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Title: The Effects of Age and Neuroticism on Stress Reactivity and Cortisol Diurnal Rhythms: Findings from the Normative Aging Study

Abstract approved:

Carolyn M. Aldwin

We examined the effect of daily stress, age, and emotional stability/neuroticism on stress reactivity, using cortisol diurnal rhythms. We used data from the Normative Aging Study (Spiro & Bossé, 2001). The 72 men in this study ranged from 67-93 (M = 79.29, SD = 4.88). Multilevel modeling showed that higher daily stress predicted flatter cortisol diurnal rhythms, $B = .09, p < .001$, as did age, $B = .01, p < .001$, while those higher in emotional stability on the emotional stability/neuroticism measure showed steeper slopes for cortisol diurnal rhythms, $B = -.04, p < .001$. These results indicate that age and emotional stability/neuroticism levels explain some of the variance in individual differences in stress reactivity and provide a basis for future research focused on the effects of psychosocial variables on physiological outcomes.
The Effects of Age and Neuroticism on Stress Reactivity and Cortisol Diurnal

Rhythms: Findings from the Normative Aging Study

by

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TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction and Literature Review</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Daily Stressors</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Measuring the Effects of Stress Reactivity</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Present Study</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Methods</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Sample and Procedure</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Measures</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Analysis</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Results</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Stress Frequencies and Descriptors</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Bivariate Relations Among the Study Variables</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Hypothesis Testing</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>Discussion</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Limitations</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Conclusion</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>Appendices</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Appendix A</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Appendix B</td>
<td>58</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cortisol Diurnal Rhythms by Number of Stressors</td>
<td>30</td>
</tr>
<tr>
<td>2. Cortisol Diurnal Rhythms by Age</td>
<td>32</td>
</tr>
<tr>
<td>3. Cortisol Diurnal Rhythms by Emotional Stability/Neuroticism</td>
<td>34</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Frequency of Total Stressors Across all People During the Four Days of the Study</td>
<td>24</td>
</tr>
<tr>
<td>2. Percentage of Reported Stressors by Stress Type</td>
<td>25</td>
</tr>
<tr>
<td>3. Correlation Matrix of Variables in Study</td>
<td>27</td>
</tr>
<tr>
<td>4. Cortisol Diurnal Rhythm by Number of Stressors</td>
<td>29</td>
</tr>
<tr>
<td>5. Cortisol Diurnal Rhythm by Age</td>
<td>31</td>
</tr>
<tr>
<td>6. Cortisol Diurnal Rhythm by Emotional Stability/Neuroticism</td>
<td>33</td>
</tr>
</tbody>
</table>
The Effects of Age and Neuroticism on Stress Reactivity and Cortisol Diurnal Rhythms: Findings from the Normative Aging Study

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Physical scientists originally coined the term “stress” to describe forces capable of straining objects to their physical limits (Seyle, 1956). Psychologists later adopted the term to describe the intensity of the effect of overwhelming environmental pressures on people’s psychological resources (Piazza, Almeida, Dmitrieva, & Klein, 2010). The body has systems in place to adjust to the challenges of stressful events with physiological changes known as stress reactivity. However, not all people respond to the same events in the same way and maladaptive reactivity to stress may lead to dangerous health outcomes (Kemeny & Schedlowski, 2007; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Mroczek & Almeida, 2004; Piazza et al., 2010; Thoits, 1995; Flier, Underhill, & McEwen, 1998; Epel, Burke, & Wolkowitz, 2007). Because the implications of poor stress responses can be serious, stress reactivity variability has led to significant interest in the predictors of the stress response. Stress reactivity is also part of a broader dialogue about the transactional relationship between individuals and their environment and may provide insights into modeling variability in the bi-directional relationship of psychosocial variables on physiological outcomes (for a review, see Aldwin, 2007).

In this thesis, we briefly review the physiological systems associated with stress reactivity, along with the health implications of an overactive stress response. We then provide justifications for the variables used in this study, which come from
data collected by the Normative Aging Study (Spiro & Bossé, 2001). We posit that the number of daily stressors a person experiences, age, and neuroticism/emotional stability level will predict variation in stress reactivity. Then, we will provide a more in-depth review of how age, daily stressors, and neuroticism/emotional stability affect individuals’ stress reactivity and the benefits of using biological markers to measure health outcomes.

**Short-Term and Long-Term Stress Responses**

The human body is extraordinary in many ways, not the least of which involves how the body maintains homeostasis under changing and sometimes challenging conditions over the lifespan (McEwen & Seeman, 1999). Some conditions and challenges can lead to an increased tension between a person’s resources and the challenges in the environment, as also called stress, and require that a person respond immediately or that a person be prepared to maintain heightened arousal for a long period of time. Two primary and interrelated mechanisms allow adaption to external stress: short-term and long-term stress responses. Both of these systems are important in sustaining homeostasis while responding to perceived or real threats.

Short-term stress responses involve a network within the sympathetic nervous system (SNS) called the sympathetic-adrenal-medullary (SAM) axis. Normal life-sustaining functions, such as maintaining blood pressure and heart rate, are regulated by the SNS (Seals & Esler, 2000), but responses to acute stress heighten SNS activity and release hormones that allow quick reaction to a threat; a phenomenon commonly called “fight or flight” (Cannon, Britton, Lewis, &
Groeneveld, 1927). In a condition that requires immediate response, such as jumping out of the way of a speeding car, the SAM axis releases the catecholamines epinephrine and norepinephrine, which increase heart rate, blood pressure, glucose levels, slow the body’s digestive systems, and enlarge the pupils (Piazza et al., 2010). SAM axis activity is important for survival, but if stressful events become more long-term, the benefits of the SAM axis become dangerous because too much catecholamine production can become toxic (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Piazza et al., 2010). Therefore, there is a “back-up” system to deal with longer-term stressors: the hypothalamic-pituitary-adrenal (HPA) axis, which will be the focus of this study.

A cognitive appraisal of events as being stressful can set into motion a cascade of chemicals leading from the hypothalamus to the pituitary and then signals the adrenal cortex to release cortisol into the bloodstream as a protective factor in response to the long-term stressor. Cortisol stimulates energy resources and anti-inflammatory and immune system responses that prepare the organism to endure long-term stress (Sapolsky, 1999). Ideally, people (and other organisms) become habituated to stressful challenges and are less affected by similar stressors in the future. However, the HPA axis is susceptible to accumulated wear and tear over time, especially if the person has experienced above-average stress across the lifespan (Seeman & Gruenwald, 2006), which is problematic because increased average cortisol levels can also be toxic (Sapolsky, 1999).
DAILY STRESSORS

There are several types of stress that can affect HPA reactivity, including trauma, life events, chronic stress, and daily stressors (see Aldwin, 2007). Past research has trended toward examining daily stressors as a preferred way of examining the impact of stress on CRT daily rhythms (see Almeida et al., 2011; Neupert, Miller, & Lachman, 2006). Almeida (2005) pointed out that major life events, such as a death in the family or a catastrophe, can have a lasting impact on the physical and emotional health of an individual, but these events are relatively rare. Daily stressors, however, are more persistent and can produce a negative cumulative health effect on individuals. Further, daily stress has a more proximal influence on health and physiological outcomes of stress can be more easily traced back to a stressful event. Examples of daily stressors are common disruptions to daily life, such as an argument with a spouse or a co-worker. The degree to which people are affected by daily stressors may be influenced by the exposure and reactivity to these events, but also individual differences in characteristics such as age and personality

Individual Differences in Reaction to Daily Stressors

Age is associated with differences in response to daily stressors in part due to age-related declines in homeostasis and may have a long-term effect on daily functioning (see Aldwin & Gilmer, 2013; Neupert, Almeida, Mroczek, & Spiro, 2006). Age may also affect the perceptions of what constitutes stress (Boeninger, Shirashi, Spiro, & Aldwin, 2009). Older individuals may also be less willing to devote a lot of attention and energy to stressful occasions as they age (Carstensen, Issacowitz, &
Charles, 1999; Charles, 2010; Mroczek & Almeida, 2004). Differences in neuroticism levels are also believed to be a source of differences in stress reactivity (Amelang, 1997). People who are higher in neuroticism tend to have a more negative view of the world, be in more stressful situations, and respond more negatively to events (Ayotte, Potter, Williams, Steffens, & Bosworth, 2009). Based on Folkman and Lazarus’ (1987) hypothesis that stress reactivity is related to how people cognitively appraise events, researchers have often assumed that individuals high in neuroticism will be more reactive in stressful circumstances (for a review, see Aldwin, 2007).

**Age and Daily Stressors**

Age explains some of the variance in the numbers of stressors reported by individuals (Mroczek & Almeida, 2004) and the level of reactivity that individuals have to those stressors (Carstensen et al., 1999; Charles, 2010). Socioemotional Selectivity Theory (SST; Carstensen et al., 1999) hypothesized that people choose emotion-focused goals as they age, as opposed to the information-gathering goals that are more common in younger adults. Greater saliency of emotion-focused goals is a result of the recognition that the time left in one’s life is getting shorter and that interaction with people who will have a positive emotional effect in their lives becomes more significant. That is, people make specific social choices and changes in perspective that will reduce the odds of being in stressful circumstances. However, negative interactions with others can be particularly stressful for older adults because of the prominence of interpersonal relationships are often the most reported stressors for older adults (Almeida, 2005).
Whether people become more susceptible to stressors as they age is a subject of debate (Diehl, Coyle, & Labouvie-Vief, 1996). Theories that assert that older adults are less susceptible to stress suggest that people are more adept at simply dismissing stressful events as they age (Stawski & Sliwinski, 2008), or that older adults tend to focus on social goals that preserve emotional health, thereby deemphasizing otherwise stressful daily events (Carstensen et al., 1999). Theories that assert that adults respond more negatively to stressful events as they age point out that older adults may report less stress than younger adults, but they tend to have stronger responses to stress when stress is unavoidable (Mroczek & Almeida, 2004). Other research suggests that repeated exposure to stress sensitizes people to stress and lowers the threshold for a negative response to stressful events and may be due to the brain’s susceptibility to stress hormones (Lupien et al., 2007a). For example, repeated stressors have the potential to affect neural networks associated with the amygdala, potentially increasing emotional reactivity to stressful events (Mroczek & Almeida, 2004).

**Emotional Stability/Neuroticism and Daily Stress**

Personality characteristics such as emotional stability and neuroticism may also affect psychological and physiological responsiveness to stress (Aldwin, 2007; Neupert, Mroczek, & Spiro, 2008). Goldberg (1992) posited that emotional stability and neuroticism reflect one bipolar construct, but most studies that examine the effect of personality characteristics on affective reactivity focus on neuroticism.

A combination of reactive differences to stressful events and ways of perceiving the world may lead to increased stress response in people higher in
neuroticism (Gerritsen et al., 2009; Gunthert, Cohen, & Armeli, 1999; Magnus, Diener, Fujit, & Pavot, 1993). Neuroticism is the enduring tendency to experience negative emotionality and respond more negatively to environmental challenges (Matthews & Dreary, 1998). People high in neuroticism are more likely to have problematic relationships with others and respond to mundane life events with more negative affect. Those high in neuroticism are also at risk of blaming themselves for poor outcomes. While there is substantial research indicating that major life events have an effect on stress reactivity in those with high levels of neuroticism, daily hassles and ordinary relationship conflicts appear to have more effect on stress outcomes for neurotic people than significant life events (Bolger & Schilling, 1991).

As noted before, neuroticism predisposes people to experience more negative events, but, interestingly, people higher in neuroticism report significantly fewer positive events despite evidence that positive events happen in their lives (Magnus, Diener, Fujita, & Pavot, 1993). These results indicated that, while good things happen to people who tend toward neuroticism, people higher in neuroticism either focus more on the negative or respond more negatively to otherwise positive situations (Folkman, Lazarus, Dunkel-Schetter, DeLongis, & Gruen, 1986). Therefore, how one responds to events is perhaps more salient than the event itself (Bolger & Schilling, 1991). Building on this theory, Mroczek and Almeida (2004) found that people with higher levels of neuroticism react with higher negative affect on stressful days than adults who are low in neuroticism. This could be due to heightened neural activity associated with stressful events over time that may make
individuals more susceptible to negative affect in stressful situations (Amelang, 1997; Ayotte et al., 2009; Bolger & Zuckerman, 1995; Eysenck, 1985; Folkman, Lazarus, Pimley, & Novacek, 1987).

A key aspect of neuroticism is that it affects how people assess a threat to their environment and perceive stressful situations (Folkman et al., 1986; Neupert et al., 2008). People tend to assess threats to their environment in one of two ways: as a challenge or as a threat. People who feel that they have the resources to handle a stressor are more likely to approach the event with a sense that they can overcome the conditions that present themselves. However, stressors can overcome people who do not feel that they have the resources to handle stressful events. People higher in neuroticism are more likely to appraise stressful situations as more harmful than people lower in neuroticism and, thus, to assess the situations as threats rather than challenges (Neupert et al., 2008). Further, those higher in neuroticism are more likely to be cognitively reactive to stressful events during the day. That is, they experience more disruptions to their executive function processes on days with more daily stressors. These results suggest that people higher in neuroticism are less capable of regulating their emotions or that they find themselves in more stressful events during a day than people lower in neuroticism. Further, those higher in neuroticism are more likely to affect performance on stressful tasks due to higher threat appraisal (Schneider, Rench, Lyons, & Riffle, 2011).
MEASURING THE EFFECTS OF STRESS REACTIVITY

Considering the variability of stress response, researchers have sought methods that could identify the effects of neuroticism and age on stress response that do not rely on self-report (for a review, see Piazza et al., 2010). Although self-report is a valid means for raters to objectively assess the effects of stress on subjects in a study, self-reports on stress by men tend to be less concordant with physiological responses than women (Alvero & Calvo, 1999). Therefore, studies suggest that researchers take measures of affective response to stressful situations as well as multiple measures of physiological response in order to better index the effects of stress reactivity. One way to assess stress reactivity and the health of the HPA axis is by measuring cortisol levels throughout the day (Granger et al., 2007; Granger, Hibel, Fortunato, & Kapelewski, 2009). Cortisol is released in daily rhythms that are distinguished by a peak in the morning, known as the cortisol awakening response (CAR), and steady decline throughout the rest of the day. These rhythms are possibly affected by life-long exposures to stress that may lead to overall increases in average daily cortisol production (Raff et al., 1999). Advances in biomarker collection and analysis have made it possible to plot daily cortisol rhythms that can be represented as a linear function, with steeper slopes indicating healthier average cortisol and flatter sloped lines indicating an impaired HPA axis, reflecting increased cortisol production throughout the day. In part, variation in CRT diurnal rhythms varies as a function of stress; nonetheless, there are individual differences in stress reactivity that may be due to differences in age and neuroticism levels. Therefore, we will measure the slopes of diurnal rhythms to assess overall
amounts of cortisol produced in each individual.

**Daily Stressors and CRT**

Dickerson & Kemeny (2004) asserted that increased cortisol response to stressors may be due to the perception of threats to self-preservation. However, research indicates that increased predictability of a stressful event can reduce stress reactivity and that not all uncontrollable events have the same cortisol response (Dickerson & Kemeny, 2004; Petrowski, Wintermann, & Siepmann, 2012). This suggests that the stressor must threaten an important goal for an individual in order for cortisol reactivity to be significant. Repeated habituation to a threat may reduce reactivity but threats to important goals that are uncontrollable will increase cortisol reactivity. Notably, most research investigating cortisol reactivity to stress has occurred in the laboratory and does not explore the relationship between stressors that adults experience in daily living and cortisol reactivity. Further, little research exists on the relationships between variables such as age, neuroticism and daily stress in a natural setting.

**Aging and CRT**

The literature often asserts that cortisol levels increase in individuals as they age, but these assertions are somewhat controversial (Almeida, Piazza, Stawski, & Klein, 2011; Ice, Katz-Stein, Himes, & Kane, 2004; Lupien et al., 2007a; Almeida, Piazza, & Stawski, 2009). For example, some longitudinal studies indicate that cortisol levels change in older adults, but there is no clear direction in change (Lupien et al., 2005). Other studies have indicated that older adults have significantly lower CAR response and reduced diurnal slopes, but overall lower
cortisol levels than younger adults (Heaney, Phillips, & Carroll, 2010). Another study indicated that older adults diurnal slopes become steeper with age (Ice et al., 2004). However, many of these studies are limited as they have small sample sizes (Lupien et al., 2007b), cross-sectional analyses (Heaney et al., 2010), and often use convenience samples (Ice et al., 2004).

Selectivity of samples may possibly confound conclusions regarding increasing cortisol levels associated with aging. For example, Wrosch, Miller, and Schultz (2009) found that increased cortisol levels in older adults may be more related to physical disability than aging per se. Significantly higher cortisol levels in older adults were attenuated among those who dedicated more time to health concerns, sought out external help, and set goals for better health. These concerns are salient when considering clinical trials that take advantage of hospitalized samples that may use more medically fragile samples (Lupien et al., 2007b). The participants in the current study were originally chosen for their good health and measurements were taken in a natural setting (Spiro & Bossé, 2001).

**Emotional Stability/Neuroticism & CRT**

Some studies show that neuroticism is associated with increased stress reactivity (Mangold & Wand, 2006), higher CAR (Portella, Harmer, Flint, Cowen, & Goodwin, 2005), and higher overall cortisol levels (Bolger & Schilling, 1991; Chida & Hamer, 2008; Nater, Hoppman, & Klumb, 2010). Laboratory studies confirm stress response differences between those higher in neuroticism and lower in neuroticism. Magnold and Wand (2006) showed that individuals divided into low and high neuroticism groups had different cortisol responses to naloxone injection, which
indicated damage to the HPA axis. Results indicating a positive association between neuroticism and appraising stressful events as more threatening are also consistent with this hypothesis (Neupert, Mrozcek, & Spiro, 2008; Mrozcek & Almeida, 2004). As noted previously, greater stress results in greater stress response and increased cortisol production in reaction to a perceived threat to an individual. These findings may be particularly important to people who may perceive threats to support as highly stressful. However, other studies indicate that cortisol response to acute stressors may be lower in high neuroticism individuals, suggesting dysregulation of the HPA axis to stressful events (Phillips, Carroll, Burns, & Drayson, 2005).

Variation in results of neuroticism and cortisol studies has made conclusions about the relationship between HPA axis response and neuroticism somewhat elusive (Nater et al., 2010). Notably, these studies may have been limited due to single point measures of cortisol (Portella et al., 2005), or the use of samples of younger adults (Hauner et al., 2008), or without the benefit of multiple measures of CRT levels associated with neuroticism (Nater et al., 2010). While several studies have examined the impact of neuroticism and negative affectivity on CRT responsiveness to daily stress (see Doane et al., 2011; Hauner et al., 2008), to our knowledge, few have done so with stress reactivity in an older sample with repeated measures of cortisol over four days.

**PRESENT STUDY**

The purpose of this study was to explore the moderating effect of age and neuroticism on individuals’ stress reactivity. The dependent variables in this study
are CRT diurnal rhythms, which will assess the physical response to daily stress. Participants in this study are a sample of older adults from the Normative Aging Study (NAS; Spiro & Bossé, 2001; see description in Sample and Procedures). Note that we will control for the effects of alcohol use, and smoking as appropriate.

**Aim 1: What is the effect of daily stress on cortisol diurnal rhythm slopes?**

1.1 **Higher numbers of daily stressors will result in more flattened cortisol diurnal rhythm slopes.** This hypothesis posits that the number of daily stressors a person experiences throughout the day will lead to increased stress reactivity. Increased levels of cortisol due to stress is evidenced by less steep declines in cortisol diurnal rhythms over the day.

**Aim 2: What is the effect of age on cortisol diurnal rhythms?**

2.1 **Cortisol diurnal rhythm slopes flatten as people age.** We will test this hypothesis in order to clarify whether cortisol levels increase with age, which may be an indication of a normative developmental decreases in the functioning of the HPA axis. Increased levels of cortisol due to age is evidenced by less steep declines in cortisol diurnal rhythms over the day.

**Aim 3. Do varying levels of Emotional Stability/Neuroticism predict more flattened cortisol diurnal rhythm slopes?**

3.1. **Individuals with higher neuroticism levels will have more flattened cortisol diurnal rhythms.**

This hypothesis asserts that individuals with higher neuroticism levels
will have higher levels of cortisol throughout the day. Increased levels of cortisol due to emotional stability/neuroticism is evidenced by less steep declines in cortisol diurnal rhythms over the day.
CHAPTER 2

METHODS

SAMPLE AND PROCEDURE

The NAS is a longitudinal project that started in the 1960’s that selected 2,280 men, aged 21-81 who were chosen from a pool of over 6,000 men to study the normal aging process. The men were screened for good health, defined as absence of chronic illness and blood pressure below 140/90. The men were also chosen for the likelihood of geographic stability, which was indicated by residence in the Boston area. The men were equally divided by blue collar and white collar workers and were representative of the racial profile of the Boston area in late 1950’s, which was mostly white. As of 2008, 981 men were still participating in the study, 1146 are deceased, 110 have left the study, and 43 were too sick to continue with the study. The current mean age of the NAS is 79 (SD = 6.6) with a range from 65 – 95. The sample in this study consisted of NAS men who participated in two previous daily diary studies that were focused on changes in stress reactivity in later life. The first diary collection was in 2002-03 and included 180 NAS men. The second assessment was in 2004-05 and included 103 men. The sample for this study consists 72 men who had data available at the time of the analyses. The mean age was 79.29 (SD = 4.88, range 67-93). The men in this study completed the third wave of diary studies in 2008-09, which also included collection of cortisol samples on four of the eight days of diary collection. All of the men were European American, and all were married. The respondents who completed the diary in this sample were significantly younger than the men who began the diary study in 2003 but did
not participate in 2008 ($M = 72.67, SD = 4.65$ vs. $M = 75.15$ years, $SD = 6.14$, $t(167) = 2.60, p < .01$). Not surprisingly, they also rated their physical health better on a standard 5-point self-rating ($1 = excellent$), ($M = 2.53, SD = .89$, vs.; $M = 2.91$, $SD = .92$, $t(169) = 2.46, p < .01$). However, there were no differences between the two groups on marital status, employment status, or self-rated emotional health compared to the previous year. Further, there were no differences between groups on the seven Goldberg Big 5 Emotional Stability/Neuroticism measure.

**Procedure**

Respondents completed the daily diaries on eight consecutive days, approximately $\frac{1}{2}$ hour before going to bed, and returned them once all eight were completed in a pre-addressed postage-paid envelope. Respondents were paid up to $65$ for their participation in the daily diaries. Emotional stability/Neuroticism was collected as part of the Well-Being and Health Survey (WBHS) that was given to the NAS men at in 2003, which is the most proximal measurement of neuroticism prior to the cortisol assays.

CRT samples were collected to assess stress response system health as part of the NAS daily diary collection in 2008/09. The saliva samples were collected on four (days 2 through 5) out of eight days of diary participation. Home saliva kits were sent to the men with instructions for collecting the saliva and directions for how to return the samples to the principal investigators in pre-addressed/paid postal materials. Sixteen salivettes per person were collected (four for each day) and linked to the time of day when the sample was collected. Wherever possible, participants were selected by the non-use of medications that are known to affect
adrenal function, such as corticosteroids, cholesterol medication, and anti-hypertensives. Saliva samples were shipped to and analyzed by the Salimetrics lab in Pennsylvania.

**MEASURES**

**Emotional Stability/Neuroticism**

The Goldberg Big 5 scale used in this study has 35 items spread across the five personality domains of Extraversion, Agreeableness, Conscientiousness, Emotional Stability, Intellect or Openness. Emotional Stability/Neuroticism is assessed through participants’ ratings of themselves on a spectrum of Emotional Stability (see Appendix A). The participants rank themselves on a continuum between two polar adjectives, such as “Angry” and “Calm,” on a 1 to 9 scale with 1 indicating low emotional stability and 9 indicating high emotional stability, and 5 indicating “Neither” high nor low. For example, participants would rate themselves somewhere on the scale between being “Angry” and “Calm,” with 1 indicating “Very Angry”, 2, 3, and 4 as “Moderately Angry,” 5 as “Neither Angry or Calm,” 6, 7, and 8 as “Moderately Calm,” and 9 as “Very Calm.” The coefficient alpha of the Goldberg Big Five measure of Emotional Stability is .86 (Goldberg, 1992). The measure used in this sample is the average response to the 7 items in the emotional stability/neuroticism measure. The average emotional stability/neuroticism rating was 6.98 (SD = 1.21) and the median score was 7.28.

**Daily Stress**

In order to assess the effect of varying amounts of stressful experiences on cortisol reactivity, this study used the NAS modified version of the Daily Inventory
to Stressful Inventory (DISE; Almeida, Wethington, & Kessler, 2002). The DISE assesses seven core stressors that people experience in a given day (see Appendix B). Participants in this study were asked to report the number of stressors they experienced each day (M = 0.85, SD = 1.10, range = 0 to 7). Examples of the questions used in the daily diary include, “In the past 24 hours, did you have an argument or disagreement with anyone?” “In the past 24 hours, did anything happen that you could have argued or disagreed about, but you decided to let it pass?” “In the past 24 hours, did anything happen in your workplace or volunteer setting (other than you have already mentioned) that most people would consider stressful?”

This study used the sum of the total number of stressors reported on each day and used that figure as a predictor for within-person variance in CRT diurnal rhythms. We centered stress at two different levels. We calculated the grand mean stress for the entire sample of the average person’s stress on an average day and we also calculated each individual’s mean variation of stress around the grand mean, indicating person i’s average stress level across all days.

**Salivary Cortisol**

All samples are assayed for salivary cortisol in duplicate using a highly sensitive enzyme immunoassay (Salimetrics LLC, State College, PA). Prior to testing, the sample’s pH was checked and corrected if outside the acceptable range (pH 4-9). The test used 25 ul of saliva per determination, with a lower limit of sensitivity of 0.003 ug/dl, standard curve range from 0.012 to 3.0 ug/dl, and average intra-and inter-assay coefficients of variation of 3.5 % and 5.1 % respectively. Method accuracy, determined by spike and recovery, and linearity, by serial dilution, was
100.8 % and 91.7 %. Values from matched serum and saliva samples showed the
expected strong linear relationship, \( r(63) = 0.89, p < 0.0001 \).

Cortisol varies in a coordinated manner with time of day, which is typically
significantly right skewed. We used the natural log of cortisol (LOGCORTISOL),
which was indicated in Stata 12 (StataCorp, 2011) as the best approximation of
normal distributions.

**Control Variables**

Smoking attenuates HPA axis activity (Rohleder & Kirschbaum, 2006) and
was controlled for in this study. Participants reported the number of cigarettes they
smoked in the previous 24 hours. Very few men in this study reported smoking
\( M = .03, SD = .17 \). Therefore, smoking was dichotomized 0 as not having smoked
in the previous 24 hours.

Alcohol intake is known to increase HPA axis activity (Boschloo et al., 2011)
and was controlled for in this study. Only about a third of the men in this study
reported having had a drink \( M = .30, SD = .46 \). Therefore, alcohol consumption
was also dichotomized, with 0 as not having a drink in the past 24 hours.

**ANALYSIS**

Multilevel modeling (MLM) in Stata 12 (Rebe-Hesketh & Skrondal, 2008;
StataCorp, 2011) was used to analyze the association of age, neuroticism and coping
on cortisol diurnal rhythms. MLM examines individual change/variability through
the use of multi-leveled hierarchical models and is appropriate for use with
longitudinal data and repeated measures nested within a person (Bryk &
Raudenbush, 1987). A key benefit of MLM is that it allows the use of predictors at
multiple levels of analysis. For example, analysis can include time-invariant predictors at one level, such as age, and time-varying predictors at another level, such as stress reports. Another advantage to MLM is that it can estimate variance around slopes and intercepts; that is, it can account for the fact that people start on at a different place on a given measure and change at different rates. However, MLM has the advantage of partitioning variance attributed to between-person variance and within-person variance, which can capture more than the aggregated mean differences in cortisol levels and reveal more about individual fluctuation throughout the day. Knowing information about within-person variation allows the attribution of differences in cortisol levels to variables such as time of day or stress level on a particular day. Perhaps most importantly, MLM captures all of this information while preserving more degrees of freedom (allowing for better model fit and future hypothesis testing) than ANOVA or regression analysis and provides enough power to detect effects based on multiple measures for each person despite lower sample sizes.

Our models included three levels. Level 1 modeled the within-person variation of cortisol levels around the mean cortisol level on a given day. Level 2 assessed the day-to-day variation around the individual's grand mean of cortisol levels and introduced time-varying control variables, such as drinking and smoking amounts for that day. At level 3, we introduced between-person time invariant variables, age and emotional stability/neuroticism. Our level 1, 2 and 3 models for our initial analysis were:
t = time, j = day, i = individual

Level 1: LOGCORTISOL_{ijt} = β_{0ij} + β_{1ij} (TIME_{ij}) + e_{ijt}

Level 2: β_{0ij} = \gamma_0 + \gamma_1 (WP STRESS [cntr’d]_{ij}) + \gamma_2 (SMOKING_{ij}) + \gamma_4 (DRINKING_{ij}) + u_{0ij}
β_{1ij} = \gamma_10 + u_{1ij}

Level 3: \gamma_{00} = \delta_{000} + \delta_{001} (BP STRESS [cntr’d]_{ij}) + v_{00i}
\gamma_{10} = \delta_{000} + v_{01i}

At level 1, the outcome, LOGCORTISOL_{ij}, is the amount of cortisol for person j at time point i. TIME_{ij} is amount of cortisol for person j at one of the four time points during the day when cortisol measures were taken and the within person error term is e_{ij}. The average time points when cortisol was measured was early in the morning at awakening, half an hour after awakening, around noon, and early evening around 8pm. The person’s own TIME intercept for cortisol levels was predicted by Level 2 as a function of β_{0ij}, the person’s own intercept for that day’s average cortisol measure, and β_{1ij} which characterizes the association between the stress that person experienced that day and the amount of cigarettes and drinks a person had that day. We centered stress around the person’s mean stress over the 4-day period, so \gamma_{01} is a person’s predicted cortisol level on a given day.

At level 3, \gamma_{00} is expressed as a function of the between-person intercept (\delta_{00}), and each individual’s variation (u_{ij}) around cortisol levels associated with the grand mean of stress (BP STRESS [cntr’d]) throughout the sample. Within-person slopes, \gamma_{10} are a function of the mean slope between persons (\delta_{00}) and the between person’s error term (u_{1ij}). In following analyses we introduced AGE and emotional stability/neuroticism (EMOT/N) into the level 3 equations and the computed
interactions of BP STRESS (cntr’d), AGE, and EMOT/N with TIME to capture the
linear slope of cortisol diurnal rhythms over the day.

1. Higher numbers of daily stressors will result in more flattened cortisol
diurnal rhythm slopes.

Level 1: LOGCORTISOL\textsubscript{ijt} = \beta_{0ijt} + \beta_{1i} (TIME)_{ijt} + e_{ijt}

Level 2: \beta_{0ij} = Y_{00} + Y_{01} (WP STRESS [cntr’d]_{ij}) + Y_{02} (SMOKING_{ij}) + Y_{04} (DRINKING_{ij}) + u_{ij}
\beta_{1ij} = Y_{10} + u_{1j}

Level 3: Y_{00} = \delta_{00} + \delta_{01} (BP STRESS [cntr’d]_{ij}) + (BP STRESS [cntr’d]_{ij} \times TIME) + u_{ij}
Y_{10} = \delta_{00} + u_{1j}

2. Cortisol diurnal rhythm slopes flatten as people age.

Level 1: LOGCORTISOL\textsubscript{ijt} = \beta_{0ijt} + \beta_{1i} (TIME)_{ijt} + e_{ijt}

Level 2: \beta_{0ij} = Y_{00} + Y_{01} (WP STRESS [cntr’d]_{ij}) + Y_{02} (SMOKING_{ij}) + Y_{04} (DRINKING_{ij}) + u_{ij}
\beta_{1ij} = Y_{10} + u_{1j}

Level 3: Y_{00} = \delta_{00} + \delta_{01} (BP STRESS [cntr’d]_{ij}) + (AGE_{ij}) + (AGE \times TIME) + u_{ij}
Y_{10} = \delta_{00} + u_{1j}

3. Individuals with higher neuroticism levels will have more flattened
cortisol diurnal rhythm slopes.

Level 1: LOGCORTISOL\textsubscript{ijt} = \beta_{0ijt} + \beta_{1i} (TIME)_{ijt} + e_{ijt}

Level 2: \beta_{0ij} = Y_{00} + Y_{01} (WP STRESS [cntr’d]_{ij}) + Y_{02} (SMOKING_{ij}) + Y_{04} (DRINKING_{ij}) + u_{ij}
\beta_{1ij} = Y_{10} + u_{1j}

Level 3: Y_{00} = \delta_{00} + \delta_{01} (BP STRESS [cntr’d]_{ij}) + (EMOT/N_{ij}) + (EMOT/N \times TIME) + u_{ij}
Y_{10} = \delta_{00} + u_{1j}
Treatment of Missing Data

Most surveys suffer from some missing data. The statistical basis for obtaining appropriate and reliable population estimates from “incomplete” data is well established. The statistical methods typically use likelihood-based estimation processes that assume that data are Missing at Random (MAR; Acock, 2005). That is, there are explanations for the missing data in the covariates and/or previous responses. Almost all missing data assumptions are based on MAR as opposed to the more conservative Missing Completely at Random assumption (MCAR) in which missing data cannot be explained by the other covariates in the model or other unobserved parameters. Examples of MCAR would be variables that are missing by design, such as waves of data that include people in different age groups where particular age groups are missing in certain years. Previous approaches to handling MAR data would use methods such as listwise deletion, pairwise deletion, or mean substitution, thereby discarding information. We used Maximum Likelihood (ML) estimation to handle missing variables, which uses all available information, such as variances and covariances, in order to estimate all parameters simultaneously without sacrificing valuable information.
CHAPTER 3

RESULTS

STRESS FREQUENCIES AND DESCRIPTORS

Table 1 provides descriptive statistics for the sum total of stressors reported across the sample for each day.

<table>
<thead>
<tr>
<th>Number of stressors per day</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>203</td>
<td>70.5</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>17.36</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>8.68</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>2.08</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1.04</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.35</td>
</tr>
</tbody>
</table>

The majority of people (70.49 %) reported no stressors each day, while 17.36 % reported 1, and about 12 % reported 2 or more. One stressor on a given day was reported 50 times. Analysis of the data indicated that 34 people reported only one stressor, but 13 of those reporting one stressor reported having one stressful event on more than one day. Two stressors on a given day were reported 25 times. Six of those people who reported two stressors reported that they had two stressors on more than one day. Six different people reported having three stressors a given day and one person reported four stressors on three consecutive days. One person reported six stressors on a given day.
Table 2 shows the percentages of the type of stressors people reported over the entire study period of the 30% of reports that did include a daily diary. As can be seen, nearly half of the stressors involved interpersonal relationships, with 25% concerning arguments or disagreements, and 24% arguments that were avoided.

<table>
<thead>
<tr>
<th>Table 2. Percentage of Reported Stressors by Stress Type (N = 85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Argument or disagreement</td>
</tr>
<tr>
<td>2. Could have argued but didn't</td>
</tr>
<tr>
<td>3. Workplace or volunteer stressor</td>
</tr>
<tr>
<td>4. Stressor at home in the past 24 hours</td>
</tr>
<tr>
<td>5. Something happen to friend or relative</td>
</tr>
<tr>
<td>6. Personal health stressor in past 24 hours</td>
</tr>
<tr>
<td>7. Anything else that happened</td>
</tr>
</tbody>
</table>
BIVARIATE RELATIONS AMONG THE STUDY VARIABLES

Table 3 shows the correlations and descriptive statistics for all the variables in the study. Time had a significant negative relationship with logged cortisol, 
\[ r = -.60, p < .001, \] indicating that, as time goes up during the day, cortisol levels go down. Logged cortisol had a positive relationship with the between person stress centered at the Grand Mean (BP Stress (cntr’d)) for the entire sample, 
\[ r = .06, p < .05, \] indicating that logged cortisol was higher for overall higher stress. Logged cortisol also had a positive relationship with centered age, 
\[ r = .08, p < .05, \] indicating that higher age was associated with higher cortisol measures. BP Stress (cntr’d) had a negative relationship with emotional stability/neuroticism, 
\[ r = -.13, p < .001, \] indicating that increased reported stress across the sample was associated with higher neuroticism. BP Stress (cntr’d) also had a negative relationship with drinking, 
\[ r = -.07, p < .05. \]

Centered age had a negative relationship to emotional stability/neuroticism, 
\[ r = -.08, p < .01, \] indicating less emotional stability with age. They were also less likely to drink, 
\[ r = -.15, p < .001. \]

Emotional stability/neuroticism had a positive relationship to drinking, 
\[ r = .18, p < .001, \] and a negative relationship to smoking, 
\[ r = -.16, p < .001. \] Drinking and smoking were modestly associated, 
\[ r = .09, p < .01. \] Thus, the bivariate correlations largely supported the hypotheses.
HYPOTHESIS TESTING

We used MLM in Stata 12 (StataCorp, 2011) to formally test all hypotheses.

This section will be organized around the hypotheses.

**Hypothesis 1: Higher Numbers of Daily Stressors Will Result in More Flattened Diurnal Rhythms**

The first hypothesis examined whether diurnal rhythms flattened with higher number of stressors reported in the day. The results of this analysis (see Table 4) showed that time had a significant negative relationship with logged cortisol, \( B = -.53, p < .001 \). This indicates that the measures of logged cortisol drop over the four time points in the day and is consistent with literature indicating a steady decline in cortisol throughout the day (for a review, see Piazza et al., 2010).
The BP Stress (cntr’d)*Time interaction was significant, $B = .09, p < .001$, which indicated that people with higher number of stressors would have more flattened cortisol diurnal rhythm slopes (see Figure 1). Neither the WP Stress (cntr’d) nor BP Stress (cntr’d) had significant main effects. This result indicated that people with higher number of stressors would have more flattened cortisol diurnal rhythm slopes (see Figure 1). The effects of the control variables cigarettes and drinking were not significant and the same pattern was evident throughout the analysis. The LR test vs. linear regression was significant, $\Delta \chi^2(2) = 275.57, p < .001$, indicating that the MLM model with random effects was a better fit to the model than one which had only fixed effects, suggesting that there were individual differences in the relations between stress and cortisol slope.
Table 4.  
Cortisol Diurnal Rhythms by Number of Stressors

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>(se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-.12</td>
<td>(0.08)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>-.05</td>
<td>(0.04)</td>
</tr>
<tr>
<td>Smoking</td>
<td>.25</td>
<td>(0.3 )</td>
</tr>
<tr>
<td>WP Stress (cntr’d)</td>
<td>-.01</td>
<td>(0.04)</td>
</tr>
<tr>
<td>BP Stress (cntr’d)</td>
<td>-.11</td>
<td>(0.11)</td>
</tr>
<tr>
<td>Time of Day</td>
<td>-.53***</td>
<td>(0.02)</td>
</tr>
<tr>
<td>BP Stress (cntr’d) x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>.09***</td>
<td>(0.03)</td>
</tr>
</tbody>
</table>

Random Effects

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>(se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>.21</td>
<td>(.04 )</td>
</tr>
<tr>
<td>Day</td>
<td>4.40E-24</td>
<td>(1.02E-23)</td>
</tr>
<tr>
<td>Residual</td>
<td>.41</td>
<td>(0.02)</td>
</tr>
</tbody>
</table>

vs. linear  \( \Delta \chi^2(2) = 275.57, p < .001 \)

***p<.001  

*Note: WP Stress (cntr’d; WP) = within person centered mean  
BP Stress (cntr’d) = between persons grand centered mean*
Figure 1 shows the effect of the number of stressors reported on a given day and time on cortisol slopes over the day. The overwhelming number of respondents in the study reported no stress, so the graph is divided into people with no stress reported and people with one or more stressors reported for illustrative purposes. Cortisol is represented as its log transformation with approximate intercepts of both groups at -.12. The slope of the stress lines indicates the drop in cortisol levels as the day progresses (time of day 1, 2, 3, & 4). As the day progresses, people with one or more stressors (dashed line) reported have a flatter diurnal rhythm slopes than people with no stress (solid line).
Hypothesis 2: Cortisol Diurnal Rhythms Flatten as People Age

The results of this analysis (see Table 5) showed that time had a significant negative relationship with logged cortisol, $B = -0.53$, $p < 0.001$. The main effect of age was not significant. However, the age*time interaction was significant, $B = 0.09$, $p < 0.001$, which reflected the linear slope of cortisol over the four time points that cortisol was measured during the day. This result indicated that people with higher age would have more flattened cortisol diurnal rhythm slopes (see Figure 2). The LR test vs. linear regression was significant, $\Delta X^2(2) = 274.98$, $p < 0.001$, indicating that the model which included the random effects terms was significantly better fit to the data than one with just fixed effect.

<table>
<thead>
<tr>
<th>Table 5. Cortisol Diurnal Rhythm by Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Effects</td>
</tr>
<tr>
<td>Constant</td>
</tr>
<tr>
<td>Alcohol</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Age (cntr’d)</td>
</tr>
<tr>
<td>WP Stress (cntr’d)</td>
</tr>
<tr>
<td>BP Stress (cntr’d)</td>
</tr>
<tr>
<td>Time of Day</td>
</tr>
<tr>
<td>Age x Time</td>
</tr>
</tbody>
</table>

Random Effects

<table>
<thead>
<tr>
<th>Individual</th>
<th>(_cons)</th>
<th>Estimate</th>
<th>(se)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>(_cons)</td>
<td>9.32e-24</td>
<td>(2.46e-23)</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>0.41</td>
<td>(0.02)</td>
</tr>
</tbody>
</table>

LR test vs. linear regression $\Delta X^2(2) = 274.98$, $p < 0.001$

***p<.001
Figure 2 shows the effect of age and time on cortisol slopes over the day. The men in the study were divided into age groups based on the median age of the sample (78) for illustrative purposes. Cortisol is represented as its log transformation with approximate intercepts of both groups at -.12. The slope of the stress lines indicates the drop in cortisol levels as the day progresses (time of day 1, 2, 3, & 4). As the day progresses, people in the older age group (dashed line) have a flatter diurnal rhythm slope than people in the younger age group (solid line).
Hypothesis 3. Cortisol Diurnal Rhythms Flatten with Higher Neuroticism

The results of this analysis shows that time had a significant negative relationship with logged cortisol, $B = -.53$, $p < .001$. However, the emotional stability/neuroticism*time interaction term was significant, $B = .09$, $p < .001$, which reflected the linear slope of cortisol over the four time points during the day. This result indicated that people with higher neuroticism had more flattened cortisol diurnal rhythm slopes (see Figure 3). The LR test vs. linear regression was significant, $\Delta X^2(2) = 247.62$, $p < 0.001$, indicating that the model which included the random effects terms was significantly better fit to the data than one with just fixed effects.

| Table 6. | 
| --- | --- | --- | --- |
| Cortisol Diurnal Rhythm by Emotional Stability/Neuroticism | Fixed Effects | Estimate | (se) |
| Constant | -.14 | (.08) |
| Alcohol | -.04 | (.03) |
| Smoking | .21 | (.3) |
| WP Stress (cntr’d) | -.01 | (.03) |
| BP Stress (cntr’d) | .08 | (.08) |
| Emot/N | .08 | (.06) |
| Time of Day | -.53*** | (.01) |
| Emot/N x Time | -.04** | (.01) |
| Random Effects | | |
| Individual | (_cons) | .19 | (.04) |
| Day | (_cons) | 6.70e-24 | 1.93e-23 |
| Residual | | .41 | (.02) |

LR test vs. linear regression: $\Delta X^2(2) = 247.62$, $p < 0.001$

**p < .01, ***p < .001;
Note: Emot/N = Emotional Stability/Neuroticism
Figure 3 shows the effect of emotional stability/neuroticism and time over the day on cortisol slopes. The median score of emotional stability/neuroticism was 7.28, so the men in the study were divided into higher emotional stability (> 7.28) and higher neuroticism (< 7.28) for illustrative purposes. Cortisol is represented as its log transformation with approximate intercepts of both groups at -.12. The slope of the lines indicates the drop in cortisol levels as the day progresses (time of day 1, 2, 3, & 4). As the day progresses, people with higher neuroticism (solid line) reported have a flatter diurnal rhythm slopes than people with higher emotional stability (dashed line), although the overall amount of cortisol is higher in the higher emotionally stable group.
We completed exploratory analyses of three-way interactions for: age, stress, and time; age, neuroticism, and time; and stress, neuroticism, and time. However, none of these interactions were significant and may be due to low sample sizes.

In summary, the results of these analyses confirmed our hypotheses that stress, age, and emotional stability/neuroticism all have significant effects on cortisol slopes throughout the day.
CHAPTER 4

DISCUSSION

We examined the inter-relationships among stress, age, personality, and cortisol diurnal rhythms in a small sample of older white men. One of the more interesting findings was the relatively few number of days on which a stress was reported (30%). However, this is not that dissimilar to a study by Almeida et al. (2002), who reported that people experienced at least one stressful event on nearly 40% of the days they participated in their first trial of the DISE. Similar to our study, nearly half of the reported stressors related to arguments and interpersonal tensions.

The results of the multi-level modeling showed that higher daily stress predicted flatter cortisol diurnal rhythms, as did age and emotional stability/neuroticism. The first hypothesis that the number of stressors reported in a day will predict higher cortisol diurnal rhythm slopes is consistent with previous studies (for a review, see Piazza et al., 2010). The number of stressors was reported at the end of the day, which would allow for the effects of stress during that day to influence the slope of cortisol rhythms. The enduring effects of stress throughout the day may explain why stressful events have a significant association with cortisol slopes throughout the day (DeLongis, Folkman, & Lazarus, 1988).

Cortisol may be a part of a system of influences that affect these common health issues due to the effect of suppressing the immune system. For example, DeLongis et al. (1988) found that daily stressors were associated with increased common health problems such as the flu and sore throat. Further, stressful events can potentially influence physiological arousal long after the cognitive effects have
worn off. That is, the body may still be responding to the stressful event long after the person has gone on to something else in their mind.

The second hypothesis is consistent with studies that showed flattened cortisol diurnal rhythms as people age (for a review, see Piazza et al., 2010). These results may be indicative of reduced functioning of the feedback system in the HPA axis due to a lifetime of use (Seeman & Gruenwald, 2006). The HPA axis is a systematic reaction to stressful events but is also a normative daily rhythm that serves to regulate the hormones that sustain our daily activities. That regulation is provided by a negative feedback response by the pituitary that monitors the amounts of circulating cortisol and shuts down the production of ACTH when the levels are too high. Overload of the HPA axis may lead to the inability to effectively regulate the amounts of cortisol in the bloodstream (McEwen & Seeman, 1999). Older adults may have restricted functioning of the HPA axis because of a greater amount of use over time due to longer use than younger adults.

Flattening of cortisol diurnal rhythms only partially confirmed results from the Douglass Hospital Longitudinal Study (Lupien et al., 2005). Note that Lupien et al. (2005) identified three different cortisol secretion level groups. One group was identified as having increasing levels of cortisol over time and that increase was “high.” A second group was found to also have increasing cortisol levels over time, but their increase was more moderate. A third group was found to have decreasing amounts of cortisol over time. While we only examined flattened trajectories with age, our random effects terms were significant, suggesting individual differences in the effects of age on trajectories.
The results in the current study are in marked contrast to Ice et al.’s (2004) results that indicates that younger adults had flatter cortisol diurnal rhythm slopes than older adults, which contradicted their own expectations. The authors hypothesized that their results may have been due to increased morning peaks of cortisol in the older group, which would affect the overall slope of cortisol during the day. However, Ice et al. pointed out that their sample was relatively small (N = 48) and that the people in their study were from upper income brackets and socially active. These characteristics may partially explain a healthier HPA axis than expected in the older group.

The third hypothesis that individuals with higher neuroticism levels will have more flattened cortisol diurnal rhythm slopes was also supported. These results are not surprising, considering that research indicates that people high in neuroticism tend to have a more negative outlook on the world and respond more negatively to normal daily events (Folkman et al., 1986). Interestingly, the levels of cortisol for older adults were lower than younger adults in the analysis of emotional stability despite a flatter slope exhibited by older adults. We attempted to analyze other covariates, such as stress and age, in this analysis, but were unable to ascertain effects due to low sample size.

The current study found results consistent with previous research that examined the effect of neuroticism on cortisol diurnal rhythms. Previous research was focused on the effects of depression and neuroticism (Doane et al., 2011) or adolescents (Hauner, 2008). Doane et al. (2011) found an association with negative emotionality and cortisol diurnal rhythms, but that effect of negative emotionality
was mediated by depression. Hauner et al. (2008) found that flatter diurnal rhythms were associated with higher neuroticism, but the study focused on adolescents. The results of the current study are different in that they employed measurements of cortisol rhythms over a longer period of days and utilized an older sample.

The results of this study illustrate how neuroticism can be more than an impact on psychological well-being and can affect potentially dangerous long-term physiological arousal (Mroczek & Almeida, 2004; Nater et al., 2009). These distinctions are worth mentioning because a person higher in neuroticism is susceptible to increased vigilance and worry in situations that would otherwise have minimal impact on a person or could actually be beneficial in terms of stress-related growth. This heightened arousal could potentially lead to increases in blood circulating cortisol, detrimental health outcomes, and more wear on the HPA axis, and might help explain the relationship between neuroticism and mortality (Mroczek & Spiro, 2007).

That older adults showed lower emotional stability and higher neuroticism is interesting in light of current theory that asserts that emotions becomes more stable as people age (Carstensen et al.,1999). This result may be indicative of a non-linear relationship between age and emotional stability. That is, emotional stability increases up to a certain age and then people may become more vulnerable due to reduction in emotional complexity that is related to reductions in cognitive complexity and emotional stability becomes less (Labouvie-Vief, Gruhn, & Studer, 2010). Adult development is typically highlighted by the integration of crystallized
intelligence, increasing cognitive complexity, and emotional stability that continually expands the thresholds of emotional resilience. However, decreasing fluid intelligence in later years may affect the ability of crystallized responses to external stimuli and older adults responses to emotionally salient events becomes more simplified to compensate for decreased cognitive functioning (i.e., emotional events become more polarized into good vs. bad instead of multifaceted problems). These emotional strategies may be adaptive in response to decreasing cognitive and emotional thresholds, but create situations where emotional stability can break down if threshold levels are breached (Carstensen et al., 1999).

The associations between lower reported stressors and higher smoking and drinking, supported evidence suggesting that people who consume moderate amounts of alcohol have better health and have more positive experiences than those who either abstain or are heavy alcohol users (for a review, see Brodsky & Peele, 1999). Further, moderate drinking can facilitate increased social experiences and that moderate drinkers report reduced stress in commonplace social interactions. This finding is in contrast to drinking behaviors of heavy drinkers who used alcohol to reduce the effects of anxiety associated with stressful circumstances. These findings suggest that alcohol has a general calming effect for moderate drinkers that may not be as available for non-drinkers and highlights coping differences of moderate and heavy drinkers where moderate drinkers are more likely to use alcohol to enhance social interaction and heavy drinkers to use alcohol to reduce tension. The results of the current study confirm some of these findings because there were no indications of heavy alcohol use among participants, but
increased drinking was associated with better emotional stability. It is surprising that the control variables – smoking and drinking – were associated with cortisol levels but were unrelated to the slopes. Future research should consider alternative control variables, including the existence of health problems (see Wrosch et al., 2009).

The primary motivation for this study was to understand psychosocial predictors of increased cortisol production, as unhealthy levels of cortisol are associated with deteriorating health with age (Dickerson & Kemeny, 2004; Eysenck, 1985; Mroczek & Almeida, 2004; Piazza et al., 2010; Sapolsky, 1999). Problematic health outcomes include hypertension, atherosclerosis, and diabetes (Lupien et al., 2007). Further, the hippocampus has many cortisol receptors, which renders this part of the brain susceptible to reduced memory function as a result of increased cortisol production, which is salient for an aging population. Mroczek and Almeida (2004) point out that higher neuroticism older adults may experience a neural tendency to respond to stressful events with more reactivity. Neural networks associated with the amygdala may be overused in higher neuroticism adults and lead to sensitization to stressful encounters that precipitates higher reactivity. Considering these results and past research, we suggest that future research look at the association between neuroticism, age, and cortisol diurnal rhythms with larger sample sizes.

LIMITATIONS

Some limitations to this study include the homogeneity of the sample, making generalizations about the data difficult. The men chosen for this study were
drawn from the same region and were generally of similar ethnic backgrounds. Also, the men in this study were originally chosen for their relative good health at the beginning of the study and the participants of this particular phase of the study may have survived this long into the study because of better overall psychosocial and physical health. These conditions may not generalize to other populations in which health is overall health is more likely to be deteriorating. Further studies should include a more diverse sample, perhaps representing various geographic locations and including women.

Another limitation is the use of a one-time measure of neuroticism and may underestimate the effect because older men tend to lower in neuroticism with age (Mroczek & Spiro, 2003). Unfortunately, there are no national norms on the Goldberg Big 5 measure used in this study. However, there was no difference in emotional stability/neuroticism from the rest of NAS men who had a previous daily diary but were not in the present study. Further, prior research on the MMPI-II (Butcher et al., 1991) showed that the NAS men in general are well within the norms for those subscales. Future research may benefit from the use of more measures of neuroticism over more time points to establish a trajectory of neuroticism.

This study is limited in scope because it measures CRT levels based on reported daily stress and does not account for lagged stress response. For example, people may experience increased CRT levels due to previous days’ stressful encounters. Also, cortisol was measured in only one wave. Repeated measures in longitudinal analysis may prove insightful because of interaction effects present in earlier analysis indicates that the effects of behavioral disposition may be vastly
different in later analysis (Segerstrom & Sephton, 2005). Original exposure to stressors may an increased effect on people who have a more optimistic approach to long-term goals but dissipate over time as the goal completion becomes more realistic. However, the opposite effect may be true for people with more negative outlooks. In other words, the relationship between neuroendocrine response and stressors may not be fully understood without repeated measures of both stress exposure and cortisol measures.

**Conclusion**

The results of this study were consistent with previous hypotheses that stress reactivity in older adults would predict higher overall cortisol levels as evidenced by cortisol diurnal rhythms and that emotional stability/neuroticism would partially explain differences in cortisol slope. Not all previous studies confirm these results and call into question the certainty that cortisol slopes flatten with age.

We expect that this research will add to the growing body of literature that employs biomarkers as a means to measure stress response. Psychosocial literature is at a crossroads in regards to biomarker testing and studies such as this one should invite other researchers to explore methods similar to the ones used here and move the field of biomarker testing in social sciences forward (M. Curran, personal communication, January 27, 2012). Future research should include alpha amylase to measure changes in short term stress response system to identify if rates of change in cortisol and alpha amylase capture the same effects of the predictor variables in the other levels of the model, or if predictor variables have different
effects on short-term and long-term stress response. Also, future research should look at the relationship between daily cortisol levels and subsequent days’ cortisol levels to ascertain if there is a lagged effect of daily stress (Mroczek & Almeida, 2004). Future studies may also want to explore the effect of morning peaks on cortisol diurnal rhythms (Ice, et al. 2004) and increased sample sizes (Lupien et al., 2005). Further, this study and previous studies relied on cross sectional analysis of cortisol diurnal rhythms. Future studies should consider longitudinal analysis of cortisol diurnal rhythms to better ascertain the effects of stress, age, and neuroticism on cortisol slopes.
REFERENCES


StataCorp (2011). *Stata statistical software: Release 12*. College Station, TX: StataCorp LP.


*Psychosomatic Medicine, 71*(9), 996–1003.
CHAPTER 5  
APPENDICES

Appendix A.  
Goldberg Neuroticism Measure

Please rate yourself on the following items with regard to how you feel usually.  
(Circle one number on each line).

<table>
<thead>
<tr>
<th>emotional stability</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angry</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9 Calm</td>
</tr>
<tr>
<td>Tense</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9 Relaxed</td>
</tr>
<tr>
<td>Nervous</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9 At Ease</td>
</tr>
<tr>
<td>Envious</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9 Not Envious</td>
</tr>
<tr>
<td>Unstable</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9 Stable</td>
</tr>
<tr>
<td>Discontented</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9 Contented</td>
</tr>
<tr>
<td>Emotional</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9 Unemotional</td>
</tr>
</tbody>
</table>

Very    Moderately    Neither    Moderately    Very
Appendix B

Daily Stress (From Normative Aging Study Diary of Daily Experiences 2008/09)

For questions 8-14, please tell us about stressful events that may have happened to you in the past 24 hours.

8. In the past 24 hours, did you have an argument or disagreement with anyone?
   ____ No   ____ Yes

9. In the past 24 hours, did anything happen (other than what you have already mentioned) that you could have argued or disagreed about, but you decided to let pass?
   ____ No   ____ Yes

10. In the past 24 hours, did anything happen in your workplace or volunteer setting (other than what you have already mentioned) that most people would consider stressful?
    ____ No   ____ Yes

11. In the past 24 hours, did anything happen at home (other than what you have already mentioned) that most people would consider stressful?
    ____ No   ____ Yes

12. In the past 24 hours, did anything happen to a close friend or relative (other than what you have already mentioned) that turned out to be stressful for you?
    ____ No   ____ Yes

13. In the past 24 hours, did anything stressful happen (other than what you have already mentioned) regarding your personal health?
    ____ No   ____ Yes

14. In the past 24 hours, did anything else happen that most people would consider stressful?
    ____ No   ____ Yes