience fewer stressors than busier people, they may be more reactive to the stressors they do encounter. In other words, this study may have found age differences in stressor reactivity if the sample of older people were not as healthy and engaged in outside activities. The assumptions associated with the SAVI model should continue to be tested with samples of post-retirement older adults who are still actively engaged in activities and who are not as actively engaged in outside activities. In addition, the SAVI model should be tested with an older sample of people, as the oldest participant in this study was 77 years old.

The life experiences that come with being older may equip older adults to successfully problem solve and manage daily hassles, even when older age is negatively associated with cognitive ability as was found in this study (Blanchard-Fields, 2007; Chen et al., 2017). Older adults have a wide range of self-regulatory strategies to choose from and effectively select strategies when they encounter challenging situations (Blanchard-Fields, 2007). Future research should continue to examine age differences in stressor exposure and reactivity. Additional studies could investigate the type of strategies people across the lifespan use in response to naturally occurring stressors and whether specific strategies tend to be more successful with regulating HPA axis and SNS activity.

4.4 Cognitive Ability and Physiological Activity

The positive association between the frequency of stressor days and cognitive ability was expected and consistent with previous research that suggests people with higher levels of cognitive ability and more education may lead lives that are more socially and professionally engaged and demanding, increasing their likelihood of encountering daily hassles (Grzywacz et al., 2004; Seeman & Crimmins, 2001; Stawski et al., 2010; Stawski, Mogle, et al., 2013). Cognitive ability may be associated with the types of environments and situations in which people find themselves.

The relationship between cognitive ability and the diurnal alpha-amylase slope was moderated by perceived stress reactivity. There was a greater average level of SNS activation across the day in individuals who considered themselves highly reactive to stressors but were low in cognitive ability. There are several explanations for this finding. First, it could be the case that individuals lower in cognitive ability have fewer cognitive resources to successfully cope with daily hassles. Negative emotions are cognitively demanding (Labouvie-Vief, 2003) and may require more cognitive resources to successfully manage the emotions. In addition, people with lower cognitive ability could use different, and potentially less effective, coping strategies compared to people with higher ability. Cognitive ability may act as a resilience factor in the stress process by helping to minimize stressor reactivity through the use of effective coping strategies when stressors occur.

Second, cognitive ability could be associated with specific lifestyles and engagement in different types of activities and experiences (Hultsch et al., 1993; Hultsch et al., 1999; Schooler & Mulatu, 2001). The types of stressful situations encountered by people with lower ability could differ from the situations more commonly encountered by individuals with higher ability. This may be particularly relevant for home- and work-related stressors (Stawski et al., 2010). Stawski et al. (2010) found that people with higher cognitive ability tended to report more frequent exposures to work- and home-related stressors, but it remains unclear whether there are qualitative differences in the types of home- and work-related stressors people of different abilities typically report. Compared to individuals with higher cognitive ability, individuals with lower ability could have occupations that require different types of demands throughout the day. In addition, the work-related demands encountered by people with lower ability could be more commonly appraised as threats (the perception that available resources are inadequate relative to the demands) rather than challenges (the perception that available resources are commensurate to the demands). In other words, cognitive ability could be associated with the frequency and types of demands people encounter each day as well as the way those demands are appraised.

Finally, individuals with lower cognitive ability could have fewer resources to control their everyday life environments and the situations in which they find themselves. Due to positive associations between cognitive ability, occupation level, and income (Schmidt & Hunter, 2004), individuals with lower ability may not have the available (or spare) financial and/or time-related resources necessary to avoid or deescalate some types of unpleasant events. For example, a person with more financial resources may be able to use those resources to avoid an anticipated stressor, whereas another person who does not have the financial resources may not have the option to avoid the stressor.

Potential differences in available cognitive resources to devote towards coping with stressors, as well as differences in life situations and experiences, could explain why people with lower cognitive ability but higher perceived stress reactivity tended to have greater SNS activation across the day. Additional research is needed to better understand the mechanism linking cognitive ability and stressor reactivity, particularly physiological reactivity. Future research should examine the roles specific types of cognitive ability, such as crystallized ability and fluid ability, may have on physiological reactivity to stressor exposures and whether this relationship depends on age.

4.5 Limitations

This study had several limitations. First, the current data cannot determine the direction of causality. For example, greater activation in the diurnal alpha-amylase slope could also predict having higher perceived stress reactivity. Longitudinally examining the relationships in this study could help address this limitation.

Although participants reported whether they had experienced a stressful event since the last randomly-prompted EMA survey, they were not asked to provide additional information about the stressor, such as the type of stressor encountered, whether it was anticipated, where the stressor occurred, or the life domain(s) impacted by the stressor. Aggregating stress-related experiences into a dichotomous stressor day versus stress-free day variable also did not allow for a deeper examination of the type of stressor encountered. It is well established that everyday life events can be qualitatively different from one event to the next, which can differentially influence well-being and the extent to which an individual reacts (Koffer et al., 2016). It could have been the case that some participants experienced the same types of stressors repeatedly (i.e., low stressor diversity) while other participants experienced a wide range of stressors (i.e., high stressor diversity). Low stressor diversity coupled with high stressor exposure is related to chronic stress and may have negative effects on health and well-being whereas high stressor diversity may be related to more positive health outcomes (Koffer et al., 2016). In addition, some types of stressors are more likely to influence cortisol levels whereas other types of stressors may more strongly influence alpha-amylase levels (Frankenhaeuser, 1982; Savla et al., 2013). Finally, participants did not indicate whether they had anticipated the stressors they reported. Anticipating potentially stressful events could influence stressor reactivity by allowing an individual to modify the upcoming event to make it less unpleasant, or providing an individual time leading up to the event to manage and regulate their response (Neupert & Bellingtier, 2019; Neupert et al., 2019). Although this study did not examine the influence stressor type, life domain impacted, or stressor forecasting have on HPA axis and SNS activity, one strength of this study is the demonstration that daily stressors in general are associated with higher daily cortisol area under the curve and steeper diurnal alpha-amylase slopes, regardless of stressor type, severity, life domain impacted, or whether the stressor was forecasted. This intraindividual effect of daily stressors on daily physiological activity was demonstrated even after adjusting for individual differences of age, perceived life stress, perceived stress reactivity, cognitive ability, and neuroticism.

Another limitation is that participants were responsible for providing the saliva samples at the designated times of day. There was no way to check if participants' saliva samples were provided some time before or after the timestamp associated with each sample and the corresponding survey submitted on the Palm Pilots. However, a similar study as the current study found a high correlation between self-reported sample times and objective sample times (measured by a "smart box" that tracked exact times the saliva samples were provided), indicating reliability in self-reported sample times (Almeida et al., 2009). Use of technology that tracks the timing of each saliva sample could improve researchers' ability to identify which samples should be considered invalid and provide more accurate estimates of the diurnal slopes.

Although participants provided seven saliva samples per day, one final limitation is that the awakening response and diurnal slopes were operationalized as the difference between two samples adjusted by the amount of time between the two samples. For example, the cortisol awakening response was calculated as the difference between the cortisol level 30-minutes post-waking and the waking cortisol level, divided by time since waking. It is recommended that researchers collect at least three saliva samples post-waking to accurately calculate the awakening response (Hellhammer et al., 2007; Stalder et al., 2016). In addition, the shape of the diurnal cortisol and alpha-amylase slopes, particularly the alpha-amylase slope, may not be linear. Future research studies should use statistical approaches that consider these complex, quadratic diurnal slopes.

4.6 Future Directions

More research attention is needed to better understand the relationship between naturally occurring stressors and alpha-amylase reactivity. Alternative methods of data collection and analysis could take a more sensitive approach to detecting the rapid increase and recovery of alpha-amylase levels following a stressor. Event-based EMA, where participants report their exposures to stressors in the moments that they occur, could be an alternative way to examine the role of naturally occurring stressors on alpha-amylase levels. Many stress studies, including the current study, use randomly-prompted EMA protocols, where participants are prompted several times a day to report their stress-related experiences and concurrently provide saliva samples. Because the SNS reacts and recovers quickly to stressor exposures, quick increases and decreases in alpha-amylase levels in response to stressors could occur in between the randomly-prompted assessment periods. Event-based EMA could be a more sensitive way to capture the immediate effect of stressors on alpha-amylase levels in everyday life.

In combination with event-based EMA reporting, the relationship between naturally occurring stressors and alpha-amylase levels could be examined using a momentary approach. Three-level hierarchical models focused on the effect of stressors, reported the moment they occur, on physiological activity would allow researchers to examine alpha-amylase reactivity to acute stress on a moment-to-moment basis within days (i.e., moments nested within days nested within people) rather than on an aggregated day-level basis as was conducted in this study. Although alpha-amylase is sensitive to acute stress, diurnal alpha-amylase measures may be relatively stable across time (Out et al., 2013). A momentary approach may be needed to examine within-day stress-related fluctuations in alpha-amylase levels due to its fast reacting and recovery profile.

This study assessed same-day relationships between daily stressors and diurnal physiological rhythms. The effects of stressors, however, could carry over and impact the next day's diurnal physiological profiles (Adam et al., 2006). This study collected 10 consecutive days of stress and physiological data, allowing lagged analyses to examine the effect of daily stressors on the following day's diurnal cortisol and alpha-amylase rhythms. Examining the relationship between naturally occurring stressors and physiological reactivity, particularly alpha-amylase reactivity, using event-based EMA reporting, three-level multilevel models, and a lagged analysis to examine the effect of stressors on next-day reactivity could provide further insight into the effects of naturally occurring stressors on physiological reactivity as well as the similarities and differences between alpha-amylase and cortisol as indicators of stress.

Although not conducted in this study, future analyses with these data could explore the usefulness and sensitivity of indexing physiological manifestations of stress using ratio methods of the biomarkers. For example, compared to examining the biomarkers on their own, recent research suggests the ratio of alpha-amylase over cortisol may be a more informative index of stress system dysregulation, specifically dysregulation associated with chronic stress and depression (Ali & Pruessner, 2012). Using ratio methods such as alpha-amylase over cortisol could allow researchers to investigate how the two biological systems function independently and together, furthering our understanding of physiological stress responses.

Researchers must continue to examine historically graded influences on stress and well-being across the lifespan. Given the recent and ongoing burdens the COVID-19 pandemic has put on individuals of all ages, it will be critical to examine how this major historical event alters trends in stress experiences and well-being over time. For example, the non-linear trend in stress and well-being found by Almeida, Charles, et al. (2020) could be exacerbated by the hardships people have faced during the pandemic. Increased levels of stress experienced by middle-aged adults may be even worse following the pandemic due to challenges many have faced throughout the pandemic, including challenges related to childcare, work, and finances. Although research studies supporting the assumptions associated with Socioemotional Selectivity Theory have produced valuable information on the experiences of stress and well-being across the lifespan, linear trends in well-being found in past cohorts may not be relevant to future cohorts. Aging researchers must consider how historically changing factors influence perceptions of stress and well-being across the lifespan (Almeida, Charles, et al., 2020).

4.7 Conclusion

In conclusion, naturally occurring stressors are prevalent among people across the lifespan. Exposure to these types of events is associated with both daily fluctuations in physiological activity as well as individual differences in physiological activity. Not only are daily stressors related to cortisol and alpha-amylase activity, but the relationships depend on individual differences in perceived stress, perceived stress reactivity, age, and cognitive ability. First, total daily cortisol output may be a sensitive measure for studying the daily stress-cortisol link, while total daily alpha-amylase output may not be a reliable indicator of daily stress. Second, the Perceived Stress Scale may be a predictor of long-term deviations in cortisol activity whereas the Perceived Stress Reactivity Scale may be a predictor of within-person fluctuations in alpha-amylase reactivity. However, the Perceived Stress Reactivity Scale should be further examined to better understand its role in the stress-alpha-amylase link. Third, the magnitude of the alpha-amylase awakening response may be associated with anticipatory stress for the upcoming day, but more research is needed to understand the purpose of the alpha-amylase awakening response. Finally, there may not be age differences in the frequency of daily stressor exposures, but additional research is needed to examine whether older adults are more physiologically reactive to daily stressors. Findings from this study provide insight into specific vulnerability and resilience factors associated with exposure to daily stressors as well as potential differences in the function of the HPA axis and SNS in response to stress.

Future research studies should continue to focus on the associations between subjective and biological measures of stressor exposure and reactivity. Investigating the psychological consequences of subjective perceptions of stress is an important aspect of understanding the stress process, but examining the connection between subjective perceptions and biological responses to stressors has important implications for health by understanding how stress gets under the skin. This study was novel in its approach in that it focused on the effect of naturally occurring stressors (rather than laboratory induced stressors) and measures of subjective stress, age, and cognitive ability on diurnal cortisol and alpha-amylase profiles in a lifespan sample of healthy adults. Keeping in mind that stress and well-being may be changing in different ways for each cohort, the stress-cortisol and stress-alpha-amylase linkages in daily life should continue to be explored across the lifespan.

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