Summer 8-15-2016

Differential Associations of Stress and Cortisol with Brain Structure and Cognition in Cognitively Normal Older Adults

Ana Kim
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST. LOUIS

Division of Biology and Biomedical Sciences
Neurosciences

Dissertation Examination Committee:
Denise Head, Chair
David Balota
Tony Buchanan
John Cirrito
Tamara Hershey

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by

Ana Kim

A dissertation presented to the
Graduate School of Arts & Sciences
of Washington University in
partial fulfillment of the
requirements for the degree
of Doctor of Philosophy

August 2016
St. Louis, Missouri

Table of Contents

List of Figures

List of Tables

Acknowledgments

Firstly, I want to thank my advisor, Dr. Denise Head, for all of her mentorship and support. I could not have done it without her expertise, patience, and dedication throughout my graduate school career. Next, I would like to thank my dissertation committee members, Drs. Tamara Hershey, David Balota, John Cirrito and Tony Buchanan for their helpful insights and guidance throughout the development and execution of my dissertation. I would also like to thank the Knight Alzheimer Disease Research Center at Washington University for the opportunity to conduct research using their rich datasets. Finally, I would like to thank my family and friends for their encouragement and support throughout my PhD study. This work was supported by NIGMS 2T32GM008151, NIGMS 5T32GM081739, NINDS 5T32NS073547, and NIA 5T32AG000030.

Dedicated to my family.

ABSTRACT OF THE DISSERTATION

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Professor Denise Head, Chair

The current literature shows discrepant findings as to the degree to which cumulative stress and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis are associated with brain structure and cognitive function in older adults, particularly in brain regions with high expression of receptors for glucocorticoid, and cognitive function reliant upon these regions. Past studies have been heavily focused on total hippocampus while limited studies have examined hippocampal subdivisions or other brain structures. In addition, one key moderator that may influence the associations of cumulative stress and cortisol on brain structure and cognition is the single-nucleotide polymorphisms (SNPs) of the stress-system genes, which has not been investigated in older adults. Therefore, in Aim 1, the current study examined the differential associations of cumulative stress and cortisol with brain structure that have high or low expression of receptors for glucocorticoid, including total hippocampus, hippocampal subdivisions, amygdala, medial prefrontal cortex, and primary visual cortex in cognitively normal older adults. In addition, the current study examined whether the genetic score from SNPs of stress-system genes moderated these associations. Aim 2 examined the differential

associations of stress and cortisol on cognitive functions, including memory, fluid intelligence, and crystallized intelligence. The moderating role of the genetic score was examined in Aim 2 as well. In general, no consistent results were found for either aim. Post-hoc analyses showed no consistent moderating role of either age or gender, but suggested timing of stress may be an important factor to consider for future studies. Overall, the current study suggests that stress and cortisol may not have robust associations with brain structure and cognition in older adults. However, future longitudinal studies with systemic incorporation of various factors, such as timing of stress and multiple cortisol measures across the day, may reveal more consistent associations of stress and cortisol.

Chapter 1: Introduction

Over two trillion dollars are spent in treating mental disorders across the globe (Insel, 2015). Anxiety disorder is one of the most common mental disorders in the United States, affecting roughly 20 percent of the U.S. adult population (National Institute of Mental Health, 2016). Stress is a contributing factor for many psychiatric disorders, particularly anxiety disorders and depression (Tottenham & Sheridan, 2010). In addition, previous studies have suggested that stress relates to other disorders as well, such as cardiovascular disorders, diabetes, diminished immune function, and cognitive decline (Lundberg, 2005). Understanding the influence of stress on brain structure and cognition in humans is important in preventing and treating stress-related disorders. Although many studies have attempted to determine this relationship, no strong conclusions can be made yet due to limitations and variations in study samples and methodologies (e.g., focus on specific types of stress only; variation in timing of the day in which cortisol measures were taken).

Furthermore, about 15 percent of the older adults are affected by anxiety disorders each year (National Institute of Health, 2016). In today's society, the older adult population is growing rapidly, therefore, finding ways to age successfully (e.g., maintaining intact cognition) is becoming one of the key areas of research. Stress is thought to be one of the multiple factors that not only contributes to psychiatric disorders, but also affect brain and cognitive aging. Specifically, researchers have predicted that cumulative exposure to stress and stress hormones (e.g., cortisol) throughout the lifespan will make neurons more vulnerable to neuronal insults, thus, possibly facilitating brain and cognitive aging (Landfield, Blalock, Chen, & Porter, 2007; Radley & Morrison, 2005). Therefore, the current study proposed to investigate how *cumulative*

stress and cortisol measures are associated with brain structure and cognition in cognitively normal older adults using available convenience samples.

1.1 Defining Stress and the Stress Response

In order to study the effect of stress, it is important to first define the term 'stress.' Unfortunately, the word 'stress' is commonly used in society without a clear definition, making this an ambiguous term. Even within the scientific research domain, there is still ongoing discussion regarding the definition of stress. To begin with, scientists have defined 'stressors' as "events and conditions that are *potentially* stressful." However, the term 'stress' cannot be defined simply as a set of events, such as war or bereavement, because this definition ignores whether these events actually trigger any psychological and biological responses. On the other hand, 'stress' cannot be defined simply based on response since events such as watching sports games, may bring about physiological alternations similar to the stress response (Contrada, 2011). In order to address this issue, Richard Lazarus developed a model which incorporated the concept of appraisal and coping. Based on the model, when an individual is exposed to a stressor, an event that can potentially cause stress, one categorizes the event into one of three categories (irrelevant, benign or stressful), a process known as primary appraisal. In addition, individuals evaluate whether they are capable of coping with the stressor, a process known as secondary appraisal (Lazarus & Folkman, 1984). However, this model has its limitation in that it does not incorporate a biological perspective of stress. Thus, Cohen, Kessler, & Gordon, (1995) developed a heuristic model in which they incorporated physiological and behavioral responses followed by appraisal process. Various stress responses work together to restore homeostasis and bring about short-term adaptation, a process known as allostasis. When the stressor is removed

and the situation is no longer perceived as threatening, the stress response is terminated (McEwen, 2000). Overall, researchers have attempted over decades to capture the complexity of the stress process from multiple perspectives, from biological to cognitive to behavioral levels, which has greatly advanced the understanding of stress process.

There are two neuroendocrine systems that are involved in stress regulation. The first response, which occurs within seconds of perceiving a threat to homeostasis, is the activation of the sympathetic nervous system (SNS). Catecholamine hormones, including adrenaline and noradrenaline, are released upon SNS activation, and this response accelerates heart rate, raises blood pressure, and increases blood glucose level in vital organs and muscles (Olff, Langeland, & Gersons, 2005). In addition, catecholamine hormones stimulate noradrenergic activity in the locus coeruleus and the nucleus of the solitary tract, which then lead to a stimulation of the amygdala (van Stegeren, 2009). The second response, which occurs relatively slower than the SNS response, involves the hypothalamic-pituitary-adrenal (HPA) axis. This second response begins with the activation of amygdala (Tottenham & Sheridan, 2010), which then initiates the release of a cascade of stress hormones from the HPA axis. The cascade of hormones includes corticotropin-releasing factor (CRF) from the hypothalamus, adrenocorticotropin (ACTH) hormone from the pituitary gland, and glucocorticoids (corticosterone in rodents and cortisol in primates) from the adrenal cortex (Chrousos & Gold, 1992). Peripheral glucocorticoids travel back to the brain via the blood-brain barrier and signal the HPA axis response to end. Specifically, glucocorticoids exert negative feedback by directly inhibiting CRF and ACTH release via glucocorticoid receptors expressed on the hypothalamus and pituitary gland. Additionally, the hippocampus and prefrontal cortex (PFC), with glucocorticoid receptors

occupied, inhibit the activity of the HPA axis (Diorio, Viau, & Meaney, 1993; Tottenham & Sheridan, 2010).

The initiation and then cessation of the neuroendocrine system in response to a stressful experience can have a protective effect in the short-term, but repeated or prolonged activation can be detrimental in the long-term. Extended exposure to adverse situations or a dysregulation of the neuroendocrine response will bring about allostatic load, which represents the cumulative negative effects on the body due to repeated allostasis (McEwen, 2000). For the current project, this notion of allostatic load will be applied to brain structures and cognition with the hypothesis that repeated allostasis due to cumulative stress, or repeated or prolonged stress accumulated throughout life, will result in changes in regional brain structure and related cognitive functions.

However, before delving into details about how cumulative stress and the HPA axis activity influence brain structure and cognition, it is important to first explore the relationship between cumulative stress and the HPA axis. Previously, researchers had thought that cumulative stress would result in greater cortisol due to a repeated activation of the HPA axis, which had been exemplified in many studies (e.g., Kunz-Ebrecht, Kirschbaum, & Steptoe, 2004; Schlotz, Hellhammer, Schulz, & Stone, 2004). However, other studies have begun to find opposite results as well (e.g., Seedat, Stein, Kennedy, & Hauger, 2003; Vedhara et al., 2002). Based on this evidence, researchers developed the idea that the onset of stress would initially lead to a greater cortisol production, but as time passes by, cortisol production would decrease below normal (Miller, Chen, & Zhou, 2007). Indeed, a meta-study by Miller et al., (2007) revealed that individuals who are currently under ongoing stress displayed a higher cortisol level whereas those with a history of stress that is no longer present showed a lower cortisol concentration. However, it is important to note that not all studies uniformly followed this pattern (e.g., Miller,

Cohen, & Ritchey, 2002; Pfeffer, Altemus, Heo, & Jiang, 2007), suggesting that variation in timing between when the stress was experienced and when the cortisol measures were taken is not the only factor that is influencing the relationship between behavioral stress and the HPA axis dysregulation (Miller et al., 2007). Also, a lack of longitudinal studies examining the relationship between behavioral stress and changes in the HPA axis activity makes it difficult to confirm the abovementioned idea.

Furthermore, cumulative stress may also be linked with a disrupted diurnal cortisol rhythm. Normally, cortisol occurs in a diurnal rhythm, reaching its peak in the morning, particularly within 30 minutes of awakening, and then gradually declining throughout the day (Pruessner et al., 1997; Vinson, Whitehouse, & Hinson, 2000). However, according to Miller et al., (2007), cumulative stress is associated with a flatter diurnal rhythm, possibly with lower morning cortisol and elevated afternoon and evening cortisol levels. Again, not all studies observed this pattern (e.g., Pfeffer et al., 2007), and this meta-study was based mostly on cross-sectional studies. In summary, the relationship between behavioral stress and the HPA axis dysregulation may not be a simple linear relationship with greater stress triggering greater cortisol production. Taking into consideration various factors, such as time interval between stress onset and cortisol measures and the timing of the cortisol assessment across the day, would be important to elucidate this complex relationship.

1.2 Stress Effects on the Brain at a Cellular Level

When stress is evaluated in relation to the brain, existing literature does not predict that cumulative stress will have an equal effect across brain structures, but rather suggests that there may be differential effects on specific brain regions. In order to understand the reasoning behind

such a prediction, it is first important to understand the stress response at a cellular level. First, there are two types of receptors for glucocorticoids, mineralocorticoid and glucocorticoid receptors (MRs and GRs, respectively) (Joels & Baram, 2009). These receptors are not evenly distributed, but have more of a localized distribution. For instance, MRs are highly expressed in limbic areas, including hippocampal, amygdalar, and PFC regions. GRs are relatively more widely expressed than MRs but GRs are still highly expressed in the hippocampus (Conrad, 2008; Patel et al., 2000), amygdala (Patel et al., 2000), and PFC (Sanchez, Young, Plotsky, & Insel, 2000). However, it is important to note that not all nonhuman primates showed consistent results (Pryce, 2008). For example, Sanchez et al., (2000) found GR to be weakly expressed in hippocampus whereas Patel et al., (2000) observed low expression of MRs in dorsomedial PFC. This may be due to differences in primate species and use of different methodologies in detecting MRs and GRs.

Furthermore, MRs and GRs play a crucial role in regulating the HPA axis response. Specifically, MRs have a ten-fold greater affinity for glucocorticoids than GRs, so glucocorticoids occupy mostly MRs at basal condition. MRs are necessary for tonic inhibition of the HPA axis activity at basal condition (van Haarst, Oitzl, & de Kloet, 1997). When glucocorticoid level rises due to stress, glucocorticoids initially bind to high-affinity, membrane located MRs, which then amplify initial stress responses. Subsequently, glucocorticoids bind to low-affinity GRs, which are responsible for preventing overshooting of the stress response and reinstating homeostasis (Oitzl, Champagne, van der Veen, & de Kloet, 2010). The underlying mechanisms of diverse glucocorticoid effects are complex and remain uncertain at the cellular level. However, a number of studies have shown that binding of glucocorticoids to MRs and GRs triggers release of glutamate, which then facilitates neuronal activity (Karst et al., 2005; Oitzl et

al., 2010). However, prolonged stress and elevation of glucocorticoids can result in over-exposure to unregulated glutamate and therefore, excitotoxicity. Therefore, it is suggested that brain regions with high expressions of MR and GR are more susceptible to receptor-mediated neuronal damage due to long-term stress experience (Conrad, 2008; Nair & Bonneau, 2006).

1.3 Stress and Corticosterone Effects on Brain Structure and Cognition in Non-Human Animals

Several non-human animal studies have explored how cumulative stress and corticosterone injection affects regions that are high in MR/GR expression, including the hippocampus, medial PFC (mPFC) and amygdala. For example, after exposure to a repeated restraint paradigm, hippocampal dendritic atrophy, including a decrease in branching point and overall branching length, has been observed in rodents (e.g., McLaughlin, Gomez, Baran, & Conrad, 2007; Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000). In addition, prolonged administration of corticosterone results in decreased hippocampal dendritic length and neuronal loss (e.g., Sapolsky, Krey, & McEwen, 1985; Woolley, Gould, & McEwen, 1990). In terms of PFC, rodents that were either cumulatively stressed (e.g., Cook & Wellman, 2004; Liston et al., 2006) or treated with corticosterone (e.g., Cerqueira et al., 2005; Wellman, 2001) showed significant dendritic atrophy in mPFC. Some researchers have suggested that dendritic retraction may be an adaptive response for protection against the exposure to glutamate bombardment (Conrad, 2008). Also, past studies have observed both chronic stress and chronic treatment with corticosterone to be associated with impairment in spatial learning and memory (e.g., Cui, Wu, & She, 2009; Dachir, Kadar, Robinzon, & Levy, 1993), which is in agreement with the findings from hippocampus and mPFC. Unfortunately, the number of studies that

examined the effect of chronic stress or corticosterone treatment in aged animals is scarce. Yet, few studies that involved middle-aged to older rodents showed reduced hippocampal neurogenesis (Borcel et al., 2008) and impaired spatial learning and memory (Arbel, Kadar, Silbermann, & Levy, 1994; Borcel et al., 2008), which are in parallel with findings in young animals. Overall, consistent effects of stress have been shown in animal studies at both neuronal and cognitive levels.

In contrast to findings for the hippocampus and mPFC, increases in both number and length of dendritic branches in the basolateral amygdala have been observed after immobilization stress (e.g., Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005; Vyas, Jadhav, & Chattarji, 2006). In terms of glucocorticoid effects, acute administration of corticosterone led to hypertrophy of amygdaloid neurons. However, neuronal changes due to chronic administration of corticosterone did not differ significantly from changes triggered by acute corticosterone treatment (Kim et al., 2014; Mitra & Sapolsky, 2008). The underlying mechanism for increased dendritic arborization in the amygdala is uncertain. Some researchers have raised the possibility that there may be critical differences in glucocorticoid-responsive neurotransmitters and transcription factors acting further downstream in the MR/GR pathway (e.g., Makino, Hashimoto, & Gold, 2002; Vyas, Pillai, & Chattarji, 2004). In addition, it has been speculated that there may be a biphasic change in amygdala structure, transitioning from hypertrophy to atrophy over time (Cordero et al., 2005). Neuronal changes that are discussed so far, however, are observations from non-human animals. Variations in stress type, magnitude, and duration between animal and human stress experience may lead to different findings between species. Moreover, because of differences in rodent and human neurobiology, determining how cumulative stress influences the *human* brain is an important next step.

1.4 Stress and Cortisol Effects on Brain Structure in Humans

1.4.1 Hippocampus

In terms of stress research in humans, the majority of studies that have examined the associations of behavioral stress or cortisol with regional brain structures focused only on the hippocampus. Thus far, studies have shown somewhat inconsistent results with the hippocampus, particularly in older adults.

For behavioral stress, the majority of studies that were done in older adults found negative associations (Gerritsen et al., 2015; Gianaros et al., 2007; Head, Singh, & Bugg, 2012; Zannas et al., 2013). However, two of these studies found varying effects within the same sample depending on the measurement of behavioral stress, or whether the association was examined cross-sectionally or longitudinally. More specifically, Gerritsen et al., (2015) found a negative relationship with early life stress (before age 18), but found a null effect with lifetime stress. Considering that the hippocampus may continue to develop through young adulthood (Gogtay et al., 2006), stress may have differential effects during development compared to adulthood since the brain may be more sensitive to environmental stress when it is still developing (Tottenham & Sheridan, 2010). Furthermore, in a study by Zannas et al., (2013), a measure of stressful events that occurred within the past year was positively associated with hippocampal volume cross-sectionally, but was associated with a longitudinal decline in volume over 2 years. These findings suggest that not only is it important to consider during what stage of life individuals experienced stress, but also the temporal relationship between stress and volume measurements. Disregarding such factors may contribute to mixed findings in the literature.

The direction of the relationship between cortisol and hippocampus has been somewhat inconsistent as well in older adults. For example, many studies that examined associations with awakening or morning cortisol noted null results (Geerlings et al., 2015; Hinterberger et al., 2013; Knoops, Gerritsen, van der Graaf, & Geerlings, 2010; Kremen et al., 2010; MacLullich et al., 2005; Sindi et al., 2014), although some studies have found negative (Beresford et al., 2006; O'Hara et al., 2007; Sindi et al., 2014; Sudheimer et al., 2014) and positive (Bruehl, Wolf, & Convit, 2009; Pruessner et al., 2005) associations. Older adult studies that measured total diurnal cortisol also failed to find significant results (Beresford et al., 2006; Bruehl et al., 2009; Kremen et al., 2010; O'Brien, Lloyd, McKeith, Gholkar, & Ferrier, 2004). In contrast, older adult studies that examined evening (Geerlings et al., 2015; Knoops et al., 2010) and 24-hour cortisol showed negative associations (Lupien et al., 1998). Overall, there seems to be a stronger association between evening cortisol and hippocampal volume in older adults compared to other times throughout the day, although only a few studies have examined evening cortisol.

In summary, the relationship of behavioral stress and cortisol with hippocampus in older adults may be more complicated than previously acknowledged, and may depend on various factors, such as timing of stress during lifespan, time interval between stress and hippocampal measures, and timing of cortisol measures across the day. Disregarding these factors may have contributed to mixed findings and confusion in interpretation of the previous findings. Furthermore, there were no systematic difference in sample sizes between studies that found significant association with hippocampus (Cohen et al., 2006; Gerritsen et al., 2015; Gorka, Hanson, Radtke, & Hariri, 2014; Zannas et al., 2013) versus those that did not (Ansell, Rando, Tuit, Guarnaccia, & Sinha, 2012; Gerritsen et al., 2015; Zannas et al., 2013). This suggest that

sample sizes may not be the significant contributing factor as with some of the other factors mentioned above for inconsistent findings with hippocampus.

Furthermore, another possible contributing factor to the inconsistent results for the hippocampus may relate to examination of the hippocampus in its entirety. Subdivisions of the hippocampus along the longitudinal axis have distinct anatomical connectivity and function. The anterior hippocampus is more strongly connected with the amygdala, hypothalamus and prefrontal cortex, regions that are involved in HPA axis regulation. Also, the anterior hippocampus is more associated with emotion and stress processing, including HPA axis reactivity in response to stress (Fanselow & Dong, 2010; Herman, Dolgas, & Carlson, 1998). In contrast, the posterior hippocampus is more strongly connected with sensory association cortices, and more associated with spatial learning (Fanselow & Dong, 2010; Moser & Moser, 1998). Indeed, past studies have demonstrated stronger negative associations of stress and cortisol with anterior compared to posterior hippocampus in children and young adults (Gunduz-Bruce et al., 2007; Szeszko et al., 2006; Wiedenmayer et al., 2006). Stress and cortisol effects across the hippocampal subdivisions, however, remain to be investigated in older adults.

1.4.2 Prefrontal Cortex

Relatively fewer studies have investigated the relationship of stress or cortisol with the PFC, and even fewer studies were done in older adults. Yet, there seems to be more consistent results with behavioral stress and PFC regions in that higher stress is associated with smaller PFC (Ansell et al., 2012; Cohen et al., 2006; Ganzel et al., 2008; Gianaros et al., 2007; Gorka et al., 2014; Papagni et al., 2011; Treadway et al., 2009; van Harmelen et al., 2010; but see Sherman, Cheng, Fingerman, & Schnyer, 2016). Many of these studies focused on examining childhood stress while brain structure was assessed in adulthood (Cohen et al., 2006; Gorka et al.,

2014; Treadway et al., 2009; van Harmelen et al., 2010). Other types of stress examined in the past studies include recent stress (Ansell et al., 2012; Sherman et al., 2016), lifetime stress (Ansell et al., 2012; Gianaros et al., 2007), and specific traumatic event (Ganzel et al., 2008). However, only two of the studies examining behavioral stress and mPFC regions involved older adults (Gianaros et al., 2007; Sherman et al., 2016), with one study observing a negative association with lifetime stress (Gianaros et al., 2007) and the other one observing a null effect with recent stress (Sherman et al., 2016). Moreover, even though some of these studies were done using large samples (n>100) (Ansell et al., 2012; Cohen et al., 2006; Gorka et al., 2014), none of these large sample studies were done in older adults. Therefore, whether the relationship between behavioral stress and PFC will remain negative in a large sample of older adults needs to be clarified.

Unlike findings with behavioral stress, inconsistent results have been found in terms of the relationship between cortisol and PFC regions. For example, some older adult studies found significant negative relationships with dexamethasone suppression test (MacLullich et al., 2006), and diurnal cortisol (Kremen et al., 2010). In contrast, null results were found in older adults with nocturnal 12-hour (Gold et al., 2005) and awakening cortisol (Kremen et al., 2010). No studies have examined evening cortisol in older adults, but a study by Carrion, Weems, Richert, Hoffman, & Reiss (2010) found a negative association between evening cortisol and PFC in a combined sample of young adults with or without PTSD. Among these studies, only one of them was completed using a large sample size (n=388) (Kremen et al., 2010). Due to limited number of studies, it is difficult to disentangle the influence of timing of cortisol assessment, age of sample, psychiatric condition, and sample size on the association of cortisol with PFC regions.

1.4.3 Amygdala

In terms of the amygdala, the majority of studies that have examined the relationship between behavioral stress and amygdala were done in children, adolescents, and young adults. Many of these studies found positive associations (Baur, Hänggi, & Jäncke, 2012; Evans et al., 2016; Holzel et al., 2010; Lupien et al., 2011; Mehta et al., 2009; Moutsiana et al., 2015; Pechtel, Lyons-Ruth, Anderson, & Teicher, 2014; Qin et al., 2014; Tottenham et al., 2010) while a few studies found negative associations (Ganzel et al., 2008; Mehta et al., 2009; Pagliaccio et al., 2014) or null results (Andersen et al., 2008; Driessen et al., 2000). These studies primarily focused on early adversities while the age at amygdala measurement varied from childhood to young adulthood. Tottenham and Sheridan (2010) hypothesized in their review a biphasic change in amygdala structure. The authors hypothesized that the amygdala undergoes significant growth when exposed to stress early in life but after an extended period, gives rise to amygdalar atrophy by adulthood. Findings thus far appear to be reflecting this hypothesis. However, no longitudinal studies have been done to examine such a trajectory. Also, insufficient research has been done in middle-aged to older adults to determine whether early stress is indeed associated with later amygdalar atrophy.

In fact, only a handful of studies were done in older adults. For example, Sherman et al., (2016) found a positive association between late-life stress and amygdala. Gerritsen et al., (2015) also found a positive association between late-life stress and amygdala, but found a negative association between early-life stress and amygdala. Findings from Sherman et al., (2016) and Gerritsen et al., (2015) may be reflecting the idea that recent stress may lead to hyperactivity of the amygdala, which in turn might have a growth effect on amygdala structure. However, prolonged hyperactivity of amygdala may have a deteriorating effect on the amygdala over time (Sherman et al., 2016). Again, a dearth of longitudinal studies makes it difficult to confirm this

conceptualization. However, the overall results seem to be in agreement with animal literature in that there may be a biphasic change in amygdala due to stress although the underlying mechanism is not yet fully understood.

Furthermore, to my knowledge, there has been only one study that examined the relationship between cortisol and the amygdala (n=4244, mean age=76), and this study found a negative association with evening cortisol but no association with morning cortisol (Geerlings et al., 2015). More replication would be required to determine whether these findings remain consistent. Yet, studies have found both patients with Cushing syndrome, which is characterized by an abnormally high secretion of cortisol, and those who are chronically treated with corticosteroid therapy show smaller amygdala volume compared to control subjects, supporting the notion that long-term elevation of cortisol will eventually lead to amygdala atrophy (Brown, Woolston, & Frol, 2008; Merke et al., 2005).

1.5 Stress and Cortisol Effects on Cognition in Humans

1.5.1 Episodic Memory

Past stress research has been interested in determining whether cumulative stress influences cognitive functions that are supported by high MR/GR expressing regions. One cognitive domain that has been examined is episodic memory. The concept of episodic memory was first introduced by Endel Tulving who described episodic memory as a type of memory that involves a person's experience, particularly “what,”“where,” and “when” (Tulving, 2002). Episodic memory is distinct from another type of memory known as semantic memory, which refers to general knowledge of facts (Tulving, 2002). Evidence as to which brain region(s) are crucial for episodic memory comes in part from lesion studies. For example, in general, patients

with hippocampal damage showed deficits in acquiring new episodic memories while showing intact short-term memory, non-declarative memory, and semantic memory (Bird & Burgess, 2008; Milner, Squire, & Kandel, 1998; Rempel-Clower, Zola, Squire, & Amaral, 1996; Scoville & Milner, 1957). Two main theories that have attempted to explain the role of the hippocampus in episodic memory are known as 'Declarative Theory' and 'Multiple-Trace Theory.' For instance, Declarative Theory proposes that the hippocampus is critical for new episodic memories whereas "older memories become consolidated to neocortical areas" (Bird & Burgess, 2008) and become independent of hippocampus (Bird & Burgess, 2008; Squire, 1986). Multiple-Trace Theory also predicts that hippocampus is important for acquiring new episodic memories, but it is different from the Declarative Theory in regards to remote episodic memories. Multiple-Trace Theory suggests that older episodic memories remain hippocampus-dependent, but can become less vulnerable to disruption with repeated retrieval of the same episode (Bird & Burgess, 2008; Nadel & Moscovitch, 1997). Although it is beyond the scope of this dissertation to discuss these theories in detail, one common aspect of these theories is that they all view the hippocampus as a crucial neural component for episodic memory (even if the precise mechanism may be elusive) (Bird & Burgess, 2008).

Based on the abovementioned evidence of the relationship between the hippocampus and episodic memory, it is logical to hypothesize that factors that damage the hippocampus will also be associated with deficits in episodic memory performance. Stress is one factor that can potentially harm the integrity of the hippocampus. Hence, previous studies have examined the relationship between behavioral stress and episodic memory. Past human studies, however, have been inconsistent in terms of relationship between behavioral stress and episodic memory performance. For instance, in older adults, some studies found negative associations (Mackenzie,

Wiprzycka, Hasher, & Goldstein, 2009; Peavy et al., 2007; Wilson et al., 2003) while others had null results (Head et al., 2012; Mackenzie, Smith, Hasher, Leach, & Behl, 2007; Oken, Fonareva, & Wahbeh, 2011; Peavy et al., 2009; Rosnick, Small, McEvoy, Borenstein, & Mortimer, 2007). Only one study, to my knowledge, has found a positive association between behavioral stress and memory in older adults (Feeney, Kamiya, Robertson, & Kenny, 2013). However, this study was different from other studies in that it involved a very large sample size (n=6912), and it did not screen for any health conditions. Also, stress type does not appear to explain the mixed results in older adults since similar types of stress have led to inconsistent findings. For example, there were mixed findings when studies examined recent stress (Peavy et al., 2007, 2009; Rosnick et al., 2007) and highly stressed caregivers (Mackenzie et al., 2007, 2009). However, the sample size may play a role in partially explaining mixed results, as the majority of studies with significant findings used medium-to-large sample sizes (n>91) while most studies with null results used small-to-medium sample sizes (n<59; but see Rosnick et al., 2007). Based on this evidence, it is reasonable to expect that the effect size for stress may be small in older adults, therefore contributing to mixed results when smaller sample sizes are being employed.

The majority of studies that investigated the relationship between cortisol and memory were done in middle-aged and older adults. Among these studies, some found negative associations with awakening or morning cortisol (Almela, van der Meij, Hidalgo, Villada, & Salvador, 2012; Beluche, Carrière, Ritchie, & Ancelin, 2010; Comijs et al., 2010; Franz et al., 2011; Geoffroy, Hertzman, Li, & Power, 2012; MacLullich et al., 2005) while others observed null results (Gaysina, Gardner, Richards, & Ben-Shlomo, 2014; Geerlings et al., 2015; Geoffroy et al., 2012; Greendale, Kritz-Silverstein, Seeman, & Barrett-Connor, 2000; Kuningas et al., 2007; MacLullich et al., 2005; Reynolds et al., 2010; Singh-Manoux et al., 2014). In general,

more consistent patterns seem to arise with diurnal and evening cortisol measures even though relatively fewer studies have been done using these measures. More specifically, negative associations with memory have been observed in older adults with measures of diurnal (Franz et al., 2011; Peavy et al., 2009; Pulopulos et al., 2014) and evening cortisol (Gaysina et al., 2014; Geerlings et al., 2015; Gerritsen et al., 2015; Li et al., 2006; but see Li et al., 2006 for incidental visual memory). This pattern with evening cortisol is in line with findings for the hippocampus in older adults even though relatively fewer number of studies have examined evening cortisol. In summary, replication with larger sample sizes and use of multiple measures of cortisol across the day may help clarify the complex relationship of behavioral stress and cortisol with memory performance in older adults.

1.5.2 Fluid Intelligence

Another cognitive domain that may be associated with cumulative stress is fluid intelligence. Raymond Cattell developed a concept of intelligence, which distinguished general intelligence into two factors: fluid intelligence and crystallized intelligence (Cattell, 1963). Fluid intelligence is defined as one's reasoning, novel problem-solving ability and processing speed whereas crystallized intelligence is referred to as one's "over-learned skills or knowledge" (Cattell, 1963; Gray & Thompson, 2004). Prefrontal cortex regions have been shown to support fluid intelligence performance (Gray, Chabris, & Braver, 2003; Gray & Thompson, 2004). Since cumulative stress and HPA axis activity may be associated with prefrontal cortex, fluid intelligence may also be associated with cumulative stress and HPA axis activity. However, similar to findings with episodic memory, previous studies have shown mixed results in terms of the relationship between behavioral stress and fluid intelligence in older adults. Mixed findings in older adults may be explained by either stress type or sample size. For example, when highly

stressed caregivers are examined in comparison to non-caregivers, most of the studies found fluid intelligence to be lower in caregivers (Mackenzie et al., 2007, 2009; Oken et al., 2011; but see Mackenzie et al., 2007 for working memory). However, when the measures of recent stress (Rosnick et al., 2007) and neuroticism (Wilson et al., 2003) were examined, no significant associations could be found. It should be noted that these two studies with null results used larger sample sizes ($n>428$) while studies that examined caregivers did not ($n<56$). Investigating caregivers in a larger sample may disentangle this issue although it will not be examined in the current study.

Another limitation in past research examining fluid intelligence is that the number of studies that tap into different aspects of fluid intelligence is still limited when examined in relation to behavioral stress, especially in older adults. There is a possibility that specific aspects of fluid intelligence may bring about different results across studies. For example, in a study by Mackenzie et al., (2007) there was a significant difference between highly stressed caregivers versus non-caregivers in attentional control but not in working memory. A similar pattern was observed in a sample of adults with a wider age range, in which there was a negative association with working memory, but null effects with executive function, psychomotor speed and attention (Majer, Nater, Lin, Capuron, & Reeves, 2010). Having multiple measures of fluid intelligence would not only clarify this issue, but would also allow for more robust estimates of the fluid intelligence domain.

In contrast to the number of studies with behavioral stress, a relatively greater number of studies has investigated the relationship between cortisol and fluid intelligence in older adults. For example, when awakening or morning cortisol measures were obtained in older adult samples, some studies found negative associations (Beluche et al., 2010; Comijs et al., 2010;

Fonda, Bertrand, O'Donnell, Longcope, & McKinlay, 2005; Geoffroy et al., 2012; Kuningas et al., 2007; MacLullich et al., 2005) while others found null effects (Comijs et al., 2010; Franz et al., 2011; Gaysina et al., 2014; Geoffroy et al., 2012; Gerritsen et al., 2015; Greendale et al., 2000; Reynolds et al., 2010; Schrijvers et al., 2011; Singh-Manoux et al., 2014) and two studies observed a positive association (Almela et al., 2012; Geerlings et al., 2015). There have been mixed results with evening cortisol as well (Gaysina et al., 2014; Geerlings et al., 2015; Gerritsen et al., 2015; Li et al., 2006). However, two studies that examined diurnal cortisol found negative associations with fluid intelligence (Franz et al., 2011; Pulopulos et al., 2014). It is possible that variability in cortisol rhythm throughout the day may be a better predictor of fluid intelligence; however, more studies are needed in order to confirm this speculation. Furthermore, one recent study that investigated the relationship between hair cortisol and fluid intelligence in young-to-middle aged nurses found no significant association between the two variables (McLennan, Ihle, Steudte-Schmiedgen, Kirschbaum, & Kliegel, 2016). However, this study involved mostly working female adults (90% of the sample), thus, whether there would be an effect in other populations (e.g., male, other age groups, individuals with no work or different occupations) remains unknown. Overall, the relationship of stress and cortisol with fluid intelligence is complicated by various factors, including age group, stress type, sample size, and use of variety of cortisol measures across the day.

1.6 Stress System Genes

One potential reason for inconsistent results in the literature may be due to the presence of moderators, such as genetic risk. Indeed, one major factor that could influence the function of the HPA axis is stress-system genes. Previous studies have identified several single nucleotide

polymorphisms (SNPs) that are associated with MR, GR or FK506 binding protein 5 (FKBP5) genes (see review by Bogdan, Hyde, & Hariri, 2013). FKBP5 is a co-chaperone protein that interacts with GR to regulate GR sensitivity. SNP refers to a genetic variation that occurs at a single specific position in a gene ("SNP," 2014). Past studies have suggested that SNPs of these genes influence proper functioning of MR and GR, which then affects the HPA axis activity. Each SNP related to MR, GR or FKBP5 is described in the following paragraphs.

The importance of MR function in the activity of the HPA axis has been well-established in animal literature. For instance, an increase in basal and stress-evoked HPA axis activity has been observed in both MR knockout (Gass et al., 2000) and MR antagonist treated mice (Ratka, Sutanto, Bloemers, & de Kloet, 1989). NR3C2 is a gene that codes for MR. Two common SNPs, known as rs5522 and rs2070951, have been identified and examined in vitro and in vivo studies. Rs5522 is characterized by an A-allele to G-allele substitution whereas rs2070951 is characterized by a C-allele to G-allele substitution (DeRijk, de Kloet, Zitman, & van Leeuwen, 2011). In vitro studies have shown that substitution of risk alleles interferes with glucocorticoid-triggered transactivation of MR (DeRijk et al., 2006; van Leeuwen et al., 2010). Furthermore, DeRijk and colleagues (2006) have demonstrated that risk allele carriers of rs5522 show greater cortisol reactivity in response to psychosocial stress compared to non-risk allele homozygotes. Also, higher basal cortisol level has been observed for individuals who are risk-allele homozygotes for rs2070951 (Kuningas et al., 2007; Muhtz, Zyriax, Bondy, Windler, & Otte, 2011).

Similar to animal studies involving MR knockout and antagonism, higher basal corticosterone and prolonged elevation of corticosterone after stress have been observed in GR knockout and GR antagonist injected rodents (Ratka et al., 1989; Tronche et al., 1999). NR3C1 is

a gene that codes for GR. Several SNPs have been identified that influence HPA axis activity, including rs10482605, rs41423247 and rs10052957. Rs10482605 produces a T-allele to C-allele substitution, rs41423247 produces a G-allele to C-allele substitution, and rs10052957 produces a G-allele to A-allele substitution. Kumsta et al., (2009) have demonstrated that the C minor allele of rs10482605 reduces transcriptional activity of GR in vitro. Additionally, in humans, risk allele carriers of rs41423247 have shown greater cortisol reactivity to psychosocial stressors (Ising et al., 2008; Wust et al., 2004). Lastly, individuals who are homozygotes for risk allele of rs10052957 have displayed the highest basal cortisol (Rosmond et al., 2000).

FKBP5 is a co-chaperone protein that reduces GR sensitivity to glucocorticoid. Specifically, when FKBP5 binds to the GR complex, glucocorticoid binds to GR with decreased affinity, which then interferes with proper functioning of negative feedback of the HPA axis (Binder, 2009). It has been demonstrated that FKBP5 knockout mice show a reduction in HPA axis reactivity in response to stressor (Touma et al., 2011). Rs1360780 is a common SNP for FKBP5 gene and is characterized by a C-allele to T-allele substitution. It has been demonstrated that in humans, TT homozygotes show higher FKBP5 levels (Binder et al., 2004) and impaired negative feedback of the HPA axis (Ising et al., 2008; Touma et al., 2011).

Many studies have examined a single SNP when examining its relationship with brain structure and/or cognition. However, focusing on only one SNP brings about one critical limitation: small effect size. The problem with small effects is that it brings about difficulty in replication of results, possibly due to previous false positive findings or variation in study samples or methodologies (Bogdan et al., 2013). In order to overcome this problem, recent studies have begun to create biologically informed, composite scores of multiple SNPs that are linked to the system of interests, and this has been shown to have stronger effects. For example,

in a study by Pagliaccio and colleagues (2014), 10 SNPs that were related to HPA axis dysregulation and/or depression were selected, and the combined genetic score of these 10 genotypes was significantly associated with cortisol in children. However, 8 out of 10 genotypes were not significantly associated with cortisol when each of them was examined individually, and 2 out of 10 genotypes predicted cortisol in females only. Therefore, composite scores of polymorphisms that are associated with the HPA axis dysregulation may be a more powerful approach to use for future studies.

1.7 Limitations of Previous Research

There are some critical characteristics and limitations to previous studies, which could have contributed to the mixed findings in literature. First, some studies did not rigorously screen for health issues or even combined both control and patient samples to examine the relationship between cumulative stress and regional brain structures. Second, many of the studies focused on only a certain type of stress (e.g., childhood abuse) or a certain time period (e.g., events that occurred in the past year) but did not rigorously measure the level and timing of stress experienced throughout the lifespan. Third, some studies only focused on particular age groups (e.g., children and adolescents). While focusing on a specific age group could be helpful in answering certain research questions, findings from a particular age range may not necessarily agree with findings in other age groups, and therefore, replication in other age groups (e.g., older adults) is warranted. Fourth, some studies had small sample sizes, one possible reason for null findings in some of these previous studies. Fifth, the use of non-specific whole-brain analyses in some previous studies may have hindered the detection of changes in smaller limbic regions. Sixth, most studies did not measure both behavioral stress and cortisol within the same sample.

Seventh, the difference in time of the day in which the cortisol measure were taken may have contributed to mixed findings as well. Eighth, variation in memory and fluid intelligence tasks, which may tap onto different aspects of memory and fluid intelligence respectively, may have led to inconsistent findings with cognitive function. Although it is difficult to address these problems all at once, my dissertation attempted to address several of these limitations by obtaining a more comprehensive measure of cumulative stress, using a larger sample size of middle-aged and older adults, examining hypothesis-drive regions of interest (ROIs), and exploring samples with measures of both cumulative stress and cortisol concentrations.

Chapter 2: Overview of Proposed Research and Specific Aims

2.1 Overview of Proposed Research

Although non-human studies have suggested that brain regions with a high expression of MR/GR may be more susceptible to cumulative stress and cortisol effects, this hypothesis has not been clarified in humans, particularly in older adults. In fact, the majority of studies have focused only on the hippocampus, and these studies have demonstrated somewhat inconsistent results with limitations. Thus, the current study was proposed to address some limitations while examining differential associations of stress and cortisol with brain structures and cognition beyond the hippocampus and hippocampal-dependent cognitive function, particularly in a convenience sample of cognitively normal older adults. The current study obtained lifetime stress measures in order to get an estimate of the cumulative stress experienced throughout the lifespan. In addition, recent stress measures were obtained for an exploratory analysis in order to compare lifetime versus recent stress effects on brain structure and cognition. Also, the current study mainly used a conveniently available morning plasma cortisol sample as an indicator of the HPA axis activity. However, to complement a single measure of cortisol obtained at one time point during the day, the current study obtained hair cortisol measures as an indicator of chronic HPA axis activity (see methods for details) for an exploratory analysis. Furthermore, since the current study is based on brain regions with high versus low expression of MR/GR, the moderating role of stress-system genes that are related to MR/GR function was also investigated.

Overall, my proposed research was designed to reveal a more clear view of the effect of cumulative stress in cognitively normal middle-aged to older adults at multiple levels, and therefore, provide a foundation for better understanding of stress-related changes that occur prior to onset of stress-related disorders.

2.2 Specific Aims and Hypotheses

2.2.1 Specific Aim 1

To determine the differential influence of cumulative stress/HPA axis activity on brain regions with a high expression of MR/GR, and whether these effects are moderated by polymorphisms in stress-related genes.

I predicted that the indicators of HPA axis activity (i.e., plasma and hair cortisol) would show negative associations with hippocampal and amygdalar volumes and mPFC thickness. Also, I predicted that the indicators of behavioral stress (i.e., lifetime and recent stress measures) would show negative associations with hippocampal and amygdalar volumes and mPFC thickness. Furthermore, I predicted that individuals with greater genetic risk scores and higher stress/HPA axis activity would show the worst structural outcomes. Lastly, I predicted that the association with target regions (i.e., hippocampus, amygdala and medial PFC) would be stronger than the association with primary visual cortex (control region).

2.2.2 Specific Aim 2

To determine the differential influence of cumulative stress/HPA axis activity on cognitive functions reliant upon brain regions with a high expression of MR/GR, and whether these effects are moderated by polymorphisms in stress-related genes.

I predicted that the indicators of HPA axis activity (i.e., plasma and hair cortisol) would show negative associations with memory and fluid intelligence. Also, I predicted that the indicators of behavioral stress (i.e., lifetime and recent stress measures) would show negative associations with memory and fluid intelligence. Furthermore, I predicted that individuals with greater genetic risk scores and higher stress/HPA axis activity would show the worst cognitive outcomes. Lastly, I predicted that the association with target cognitive functions (i.e., episodic memory and fluid intelligence) would be stronger than the association with crystallized intelligence (control).

Chapter 3: Associations of Stress and Cortisol with Brain Structure

3.1 Methods

3.1.1 Participants

The participants, aged from 58 to 92, were recruited from Knight Alzheimer Disease Research Center (ADRC) at Washington University. They were screened for neurological conditions that may interfere with completion and/or interpretation of the results (e.g., stroke, transient ischemic attack, seizure, Parkinson's disease). Based on the Clinical Dementia Rating (CDR), a highly reliable and validated protocol for staging dementia severity (Morris, 1993), all participants were classified as cognitively normal (CDR=0).

The first sample consisted of existing ADRC participants who already had morning plasma cortisol and neuroimaging data collected (n=152) through the ADRC (see Table 3.1 for demographic information). The second sample consisted of ADRC participants for whom lifetime stress measures were collected (n=89) (see Table 3.2 for demographic information) in the Head Research Laboratory (HRL). Some individuals from the second sample (n=70) overlapped with the first sample and thus, had plasma cortisol data. However, because of a large time interval between plasma cortisol and lifetime stress assessments (mean=7.5 years), the two samples were treated as two independent samples, and MR scan dates closest to the lifetime stress assessment date were chosen for the overlapping subsample instead of using the same scan as in the first sample. In addition, the MINI International Neuropsychiatric Interview was

administered to the second sample, in order to be aware of their potential psychiatric conditions, such as post-traumatic stress disorder and social phobia. While no participant was excluded based on the MINI, none of the participants met the criteria for current panic disorder, social phobia, post-traumatic stress disorder, or general anxiety disorder. The third sample consisted of a subset of the second sample for whom recent stress measures (n=25) (see Table 3.3 for demographic information) and hair samples (n=23) (see Table 3.4 for demographic information) were collected in the HRL. Because the third sample was underpowered, the findings from this sample were treated as exploratory.

Table 3.1 Descriptive statistics for the plasma cortisol-MRI sample

N	152
Age, years (mean (SD))	71 (7)
Female, n (%)	99 (65)
Education, years (mean (SD))	16 (3)
Plasma cortisol, ng/ml (mean (SD))	159 (56)
Genetic profile scores (mean (SD))	4.37 (1.92)
Total hippocampus, mm^3 (mean (SD))	7596 (964)
Amygdala, mm^3 (mean (SD))	2824 (429)
Medial PFC, mm^2 (mean (SD))	2.55 (.17)
Primary visual cortex, mm^2 (mean (SD))	1.56 (.10)
Hippocampus head, mm^3 (mean (SD))	4453 (699)
Hippocampus body, mm^3 (mean (SD))	2463 (433)
Hippocampus tail, mm^3 (mean (SD))	679 (261)

Table 3.2 Descriptive statistics for the lifetime stress-MRI sample

N	89
Age, years (mean (SD))	75 (6)
Female, n (%)	58 (65)
Education, years (mean (SD))	15 (3)
Lifetime stress - log transformed (mean (SD))	0.64 (.28)
Genetic profile scores (mean (SD))	4.26 (1.93)
Total hippocampus, mm^3 (mean (SD))	7384 (893)
Amygdala, mm^3 (mean (SD))	2990 (392)
Medial PFC, mm^2 (mean (SD))	2.45 (.15)
Primary visual cortex, mm^2 (mean (SD))	1.57 (.11)
Hippocampus head, mm^3 (mean (SD))	4650 (677)
Hippocampus body, mm^3 (mean (SD))	2459 (352)
Hippocampus tail, mm^3 (mean (SD))	552 (182)

Table 3.3 Descriptive statistics for the recent stress-MRI sample

N	25
Age, years (mean (SD))	73 (7)
Female, n (%)	12 (48)
Education, years (mean (SD))	16 (3)
Perceived Stress Scale - square root transformed (mean (SD))	2.74 (1.23)
Elders' Life Stress Inventory - square root transformed (mean (SD))	2.63 (1.47)
Recent stress - standardized and averaged (mean (SD))	0.00 (.96)
Total hippocampus, mm^3 (mean (SD))	7847 (865)
Amygdala, mm^3 (mean (SD))	3144 (325)
Medial PFC, mm^2 (mean (SD))	2.49 (.12)
Primary visual cortex, mm^2 (mean (SD))	1.56 (.10)

Table 3.4 Descriptive statistics for the hair cortisol-MRI sample

N	23
Age, years (mean (SD))	73 (8)
Female, n (%)	12 (52)
Education, years (mean (SD))	16 (3)
Hair cortisol - log transformed, pg/mg (mean (SD))	1.15 (.60)
Total hippocampus, mm^3 (mean (SD))	7935 (810)
Amygdala, mm^3 (mean (SD))	3154 (331)
Medial PFC, mm^2 (mean (SD))	2.50 (.13)
Primary visual cortex, mm^2 (mean (SD))	1.55 (.11)

3.1.2 Plasma Cortisol

Blood samples were collected in the morning after an overnight fast, centrifuged to prepare plasma, aliquotted and frozen on dry ice. Samples underwent a single free-thaw cycle prior to analysis. Cortisol concentration was analyzed using the multiplex immunoassay panel, which is based upon Luminex's xMAP Technology by Rules Based Medicine (RBM, Austin, TX). QC was performed on all samples. Plasma cortisol assessment was within +/- 2 years of MR scans.

3.1.3 Hair Cortisol

Approximately 50 strands of hair were collected from the ADRC participants who visited the HRL. One advantage of using hair cortisol is that it can estimate past month(s) of cortisol production, unlike plasma and saliva measurements that are taken at one time point (Meyer & Novak, 2012). Thus, hair cortisol may be a better indicator of chronic dysregulation of HPA axis than the morning plasma cortisol measure. Hair samples were shipped to Dr. Mark Laudenslager's laboratory at the University of Colorado for hair cortisol assay service.

3.1.4 Lifetime Stress

The Life Stressor Checklist - Revised (LSC-R) was used to assess lifetime stress. LSC-R consists of 31 traumatic or stressful life events, and each event was followed by one to three additional questions, asking about whether the event happened within the previous 6 months, age at the time of event, and/or frequency of the event (Wolfe, Kimerling, Brown, Chrestman, & Levin, 1996). The total score was derived by summing the total number of events experienced so the possible total score range was 0 to 31. As the total score was highly skewed, a log transform was applied. The estimated test-retest reliability for the LSC-R ranged from 0.52 to 0.97 (by

items), with an average of 0.70 (McHugo et al., 2005). The mean interval between LSC-R and MRI assessments was +/- 16.8 months (*SD*=22.5).

3.1.5 Recent Stress

The Perceived Stress Scale (PSS) and the Elder's Life Stress Inventory (ELSI) were used to assess recent stress. The PSS consists of 10 questions regarding appraisal of life situations during the last 3 months (Cohen, Kamarck, & Mermelstein, 1983). Participants respond using a Likert scale on the frequency of particular thoughts or feelings, with scores ranging from 0 (never) to 4 (very often). Thus, the possible total score range was 0 to 40. PSS scores were derived by following the PSS scoring guideline. Specifically, the scores from four positive items were reversed, and then the scores from each question were summed to derive the total scores. Cronbach's alpha varied from .74 to .91 for 1-month perceived stress measure (Lee, 2012). However, I extended the length of time to 3 month because hair cortisol represents approximately 3-months of cortisol production. Cronbach's alpha based on my data was .91 for the 3-month perceived stress measure.

The ELSI consists of 31 stressful events. Participants were asked to indicate whether they experienced these events in the past year, and to rate the extent of stressfulness of each event on a scale from 1 to 5, with 1 representing 'not at all stressful' and 5 representing 'extremely stressful'. They were asked to choose 0 if the event did not occur in the past year (Aldwin, Levenson, Spiro, & Bosse, 1989). The ELSI total scores were derived by summing across the scale items, and the possible total score ranged from 0 to 155. The standardized alpha coefficient of the ELSI was 0.70 (VonDras, Powless, Olson, Wheeler, & Snudden, 2005).

As the total scores from both questionnaires were highly skewed, a square root transformation was applied to improve normality. PSS scores were significantly correlated with

the ELSI scores (r=.838, p<.0001). Therefore, scores from each questionnaire were standardized and averaged to obtain a composite score of recent stress. The mean interval between recent stress and MRI assessments was +/- 3.8 months (*SD*=1.3).

3.1.6 Genotyping

DNA samples from the ADRC participants were genotyped to obtain SNPs that were used for this study. The technical details of genotyping procedure are described in prior publications (Cruchaga et al., 2012, 2013). Six SNPs were selected based on their associations with HPA axis activity. The six SNPs included rs5522, rs2070951, rs41423247, rs10482605, rs10052957 and rs1360780 (see Table 3.5 for SNP frequency data). All SNPs were consistent with Hardy-Weinberg Equilibrium except for rs2070951 in lifetime stress sample. The composite score of these genotypes were calculated by summing the number of risk alleles that each individual possessed, dividing by the total number of non-missing alleles, and then multiplying by the total possible number of alleles (in this case, the total possible number of alleles would be 12) (see Table 3.6 for genetic risk score coding data). This method has been used in previous studies (Cornelis et al., 2009; David et al., 2013; McGeary et al., 2012).

Table 3.5 Single nucleotide polymorphism (SNP) frequency data for Aim 1

	Plasma cortisol-MRI sample				Lifetime stress-MRI sample			
SNP	Major homozygote, n (%)	Heterozygote, n (%)	Minor homozygote, n (%)	Missing, n (%)	Major homozygote, n (%)	Heterozygote, n (%)	Minor homozygote, n (%)	Missing, n (%)
rs5522	122 (80)	29 (19)	1 (1)	0 (0)	70 (79)	17 (19)	0 (0)	2 (2)
rs2070951	42 (28)	70 (46)	39 (26)	1 (1)	18 (20)	52 (58)	15 (17)	4 (4)
rs41423247	63 (41)	71 (47)	18 (12)	0 (0)	35 (39)	47 (53)	7 (8)	0 (0)
rs10482605	98 (64)	42 (28)	8 (5)	4 (3)	56 (63)	28 (31)	3 (3)	2 (2)
rs10052957	63 (41)	66 (43)	22 (14)	1 (1)	36 (40)	44 (49)	9 (10)	0 (0)
rs1360780	63 (41)	65 (43)	21 (14)	3 (2)	36 (40)	43 (48)	9 (10)	1 (1)

Table 3.6 Single nucleotide polymorphism genetic risk coding data

Gene	SNP	Coding
MR	rs5522	GG=2, AG=1, AA=0
MR	rs2070951	GG=2, CG=1, CC=0
GR	rs41423247	GG=2, CG=1, CC=0
GR	rs10482605	CC=2, CT=1, TT=0
GR	rs10052957	AA=2, AG=1, GG=0
FKBP5	rs1360780	TT=2, CT=1, CC=0

3.1.7 MR Acquisition.

Imaging was performed using a Siemens Vision 1.5T scanner, a Siemens Trio 3T scanner, or a Siemens Biograph mMR 3T scanner. For the Vision 1.5 scans, two to four T1-weighted sagittal MP-RAGE scans (TR=9.7 ms, flip angle=10º, TI=20 ms, 1 × 1 × 1.25 mm resolution) were acquired for each participant. For the Trio 3T scans, up to two T1-weighted sagittal MP-RAGE scans (TR=2400ms, flip angle=8º, TI=1000 ms, 1 × 1 × 1 mm resolution) were acquired for each participant. For the Biograph mMR 3T scans, one T1-weighted sagittal MP-RAGE scans (TR=2300ms, flip angle=9º, TI=900ms, 1 × 1 × 1.2 mm resolution) were acquired for each participant.

3.1.8 Regional Brain Structure

Regional volume and thickness estimates were obtained using the Freesurfer image analysis suite. For the plasma cortisol - MRI sample, the Vision 1.5T scans were processed using Freesurfer v5.0, whereas the Trio 3T scans were processed using Freesurfer v5.1. For all other MRI samples, including lifetime stress and recent stress/hair cortisol samples, the Trio and Biograph mMR 3T scans were processed using Freesurfer v5.3. The technical details of these procedures are described in prior publications (Fischl et al., 2002, 2004). Volumetric estimates for hippocampus and amygdala as well as thickness estimates of primary visual cortex and mPFC were obtained using Freesurfer. For the mPFC, the average cortical thickness of the rostral anterior cingulate and medial orbitofrontal cortex was used.

While there is evidence of reliability of Freesurfer-derived estimates across scanner upgrades, different manufacturers, and number of MP-RAGE acquisitions, variation in field strength and Freesurfer version may introduce slight bias (Fennema-Notestine et al., 2007;

Gronenschild et al., 2012; Han et al., 2006; Jovicich et al., 2009). To address potential biases, scanner type/Freesurfer version was considered as a covariate in the analyses.

As there were no *a priori* hypotheses regarding laterality effects, estimated regional volumes were summed across hemispheres, and cortical thickness was averaged across hemispheres. Estimated total intracranial (ICV; Buckner et al., 2004) was used to adjust regional volumes for body size differences via a formula based on the analyses of covariance approach: Adjusted volume=raw volume-(*b* x (ICV – mean ICV)), where *b* is the slope of the regression of the ROI volume on ICV (Jack et al., 1989; Mathalon, Sullivan, Rawles, & Pfefferbaum, 1993). Adjusted regional volumes were used as the dependent variable in analyses.

3.1.9 Delineation of Subdivisions Along the Longitudinal Axis of the Hippocampus

First, images were placed into Talairach stereotaxic space to align all of the hippocampi to the same orientation before division along the longitudinal axis. Next, boundary slices between hippocampal subdivisions were manually determined. The procedure for identifying the boundaries was based on Malykhin et al., (2007) and has been successfully used in a prior publication (Gordon, Blazey, Benzinger, & Head, 2013). Coronal images were viewed while moving in an anterior-to-posterior direction. The boundary slice between hippocampal head and body was determined as the first slice that showed the complete disappearance of the uncus. The boundary slice between hippocampal body and tail was determined as the first slice that showed a clear separation of the fornix and the pulvinar. Boundary slices were identified for each hemisphere separately, and the y-coordinates of the identified slices were recorded. A locally generated algorithm used this boundary information to parse the Freesurfer-delineated hippocampus into regions representing the head, body and tail by automatically assigning a

unique categorical value to voxels in each region. The hippocampal mask was placed back into native space providing subdivision labeling. As the voxels were 1mm isotropic, summing the number of voxels with each label provided volumetric estimates for that hippocampal subdivision (Gordon et al., 2013).

3.1.10 Statistical Analysis

Age, gender, education, scanner type and health status were included as covariates in all analyses. A series of robust regression analyses were conducted to examine the main effects of cortisol (or stress) and the composite genotype score, and the interactive effect of cortisol (or stress) and composite genotype score on regional volumes and thickness. In the regression analyses, covariates were entered in the first step, cortisol (or stress) was entered in the second step, the composite genotype score was entered in the third step, and the cortisol (or stress) × genotype score interaction was entered in the last step.

3.2 Results

3.2.1 Morning Plasma Cortisol

Total Hippocampal Volume

There was a non-significant trend for a positive association between morning plasma cortisol and total hippocampal volume (β=.131, p=.084, 95%CI:-.018-.279). In addition, the genetic score was not significantly associated with hippocampal volume (β=-.018, p=.800, 95%CI:-.162-.125), nor was the plasma cortisol × genetic score interaction (β=.006, p=.932, 95%CI:-.138-.150) (see Figure 3.1A).

Amygdala Volume

Morning plasma cortisol and amygdala volume were not significantly associated (β=.081, p=.299, 95%CI:-.072-.233). In addition, the genetic score was not significantly associated with amygdala volume (β=-.022, p=.768, 95%CI:-.169-.125), nor was the plasma cortisol × genetic score interaction (β=.121, p=.105, 95%CI:-.026-.268) (see Figure 3.1B).

Medial PFC Thickness

Morning plasma cortisol and mPFC thickness were not significantly associated (β=-.111, p=.198, 95%CI:-.280-.059). In addition, the genetic score was not significantly associated with mPFC thickness (β=-.090, p=.277, 95%CI:-.253-.073), nor was the plasma cortisol × genetic score interaction (β=-.088, p=.284, 95%CI:-.251-.074) (see Figure 3.1C).

Primary Visual Cortical Thickness

Morning plasma cortisol and primary visual cortical thickness were not significantly associated (β=-.026, p=.761, 95%CI:-.193-.141). In addition, the genetic score was not significantly associated with primary visual cortical thickness (β=-.117, p=.144, 95%CI:-.275-.041), nor was the plasma cortisol × genetic score interaction (β=-.052, p=.517, 95%CI:-.209-.106) (see Figure 3.1D).

Hippocampal Subdivisions: Hippocampal Head

Morning plasma cortisol was significantly and positively associated with hippocampal head volume (β=.188, p=.012, 95%CI:.042-.334). However, the genetic score was not significantly associated with hippocampal head volume (β=.000, p=.999, 95%CI:-.141-.141). In addition, the plasma cortisol × genetic score interaction was not significant (β=.077, p=.274, 95%CI:-.062-.217) (see Figure 3.2A).

Hippocampal Subdivisions: Hippocampal Body

Morning plasma cortisol was not significantly associated with hippocampal body volume (β=-.053, p=.502, 95%CI:-.210-.103). In addition, the genetic score was not significantly associated with hippocampal body volume (β=.056, p=.459, 95%CI:-.094-.207). There was a non-significant trend for the plasma cortisol × genetic score interaction (β=-.132, p=.084, 95%CI:-.281-.018) (see Figure 3.2B). Specifically, there was a non-significant trend for a negative association between plasma cortisol and hippocampal body in the context of high genetic score (β=-.206, p=.069, 95%CI:-.427-.016). The association was not significant in the context of low genetic score (β=.054, p=.612, 95%CI:-.155-.262).

Hippocampal Subdivisions: Hippocampal Tail

Morning plasma cortisol was not significantly associated with hippocampal tail volume (β=.058, p=.521, 95%CI:-.121-.237). In addition, the genetic score was not significantly associated with hippocampal tail volume (β=-.114, p=.191, 95%CI:-.285-.057). The plasma cortisol × genetic score interaction was not significant (β=-.034, p=.692, 95%CI:-.206-.137) (see Figure 3.2C).

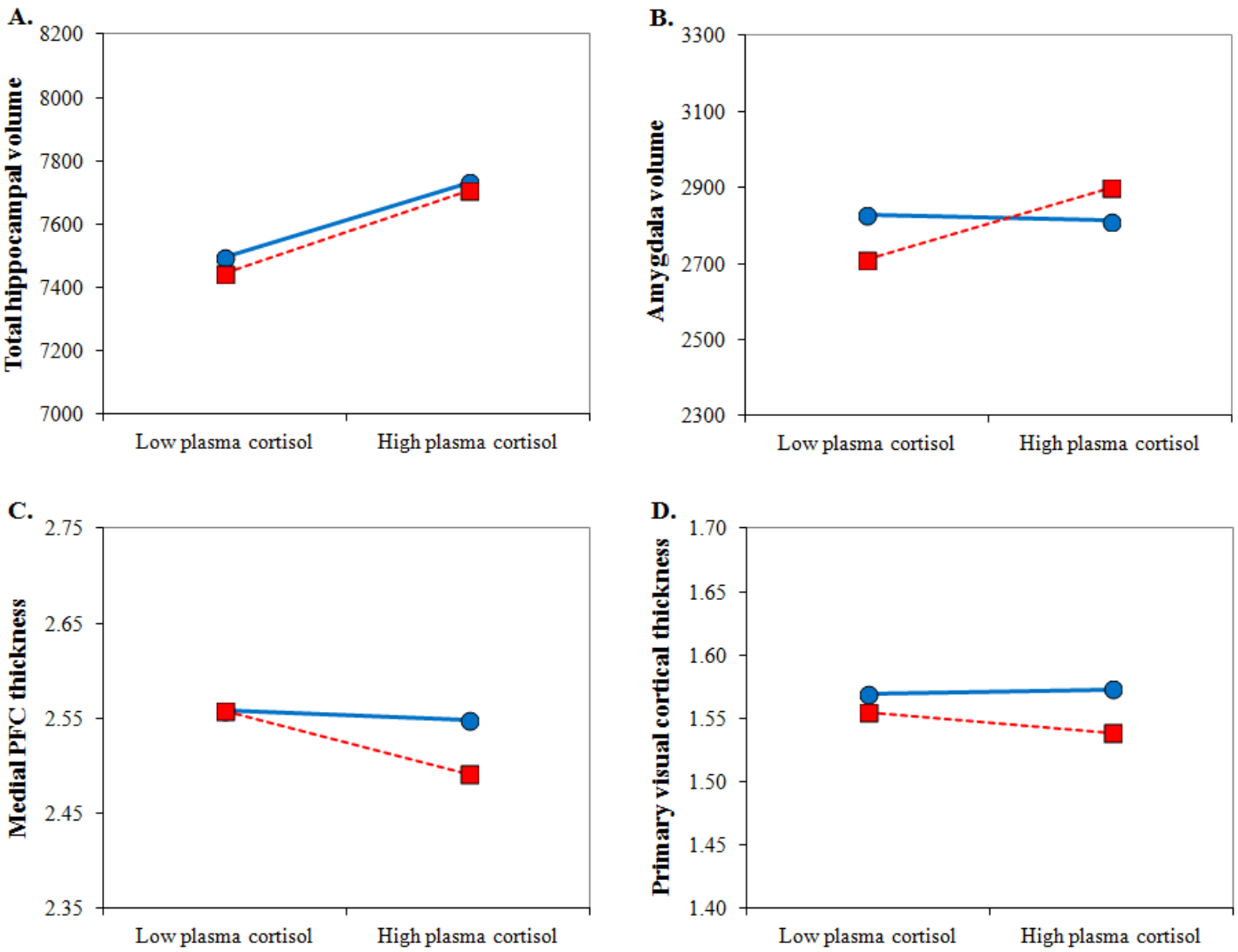

Figure 3.1 Plasma cortisol and regional volume or thickness. A) Hippocampus; B) Amygdala; C) Medial prefrontal cortex; D) Primary visual cortex. The blue, solid line represents low genetic risk and the red, dotted line represents high genetic risk.

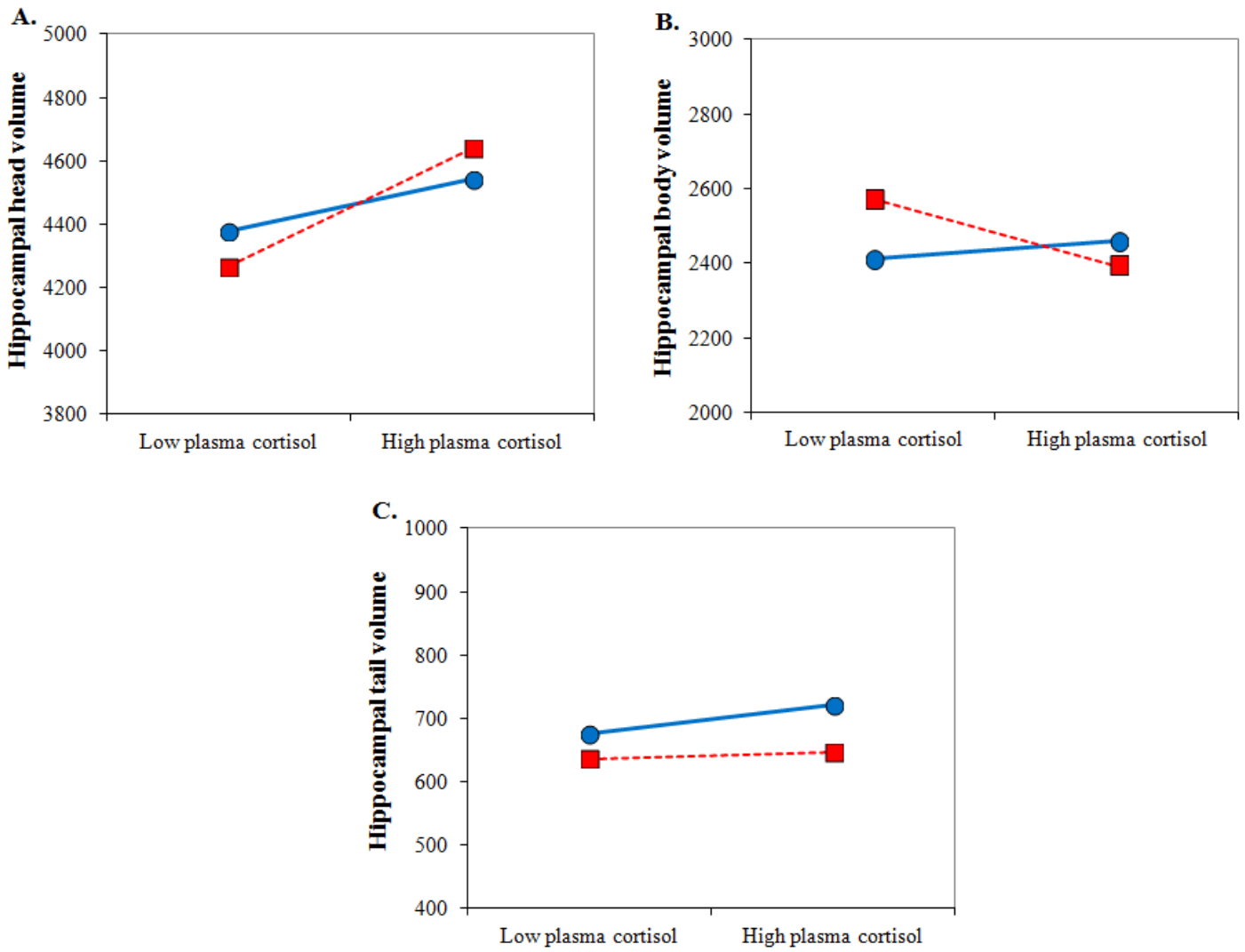

Figure 3.2 Plasma cortisol and hippocampal subdivisions. A) Hippocampal head; B) Hippocampal body; C) Hippocampal tail. The blue, solid line represents low genetic risk and the red, dotted line represents high genetic risk.

3.2.2 Lifetime Stress

Hippocampal Volume

Lifetime stress was not significantly associated with total hippocampal volume (β=.106, p=.316, 95%CI:-.104-.316). There was a non-significant trend for a negative association between genetic score and total hippocampal volume (β=-.195, p=.055, 95%CI:-.394-.004). The lifetime stress × genetic score interaction was not significant (β=-.072, p=.513, 95%CI:-.291-.146) (see Figure 3.3A).

Amygdala Volume

Lifetime stress and amygdala volume were not significantly associated (β=.022, p=.841, 95%CI:-.197-.241). In addition, the genetic score was not significantly associated with amygdala volume (β=-.046, p=.672, 95%CI:-.261-.169), nor was the lifetime stress × genetic score interaction (β=-.021, p=.859, 95%CI:-.256-.214) (see Figure 3.3B).

Medial PFC Thickness

Lifetime stress and mPFC thickness were not significantly associated (β=.161, p=.150, 95%CI:-.060-.382). In addition, the genetic score was not significantly associated with mPFC thickness (β=-.066, p=.548, 95%CI:-.282-.151), nor was the lifetime stress × genetic score interaction (β=.110, p=.350, 95%CI:-.123-.343) (see Figure 3.3C).

Primary Visual Cortical Thickness

Lifetime stress and primary visual cortical thickness were not significantly associated (β=.014, p=.907, 95%CI:-.222-.249). In addition, the genetic score was not significantly associated with primary visual cortical thickness (β=-.023, p=.842, 95%CI:-.255-.208), nor was the lifetime stress × genetic score interaction (β=.156, p=.213, 95%CI:-.091-.403) (see Figure 3.3D).

Hippocampal Subdivisions: Hippocampal Head

Lifetime stress was not significantly associated with hippocampal head volume (β=.049, p=.657, 95%CI:-.169-.267). In addition, the genetic score was not significantly associated with hippocampal head volume (β=-.165, p=.117, 95%CI:-.373-.043). The lifetime stress × genetic score interaction was not significant (β=-.091, p=.421, 95%CI:-.315-.133) (see Figure 3.4A).

Hippocampal Subdivisions: Hippocampal Body

Lifetime stress was not significantly associated with hippocampal body volume (β=.149, p=.191, 95%CI:-.076-.374). In addition, the genetic score was not significantly associated with hippocampal body volume (β=-.073, p=.511, 95%CI:-.292-.146). The lifetime stress × genetic score interaction was not significant (β=.112, p=.346, 95%CI:-.124-.348) (see Figure 3.4B).

Hippocampal Subdivisions: Hippocampal Tail

Lifetime stress was not significantly associated with hippocampal tail volume (β=.089, p=.467, 95%CI:-.153-.332). The genetic score was significantly and negatively associated with hippocampal tail volume (β=-.229, p=.049, 95%CI:-.457--.001). The lifetime stress × genetic score interaction was not significant (β=-.154, p=.223, 95%CI:-.404-.096) (see Figure 3.4C).

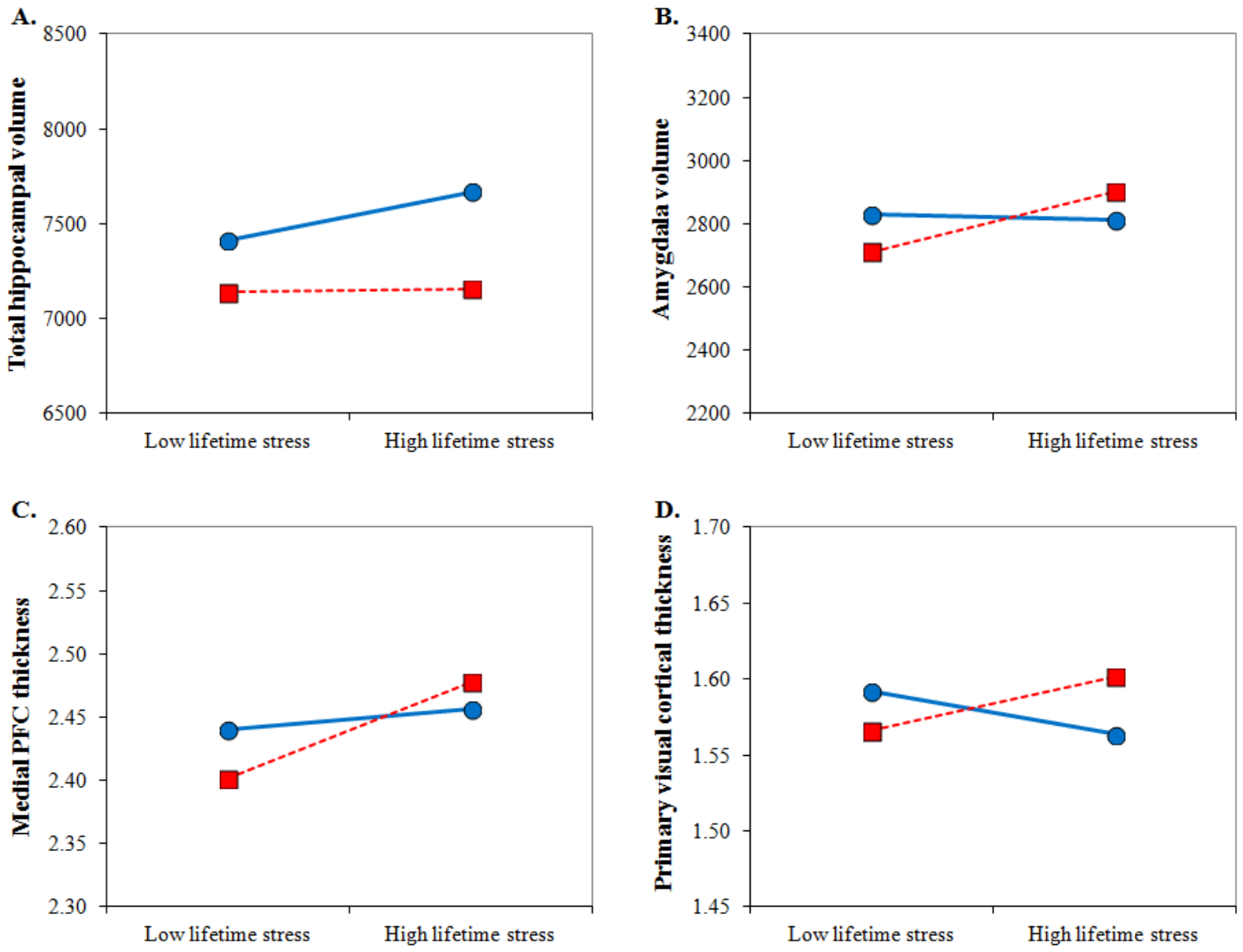

Figure 3.3 Lifetime stress and regional volume or thickness. A) Hippocampus; B) Amygdala; C) Medial prefrontal cortex; D) Primary visual cortex. The blue, solid line represents low genetic risk and the red, dotted line represents high genetic risk.

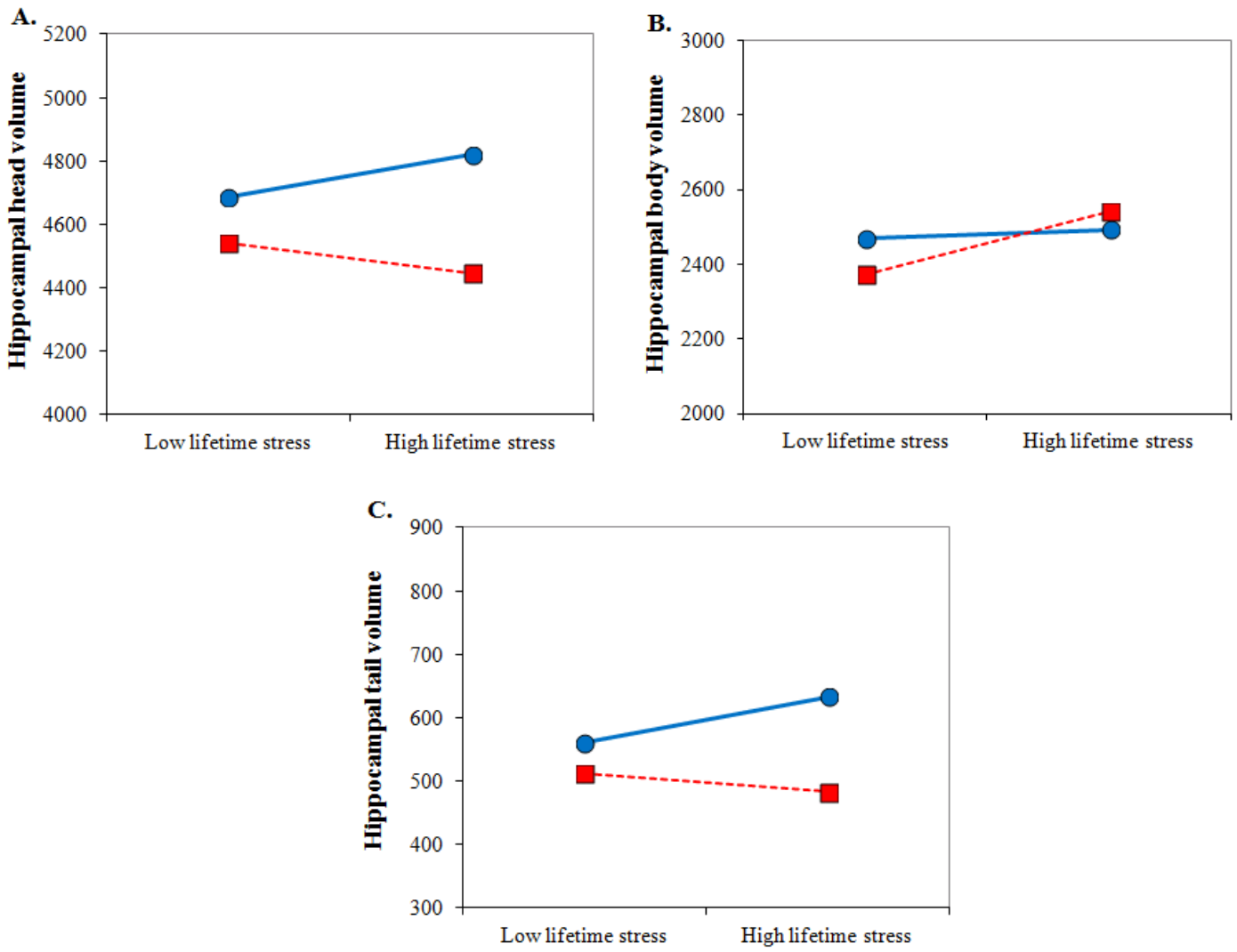

Figure 3.4 Lifetime stress and hippocampal subdivisions. A) Hippocampal head; B) Hippocampal body; C) Hippocampal tail. The blue, solid line represents low genetic risk and the red, dotted line represents high genetic risk.

3.2.3 Exploratory Analyses: Hair Cortisol

Hair cortisol was not significantly associated with hippocampal volume (β=-.071, p=.756, 95%CI:-.551-.408) (see Figure 3.5A), amygdala volume (β=-.401, p=.156, 95%CI:-.972-.170) (see Figure 3.5B), or medial PFC thickness (β=.154, p=.549, 95%CI:-.380-.688) (see Figure 3.5C). However, hair cortisol was significantly and positively associated with primary visual cortical thickness (β=.276, p=.049, 95%CI:.001-.551) (see Figure 3.5D).

3.2.4 Exploratory Analyses: Recent Stress

Recent stress was not significantly associated with hippocampal volume (β=-.118, p=.609, 95%CI:-.596-.359) (see Figure 3.6A), amygdala volume (β=-.051, p=.868, 95%CI:-.686-.584) (see Figure 3.6B), or medial PFC thickness (β=.000, p=.999, 95%CI:-.514-.515) (see Figure 3.6C). However, recent stress was significantly and negatively associated with primary visual cortical thickness (β=-.429, p=.006, 95%CI:-.718--.139) (see Figure 3.6D).

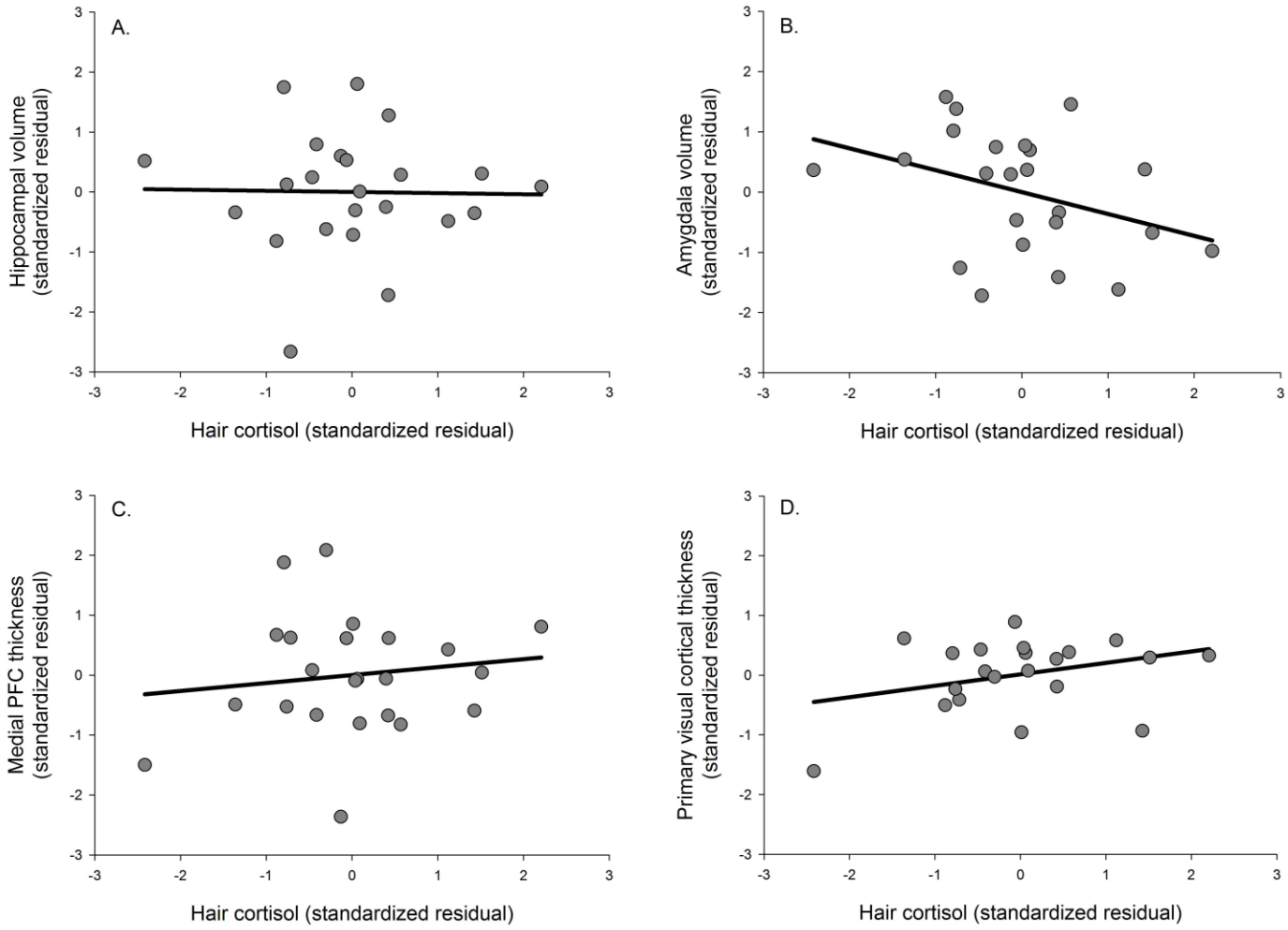

Figure 3.5 Hair cortisol and regional volume or thickness. A) Hippocampus; B) Amygdala; C) Medial prefrontal cortex; D) Primary visual cortex.

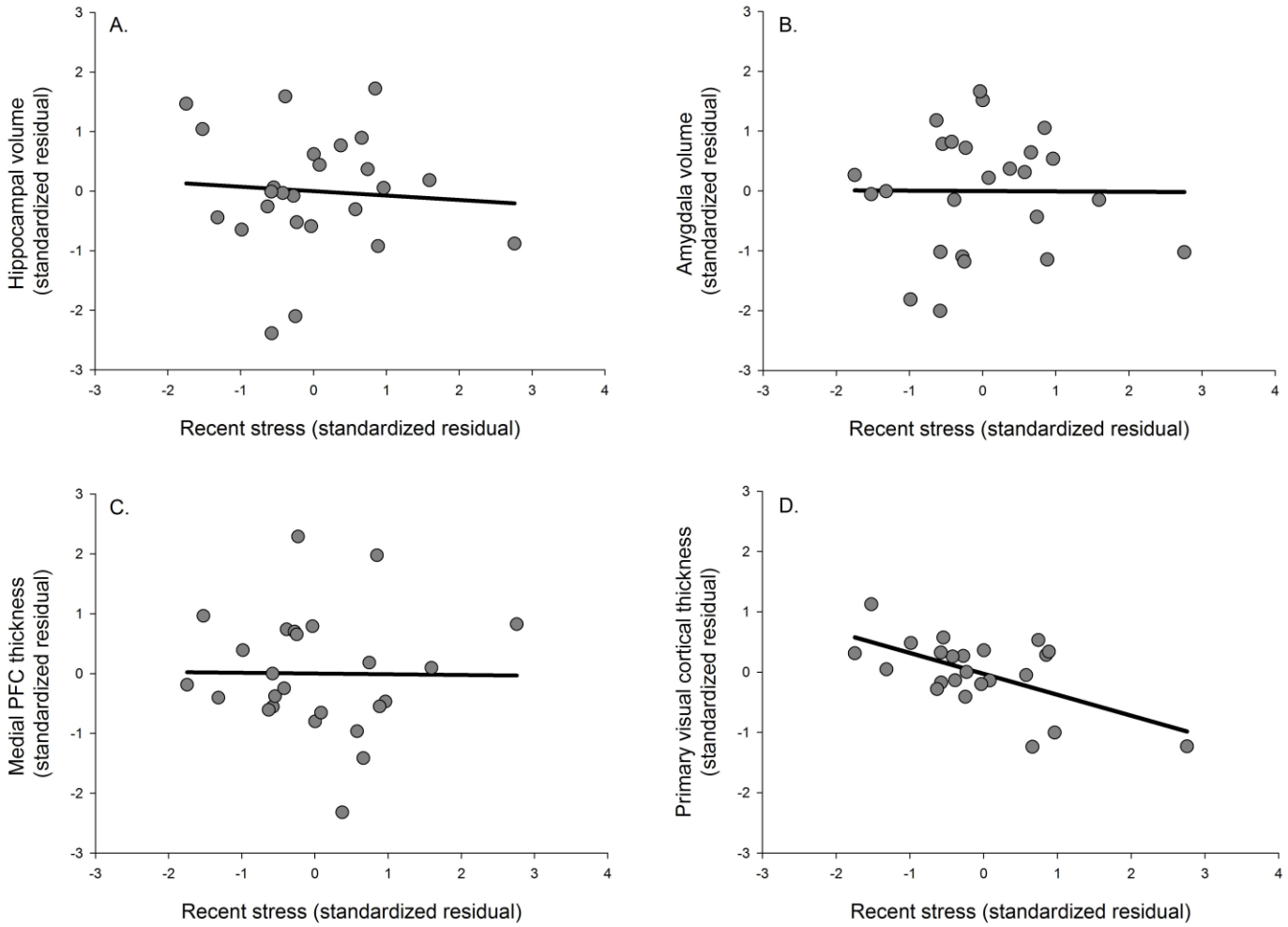

Figure 3.6 Recent stress and regional volume or thickness. A) Hippocampus; B) Amygdala; C) Medial prefrontal cortex; D) Primary visual cortex.

3.3 Discussion

3.3.1 Summary

This chapter investigated the differential associations of plasma cortisol or lifetime stress with brain structures with a high versus low expression of MR/GR, and whether or not these associations were moderated by genetic scores from stress-system genes. Also, as an exploratory analysis, the associations of hair cortisol or recent stress with brain structure were examined as well. Overall, the main hypothesis was not confirmed since most of the associations were not significant except for a positive association between plasma cortisol and hippocampal head volume.

3.3.2 Plasma Cortisol and Brain Structure

Animal studies have suggested that stress and emotion regulation are more closely linked to the anterior portion of the hippocampus (see review Fanselow & Dong, 2010). Consistent with this observation, the present investigation revealed a significant association with the hippocampal head, a subdivision that represents the more anterior portion of the hippocampus, but not with hippocampal body and tail volumes. A similar pattern has been observed for stress and cortisol in children (Szeszko et al., 2006; Wiedenmayer et al., 2006), and for cortisol in a sample of young adults with schizophrenia and healthy controls (Gunduz-Bruce et al., 2007). Thus, the discrepant findings in past examinations of the hippocampus in older adults may indeed relate in part to examination of the total volume.

However, it is important to note that the direction of the association between cortisol and the anterior hippocampus was positive rather than negative. One possibility is that this relates to the timing of the cortisol measurement. Cortisol secretion follows a diurnal pattern such that it

peaks in the morning, declines throughout the day and reaches a nadir in the late evening. One component of this pattern is the cortisol awakening response (CAR), during which cortisol markedly rises within the 30 minutes after waking. One characteristic of HPA axis dysregulation may be an altered CAR (Fries, Dettenborn, & Kirschbaum, 2009). In addition, there is evidence that hippocampal damage may reduce the cortisol awakening response (Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004). Prior studies have found positive associations of CAR or morning cortisol with hippocampal volume in various cohorts, including healthy children (Wiedenmayer et al., 2006), pre-diabetic adolescents (Ursache, Wedin, Tirsi, & Convit, 2012), healthy young (Pruessner, Pruessner, Hellhammer, Bruce Pike, & Lupien, 2007) and middle-aged/older (Bruehl et al., 2009) adults, and young adult patients with post-traumatic stress disorder (Lindauer, Olff, van Meijel, Carlier, & Gersons, 2006). Based on these previous investigations, the positive direction in the current results is not without precedent, and may still reflect an association between HPA axis dysregulation and anterior hippocampal volume in older adults.

However, it should also be noted that, as detailed out in Chapter 1, there has been inconsistent findings among studies that have specifically examined middle-aged and older adults, especially with awakening and morning cortisol measures (e.g., Kremen et al., 2010; O'Hara et al., 2007). Nonetheless, the positive association with hippocampus has been observed only in the case of awakening/morning cortisol and for no other time points during the day in older adults. Clearly, more systematic research is needed to understand this heterogeneity. Longitudinal work with sufficiently large samples should examine whether associations with hippocampal subdivisions differ based on longitudinal increases and decreases in cortisol, and incorporate multiple measures of HPA axis activity taken at different time points throughout the day.

Another potential reason for this positive association may relate to the conceptualization that dysregulation of the HPA axis is first characterized by hypercortisolism, but chronic hyperactivity of the HPA axis activity would eventually lead to hypoactivity of the HPA axis (Miller et al., 2007). It is possible that the participants' distant stress experiences resulted in hypocortisolism within these individuals, possibly explaining why the current study observed lower cortisol to be associated with lower hippocampal volume. However, a lack of longitudinal studies makes it difficult to draw a firm conclusion at this point.

Furthermore, the current project did not observe any significant associations of morning cortisol with either the amygdala or mPFC. Only one prior study has examined amygdala volume in relation to cortisol, and found a negative association with evening cortisol but null effects with morning cortisol (Geerlings et al., 2015). In addition, only a limited number of studies has examined PFC regions in association with cortisol (e.g., Carrion et al., 2010; Gold et al., 2005; Kremen et al., 2010; Treadway et al., 2009; Wolf, Convit, de Leon, Caraos, & Qadri, 2002). The associations were significant only in samples that were composed of either a large sample size (n=388; Kremen et al., 2010) or individuals with mood disorders, such as major depressive disorder (Treadway et al., 2009) or post-traumatic stress disorder (Carrion et al., 2010). One past study that specifically examined awakening cortisol and mPFC found no significant associations (Kremen et al., 2010). Our null findings with morning cortisol are in line with these studies. However, this does not necessarily indicate that HPA axis activity has no association with amygdala and mPFC structure in healthy adults, but instead suggests that systematic examinations with multiple components of HPA axis activity in large samples are necessary before strong conclusions can be made.

There were neither main effects of genetic score nor interactive effects of cortisol and genetic score for any target regions, except for a non-significant trend for an interaction with hippocampal body. It is unclear as to why this trend was observed only in the hippocampal body. Since the effect size was small and not statistically significant, and there are no clear reasons for the interaction to be observed specifically in the hippocampal body, this finding needs further replication.

3.3.3 Lifetime Stress and Brain Structure

Lifetime stress was not associated with any of the brain structure measures. Although these associations were not significant, all of them were in a slightly positive direction. This is in contrast to previous findings that behavioral stress is generally negatively associated with the hippocampus and prefrontal cortex as mentioned in Chapter 1. However, Zannas et al., (2013) found a positive association between the hippocampus and stress over the last year in older adults when examined cross-sectionally. Also, it should be noted that some past studies have found positive associations with amygdala, even though most of them involved children and young adults (e.g., Lupien et al., 2011; Moutsiana et al., 2015). A U-shaped relationship between stress and brain structure has been conceptualized previously, with the idea that a low level of stress is beneficial (McEwen et al., 2015). The Life Stressor Checklist-Revised, the questionnaire that was used to assess lifetime stress, consisted of 31 stressful events total. The mean value for the current sample was 5 (range 1-14). It is possible that the current study's measure of lifetime stress did not capture the entire spectrum of stress continuity but captured those who are in the lower range of the spectrum. Indeed, the majority of participants were Caucasians (n=83, 94.3%) and were categorized into high-privilege to middle socioeconomic status groups (n=77, 86.5%). Therefore, it may have been less likely that they were exposed to racial discrimination and/or

financial crisis, some of the stressful events that were included in this particular questionnaire. However, measuring a wider spectrum of stress within individuals who do not have psychiatric conditions may be challenging in humans, yet necessary in order to confirm the importance of incorporating nonlinearity in understanding the complex relations between stress and brain.

Furthermore, neither main effects of genetic score nor interactive effects between lifetime stress and genetic score were observed, except for the hippocampal tail, in which greater genetic risk was associated with smaller hippocampal tail. However, this pattern was not observed in a larger plasma-MRI sample. Therefore, it is questionable as to whether or not this association is robust. To my knowledge, only one past study has created a genetic profile score of stress-system genes and examined interactions with lifetime stress on brain structure, specifically hippocampal and amygdalar volumes (Pagliaccio et al., 2014). Similar to the current finding, this past study also did not find significant main effects of genetic scores on any of the brain volumes. However, in contrast to the current study, Pagliaccio et al., (2014) did observe an interactive effect of genetic profile score and lifetime stress on left hippocampal and left amygdala volume (but not with the right volumes). However, there were a few differences between the study by Pagliaccio et al., (2014) and the current project. This past study was done in children whereas the current study was done in middle-aged to older adults. Although some stressful experiences were similar in both age groups (e.g., physical abuse), other stressful life events reported by these children (e.g., change in daycare) were different from those that were reported by older adults in the current study (e.g., responsible for taking care of someone with handicap). Also, there may be differential effects of stress on brains during developmental phase versus normal-aging phase. Furthermore, Pagliaccio et al., (2014) used 10 different genotypes, including those related to depression, whereas the current study focused on 6 genotypes that were related to MR and GR

functioning. Lastly, because the current study did not have a priori hypothesis in terms of lateralization effect, only the combined volumes were examined for the current study. Because of these differences and the limited number of studies that investigated the effects of stress-system genes on brain structure, replication in large independent samples would be necessary.

3.3.4 Hair Cortisol and Recent Stress with Brain Structure

Although hair cortisol was not significantly associated with any of the target brain structures, there was a moderate effect size for the association with amygdala volume (beta=-.401). To my knowledge, no study has examined the relationship between hair cortisol and brain structure in humans. But based on the observed effect sizes, it may be worthwhile for future studies to investigate the relationship between hair cortisol and brain volumes, particularly amygdala, in a larger sample of participants. Moreover, hair cortisol was significantly and positively associated with primary visual cortical thickness. This is in contrast to the negative association found with amygdala. Also, the effect size for primary visual cortical thickness (beta=.276) was smaller than the effect size for the amygdala. In addition, this positive association with hair cortisol was in the opposite direction from the association between recent stress and primary visual cortex. Yet, there was no significant correlation between recent stress and hair cortisol (see Chapter 5 for more details). Overall, it is unclear as to why the direction of the relationship is not consistent, but having a larger sample size may help to resolve this issue.

There were no significant relationships between recent stress and target brain structures. It is possible that recent stress may not have immediate large effects at a macroscopic level. The current study was not designed to examine the relationship at a microscopic level, but it is possible that recent stress is more related to dendritic changes in these brain regions, as examined in animal studies, yet these changes may not be immediately detectable at a macroscopic level.

Unexpectedly, there was a significant negative association between recent stress and the primary visual cortex, in contrast to null effects with target regions. These target regions are more prone to aging whereas primary visual cortex is relatively more robust against aging effect (Raz et al., 2005). In fact, there was no significant association between age and primary visual cortex in the recent stress sample (r=.005, p=.980). Thus, it is possible that the aging effect accounted for a large portion of the variance in target regions but not in primary visual cortex, causing a unique effect that recent stress could contribute to these target regions above and beyond the aging effects to be very small.

Chapter 4: Associations of Stress and Cortisol with Cognition

4.1 Methods

4.1.1 Participants

The participants, aged from 52 to 92, were recruited from the Knight Alzheimer Disease Research Center (ADRC) at Washington University. The same screening criteria that were applied for Specific Aim 1 were used. The first sample consisted of existing ADRC participants who already had morning plasma cortisol and cognitive data collected (n=203) (see Table 4.1 for demographic information). The plasma cortisol assessment was within +/- 2 years of cognitive assessment. The second sample consisted of ADRC participants for whom the lifetime stress measure was collected (n=92) through the HRL (see Table 4.2 for demographic information). The ADRC cognitive data were used for the second sample. Some individuals from the second sample (n=72) also had plasma cortisol data, but because of a large time interval between plasma cortisol and lifetime stress (mean=7.4 years) assessments, cognitive assessment dates that are closest to the lifetime stress assessment date were chosen instead of using the same cognitive data as in the first sample. The third sample consisted of a subset of the second sample for whom recent stress measures and additional cognitive data (n=32) (see Table 4.3 for demographic information), as well as hair samples (n=27) (see Table 4.4 for demographic information) were collected in the HRL. Because the third sample was underpowered, the findings from this sample were treated as exploratory.

Plasma cortisol, hair cortisol, lifetime stress, recent stress, and genotype measures (see Table 4.5 for SNP frequency data) were the same as reported in Chapter 3.

Table 4.1 Descriptive statistics for the plasma cortisol-cognitive sample

N	203
Age, years (mean (SD))	71 (7)
Female, n (%)	131 (65)
Education, years (mean (SD))	16 (3)
Plasma cortisol, ng/ml (mean (SD))	165 (58)
Genetic profile scores (mean (SD))	4.28 (1.96)
Free and Cued Selective Reminding Test (mean (SD))	30 (6)
WMS Associate Learning (n=174) (mean (SD))	14 (4)
WMS-III Verbal Paired Associates (n=27) (mean (SD))	18 (9)
WMS Logical Memory - immediate (n=59) (mean (SD))	10 (4)
WMS Logical Memory - delayed (n=59) (mean (SD))	8 (4)
WMS-Revised Logical Memory - immediate (n=116) (mean (SD))	13 (4)
WMS-Revised Logical Memory - delayed (n=116) (mean (SD))	12 (4)
WMS-III Logical Memory - immediate (n=27) (mean (SD))	28 (8)
WMS-III Logical Memory - delayed (n=27) (mean (SD))	22 (9)
Memory - standardized and averaged (mean (SD))	0.00 (.78)
Trailmaking A, seconds (mean (SD))	32 (10)
Trailmaking B, seconds (mean (SD))	81 (31)
Fluid intelligence - standardized and averaged (mean (SD))	0.00 (.88)
WAIS Information (n=176) (mean (SD))	22 (4)
WAIS-III Information (n=27) (mean (SD))	22 (3)

Table 4.2 Descriptive statistics for the lifetime stress-cognitive sample

N	92
Age, years (mean (SD))	75 (6)
Female, n (%)	60 (65)
Education, years (mean (SD))	15 (3)
Lifetime stress - log transformed (mean (SD))	.64 (.27)
Genetic profile scores (mean (SD))	4.18 (1.94)
Free and Cued Selective Reminding Test (mean (SD))	33 (6)
WMS Associate Learning (n=76) (mean (SD))	16 (3)
WMS-III Verbal Paired Associates (n=14) (mean (SD))	23 (6)
WMS-Revised Logical Memory - immediate (n=77) (mean (SD))	16 (4)
WMS-Revised Logical Memory - delayed (n=77) (mean (SD))	15 (3)
WMS-III Logical Memory - immediate (n=15) (mean (SD))	32 (5)
WMS-III Logical Memory - delayed (n=15) (mean (SD))	28 (5)
Memory - standardized and averaged (mean (SD))	0.00 (.77)
Trailmaking A, seconds (mean (SD))	29 (9)
Trailmaking B, seconds (mean (SD))	76 (36)
Fluid intelligence - standardized and averaged (mean (SD))	0.00 (.90)
WAIS Information (n=77) (mean (SD))	22 (4)
WAIS-III Information (n=15) (mean (SD))	21 (5)

Table 4.3 Descriptive statistics for the recent stress-cognitive sample

N	32
Age, years (mean (SD))	73 (7)
Female, n (%)	16 (50)
Education, years (mean (SD))	16 (3)
Perceived Stress Scale - square root transformed (mean (SD))	2.83 (1.21)
Elders' Life Stress Inventory - square root transformed (mean (SD))	2.71 (1.43)
Recent stress - standardized and averaged (mean (SD))	0.00 (.96)
Visual Auditory Learning - immediate (mean (SD))	89 (16)
Visual Auditory Learning - delayed (mean (SD))	104 (14)
Cattell Culture Fair Intelligence Test (mean (SD))	8 (2)
Shipley Vocabulary Test (mean (SD))	35 (4)

Table 4.4 Descriptive statistics for the hair cortisol-cognitive sample

N	27
Age, years (mean (SD))	72 (7)
Female, n (%)	14 (52)
Education, years (mean (SD))	16 (3)
Hair cortisol - log transformed, pg/mg (mean (SD))	1.24 (.72)
Visual Auditory Learning - immediate (mean (SD))	93 (12)
Visual Auditory Learning - delayed (mean (SD))	108 (10)
Cattell Culture Fair Intelligence Test (mean (SD))	8 (2)
Shipley Vocabulary Test (mean (SD))	35 (4)

Table 4.5 Single nucleotide polymorphism (SNP) frequency data for Aim 2

	Plasma cortisol-cognitive sample				Lifetime stress-cognitive sample			
SNP	Major homozygote, n (%)	Heterozygote, n (%)	Minor homozygote, n (%)	Missing, n (%)	Major homozygote, n (%)	Heterozygote, n (%)	Minor homozygote, n (%)	Missing, n (%)
rs5522	149 (73)	35 (17)	1 (1)	18 (9)	70 (76)	17 (18)	0 (0)	5 (5)
rs2070951	50 (25)	86 (42)	48 (24)	19 (9)	18 (20)	52 (56)	15 (16)	7 (8)
rs41423247	81 (40)	97 (48)	25 (12)	0 (0)	35 (38)	48 (52)	9 (10)	0 (0)
rs10482605	134 (66)	56 (28)	9 (4)	4 (2)	59 (64)	28 (30)	3 (3)	2 (2)
rs10052957	86 (42)	91 (45)	25 (12)	1 (1)	38 (41)	45 (49)	9 (10)	0 (0)
rs1360780	82 (40)	94 (46)	24 (12)	3 (1)	37 (40)	45 (49)	9 (10)	1 (1)

4.1.2 ADRC Memory Assessment

The episodic memory domain was assessed using the Free and Cued Selective Reminding Test, and the Associate Learning and Logical Memory (immediate and delay) subtests from the Wechsler Memory Scale (WMS). A composite measure of memory was created by standardizing scores for each task (a total of 4 variables) and averaging the standardized scores.

Free and Cued Selective Reminding Test

The participants were required to name a pictured item (e.g., grape) when they were presented with the category cue (e.g., fruit). Once they had learned all 16 items, they were asked to recall items. Then they were given the category cue for items that were not recalled. The scores were derived by counting the number of items correctly recalled on three trials (Grober, Buschke, Crystal, Bang, & Dresner, 1988).

Associate Learning

Either the WMS Associate Learning (Wechsler & Stone, 1973) or WMS-III Verbal Paired Associates (Wechsler, 1997b) was administered to each participant. The participants were required to learn eight word pairs over 4 trials. The scores were derived by counting the number of correct responses over 4 trials. Because two different versions were administered, the scores on the respective test versions were standardized to obtain an estimate of each individual's ranking, and compiled into one Associate Learning variable.

Logical Memory

One of the three versions of Logical Memory, WMS Logical Memory (Wechsler & Stone, 1973), WMS-Revised Logical Memory (Wechsler, 1987) or WMS-III Logical Memory (Wechsler, 1997b), was administered to each participant. Participants were read two stories and then they were asked to recall details, both immediately and after delay. However, only one story

was read to participants who were administered with WMS-Revised Logical Memory. Because three different versions were administered, the same approach as for Associate Learning was used to generate one Logical Memory variable.

4.1.3 ADRC Fluid Intelligence Assessment

The fluid intelligence variable included scores from Trailmaking A and B. A composite measure of fluid intelligence was created by standardizing scores for each test and averaging the standardized scores.

Trailmaking A

The participants were required to draw lines to connect 25 numbered circles in sequential order as quickly as they can (Armitage, 1945). The raw scores equaled the total seconds that each participant spent on completing the task. The scores were reversed so that the direction of all variables was the same (i.e., a higher score reflects better performance).

Trailmaking B

The participants were required to draw lines to connect numbered circles to lettered circles in alternating sequential order (Armitage, 1945). The raw scores equaled the total seconds that each participant spent on completing the task. The scores were reversed so that the direction of all variables was the same (i.e., a higher score reflects better performance).

4.1.4 ADRC Crystallized Intelligence Assessment

WAIS Information

Either WAIS Information (Wechsler, 1955) or WAIS-III Information (Wechsler, 1997a) was administered to each participant. The participants were required to answer questions about factual information. The scores were derived by counting the total number of correct answers.

Because two different versions were administered, the same approach as for Associate Learning and Logical Memory was used to generate one Information variable.

4.1.5 HRL Cognitive Assessment

Additional cognitive measures were obtained from the ADRC participants who visited the laboratory. These cognitive measures were used in the analyses with recent stress and hair cortisol since they were all measured on the same day.

Visual-Auditory Learning-Delayed

This test is part of the Woodcock-Johnson III Tests of Cognitive Abilities (WJ III COG), and was used to assess episodic memory. The participants were asked to learn and recall pictorial representations of words, immediately and after a >30 minute delay (Mather & Woodcock, 2001). The scores were derived by counting the total number of correct answers for both immediate and delay. A composite measure of memory was created by standardizing scores for each test and averaging the standardized scores.

Cattell Culture Fair Intelligence Test

The 3B-2 subtest, which consists of 14 items, was administered to assess fluid intelligence. For each item, the participants were presented with five pictures, and they were asked to choose the two that were different from the other three (Cattell & Cattell, 1973). The scores were derived by counting the total number of correct answers.

Shipley Vocabulary Test

This test, which consists of 40 vocabulary questions, was administered to assess fluid intelligence. Participants were given the first word and four other words from which they had to choose one word that was the synonym of the first word (Shipley, 1940). The scores were derived by counting the total number of correct answers.

4.1.6 Statistical Analysis

The same covariates (except for scanner type) and statistical methods were used as described in Chapter 3 to analyze the data for Specific Aim 2

4.2 Results

4.2.1 Morning Plasma Cortisol

Memory

There was a non-significant trend for a negative association between morning plasma cortisol and memory (β=-.121, p=.072, 95%CI:-.254-.011). In addition, the genetic score was not significantly associated with memory (β=-.071, p=.291, 95%CI:-.202-.061), nor was the plasma cortisol × genetic score interaction (β=-.016, p=.818, 95%CI:-.148-.117) (see Figure 4.1A).

Fluid Intelligence

Morning plasma cortisol and fluid intelligence were not significantly associated (β=-.071, p=.252, 95%CI:-.193-.051). In addition, the genetic score was not significantly associated with fluid intelligence (β=.069, p=.257, 95%CI:-.051-.190). There was a significant plasma cortisol × genetic score interaction (β=-.151, p=.014, 95%CI:-.270--.031) (see Figure 4.1B). Specifically, a significant, negative association was observed between plasma cortisol and fluid intelligence in the context of high genetic score (β=-.208, p=.014, 95%CI:-.374--.042). The association was not significant in the context of low genetic score (β=.064, p=.421, 95%CI:-.093-.221).

Crystallized Intelligence

Morning plasma cortisol and crystallized intelligence were not significantly associated (β=.013, p=.842, 95%CI:-.114-.140). In addition, the genetic score was not significantly associated with crystallized intelligence (β=-.105, p=.101, 95%CI:-.230-.021), nor was the

plasma cortisol × genetic score interaction (β=-.051, p=.432, 95%CI:-.177-.076) (see Figure 4.1C).

4.2.2 Lifetime Stress

Memory

Lifetime stress and memory were not significantly associated (β=.050, p=.628, 95%CI:-.154-.253). In addition, the genetic score was not significantly associated with memory (β=-.072, p=.474, 95%CI:-.272-.127), nor was the lifetime stress × genetic score interaction (β=.020, p=.853, 95%CI:-.194-.234) (see Figure 4.2A).

Fluid Intelligence

Lifetime stress and fluid intelligence were not significantly associated (β=.088, p=.220, 95%CI:-.054-.230). In addition, the genetic score was not significantly associated with fluid intelligence (β=-.016, p=.822, 95%CI:-.154-.123), nor was the lifetime stress × genetic score interaction (β=.055, p=.466, 95%CI:-.094-.203) (see Figure 4.2B).

Crystallized Intelligence

Lifetime stress and crystallized intelligence were not significantly associated (β=-.096, p=.342, 95%CI:-.296-.104). In addition, the genetic score was not significantly associated with crystallized intelligence (β=-.139, p=.156, 95%CI:-.332-.054), nor was the lifetime stress × genetic score interaction (β=-.043, p=.682, 95%CI:-.250-.164) (see Figure 4.2C).

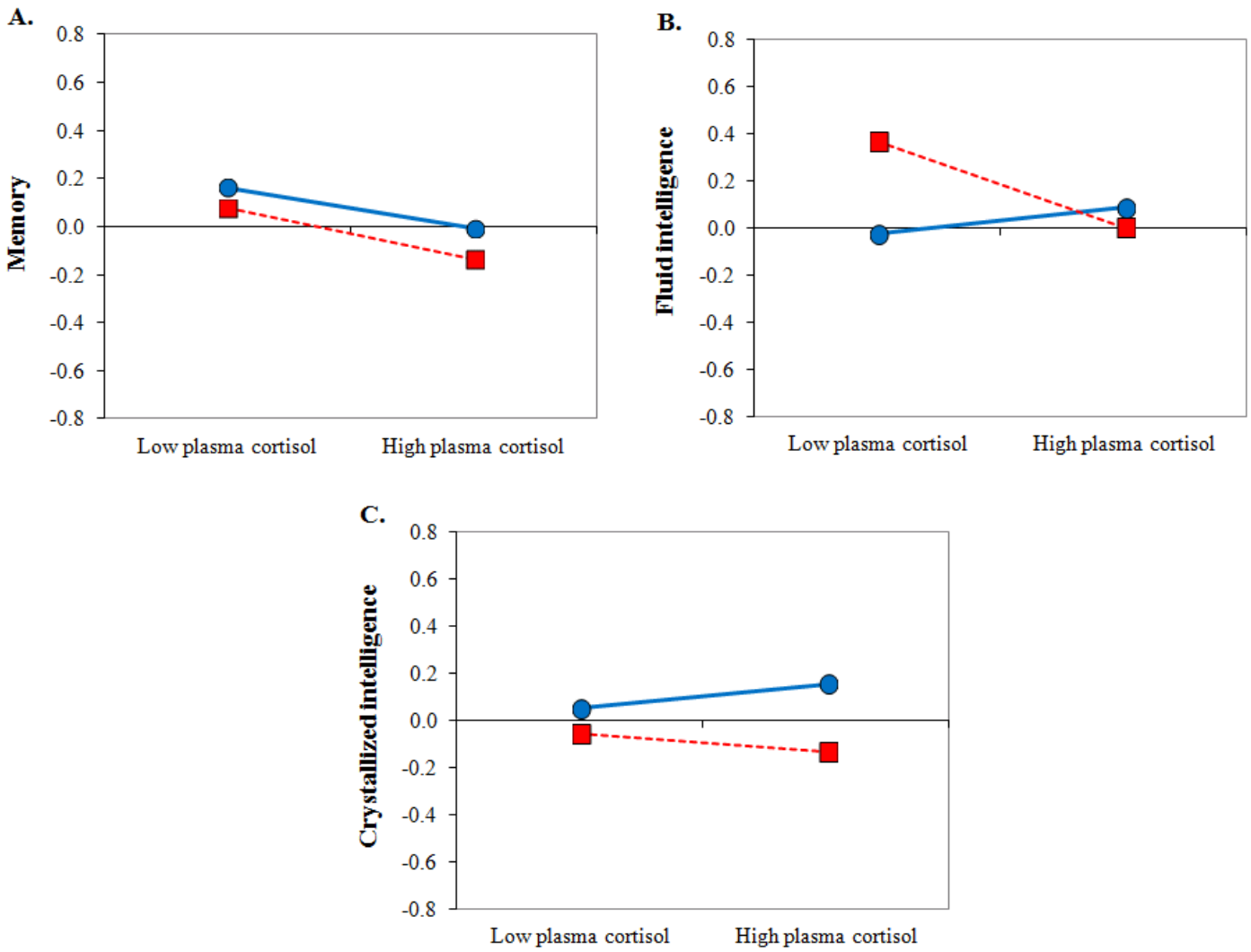

Figure 4.1 Plasma cortisol and cognition. A) Memory; B) Fluid intelligence; C) Crystallized intelligence. The blue, solid line represents low genetic risk and the red, dotted line represents high genetic risk.

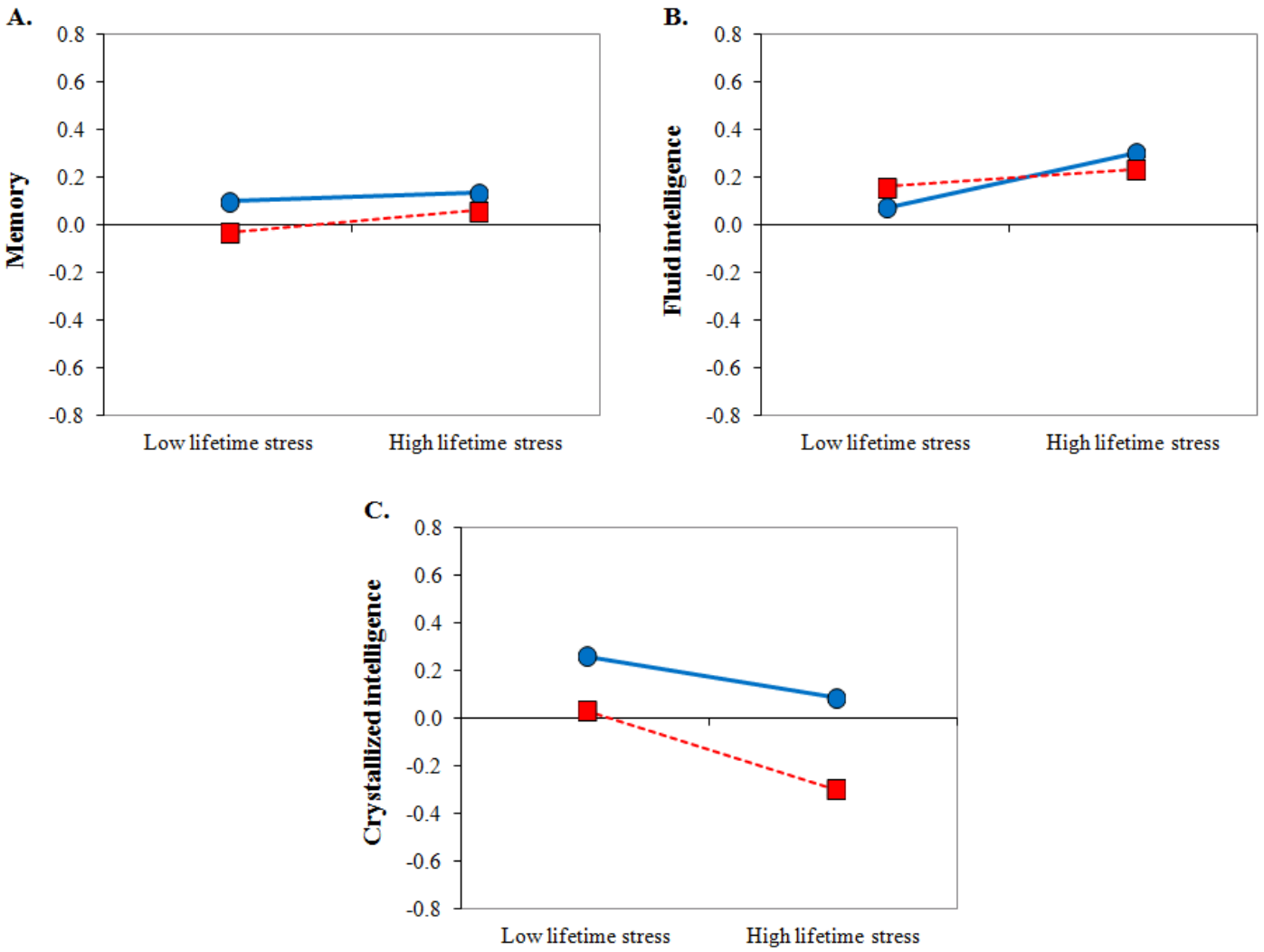

Figure 4.2 Lifetime stress and cognition. A) Memory; B) Fluid intelligence; C) Crystallized intelligence. The blue, solid line represents low genetic risk and the red, dotted line represents high genetic risk.

4.2.3 Exploratory Analyses: Hair Cortisol

Hair cortisol and memory were not significantly associated (β=-.250, p=.247, 95%CI:-.686-.186) (see Figure 4.3A). In contrast, hair cortisol was significantly and negatively associated with fluid intelligence (β=-.480, p=.045, 95%CI:-.949--.011) (see Figure 4.3B). Hair cortisol and crystallized intelligence were not significantly associated (β=.281, p=.132, 95%CI:-.092-.653) (see Figure 4.3C).

4.2.4 Exploratory Analyses: Recent Stress

Recent stress was not significantly associated with memory (β=.148, p=.287, 95%CI:-.131-.427) (see Figure 4.4A), fluid intelligence (β=-.128, p=.571, 95%CI:-.586-.330) (see Figure 4.4B), or crystallized intelligence (β=.050, p=.736, 95%CI:-.251-.351) (see Figure 4.4C).

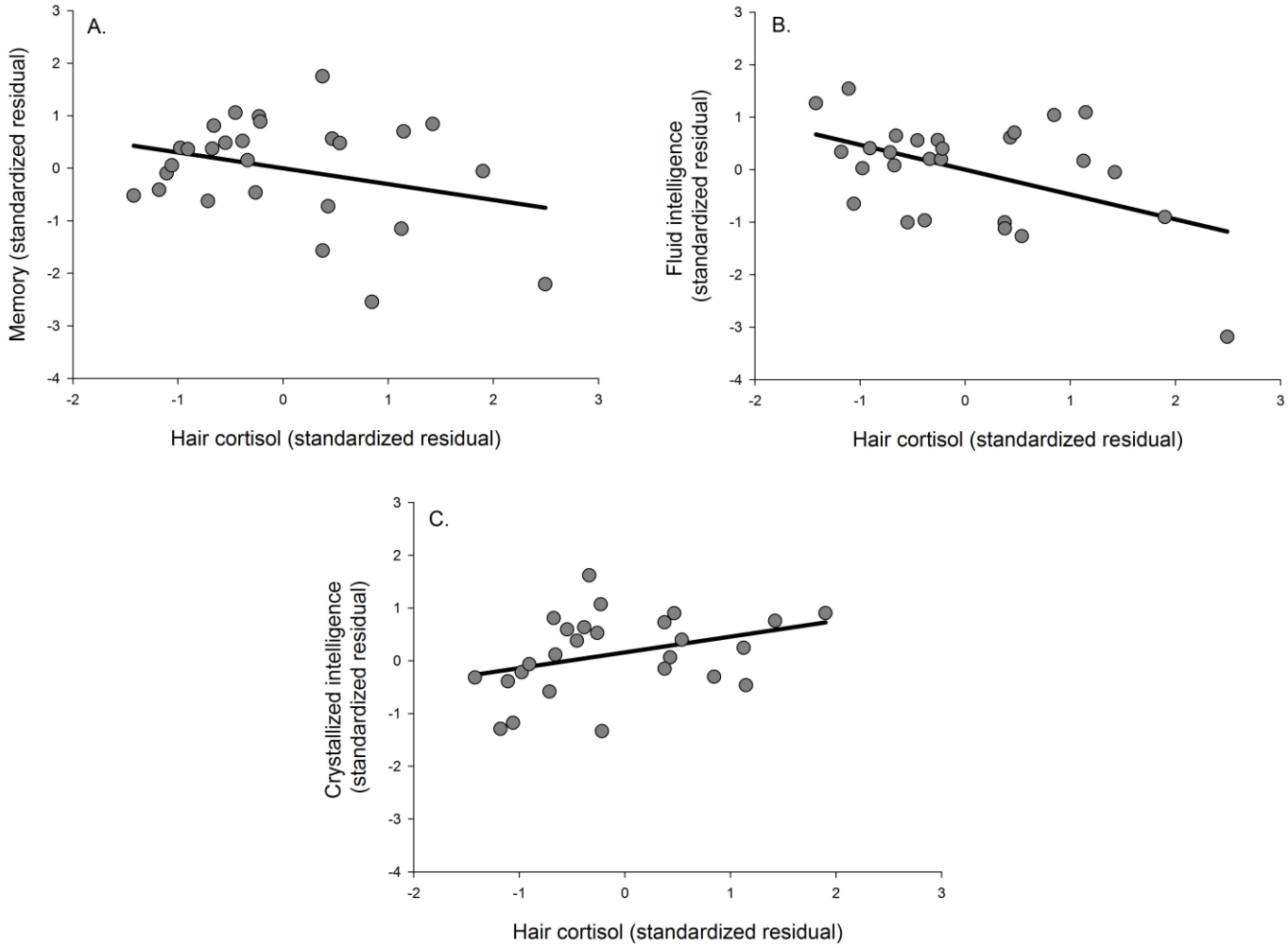

Figure 4.3 Hair cortisol and cognition. A) Memory; B) Fluid intelligence; C) Crystallized intelligence.

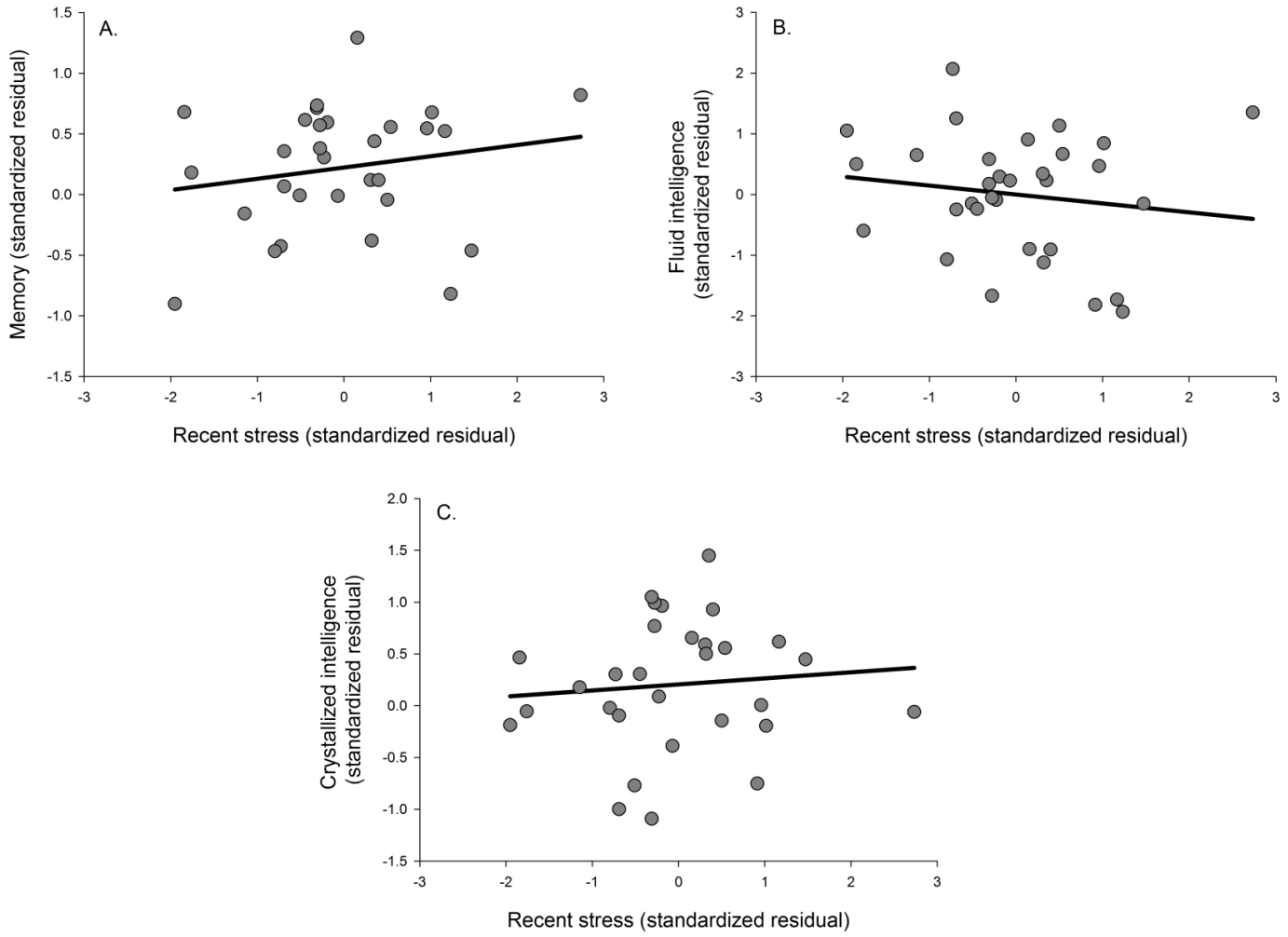

Figure 4.4 Recent stress and cognition. A) Memory; B) Fluid intelligence; C) Crystallized intelligence.

4.3 Discussion

4.3.1 Summary

This chapter investigated the differential effects of plasma cortisol or lifetime stress on cognitive functions that are reliant upon brain regions with high versus low expression of MR/GR, and examined whether the associations were moderated by genetic scores from stress-system genes. The main hypotheses were largely not confirmed since most of the associations were not significant. However, there was a significant interactive effect between plasma cortisol and genetic scores on fluid intelligence. Also, a significant negative association between hair cortisol and fluid intelligence was observed in the exploratory analysis.

4.3.2 Plasma Cortisol and Cognition

The present study found a trend for a negative association between plasma cortisol and memory performance, which is in agreement with my hypothesis that higher cortisol would be associated with worse memory. Considering the conceptualization that the hippocampus is positively associated with memory (e.g., Kaup, Mirzakhanian, Jeste, & Eyler, 2011), it might have been expected that cortisol would be associated with memory and hippocampal volume in the same direction (e.g., higher cortisol associated with both smaller hippocampus and lower memory). However, I observed that higher cortisol was associated with a larger hippocampal head volume, and there was a positive trend for total hippocampus (see Chapter 3.2.1). One possible explanation for cortisol having opposite associations with the hippocampus and memory may be due to variation across hippocampal subdivisions. However, none of the associations between hippocampal subdivisions and memory remained significant when controlled for age (all ps>.259). It is important to note that hippocampal volume and memory may not be as

strongly associated in older adults as noted in a meta-analysis by Van Petten (2004) and in the current work. Therefore, cortisol having opposite associations for hippocampus versus memory may be due to weak association between hippocampus and memory. This warrants consideration of more complex conceptualizations of associations of cortisol with hippocampal structure and memory.

Also, there is a possibility of a compensatory response playing a role. For instance, functional MRI studies have revealed increased activation of frontal regions in aging individuals who perform well on memory tasks (see review by Buckner, 2004). Similarly, there is evidence of stronger recruitment of frontal regions in cognitively normal older adults who are genetically at risk for Alzheimer's disease compared to a non-risk control group (Bookheimer et al., 2000). Based on these findings, researchers have hypothesized that compensation by other structures, particularly frontal regions, may be taking place to maintain memory performance in response to a threat to hippocampal integrity (e.g., during initial stages of dementia). Similarly, hippocampal deterioration due to dysregulated cortisol rhythm may have led to an activation of compensatory mechanism, resulting in opposite findings for hippocampal volume and memory. Yet, there was no significant association between medial PFC and memory in the current study when controlled for age ($r=.133$, $p=.106$). The current study did not examine frontal regions beyond medial PFC; thus, it is possible that other PFC regions may be related to this compensatory mechanism. Furthermore, factors such as coping strategies may have led some individuals to be more resilient to the effects of hippocampal damage, possibly contributing to why the same pattern was not observed for hippocampus versus memory. Overall, the relationship among cortisol, hippocampus and memory is unlikely to be simple but may need to systematically incorporate

hippocampal subdivision variation, contribution of other brain regions, and individual differences in resiliency.

The current study observed a significant interaction of plasma cortisol and genetic score on fluid intelligence. This finding is in agreement with my prediction that individuals with higher cortisol and greater number of risk alleles would evidence the worst cognitive outcome. Such an interactive effect may explain why some studies found significant associations with fluid intelligence measures while others found null results. To my knowledge, no study has examined the interactive effects of composite genetic score of stress system genes and HPA axis activity on fluid intelligence. Since the current study utilized scores from Trailmaking A and B only, whether this pattern of interaction will remain consistent with other fluid intelligence measures needs to be investigated in future studies. Furthermore, it is unclear as to why similar association was not observed for memory. Yet, the association between plasma cortisol and memory was in the same direction as with fluid intelligence in the context of high genetic risk although it did not approach significance. As mentioned above, replication with diverse cognitive measures may be necessary to determine the robustness and generalizability of the findings with stress-system genes.

4.3.3 Lifetime Stress and Cognition

There were no significant associations between lifetime stress and any of the cognitive measures. However, similar to the findings with lifetime stress and brain structure described in Chapter 3, the associations with memory and fluid intelligence were generally in a positive direction. Also, neither main effects of genetic score nor interactive effects of lifetime stress and genetic score were significant for any of the cognitive measures. As mentioned in Chapter 3, the current measure of lifetime stress is likely to have captured the lower range of the stress exposure

spectrum rather than a full range. If stress effects are in a U-shaped curve as some researchers have suggested (McEwen et al., 2015), then a lower level of stress may have beneficial effects on cognition, possibly explaining why the results with memory and fluid intelligence tended to be in a positive direction.

To my knowledge, no study has examined the effect of stress-system genes on cognitive function, thus it is difficult to interpret the null findings at this point. One speculation may be that the age-related changes that are taking place independent of stress may be lowering the penetrance of the stress-system genes in older adults (Erickson, Miller, & Roecklein, 2012). However, more replication is necessary, particularly in older adults, before drawing any conclusion as to the role of stress-system genes on cognition.

4.3.4 Hair Cortisol and Recent Stress with Cognition

Hair cortisol was significantly negatively associated with fluid intelligence, which is in agreement with my hypotheses that more chronic cortisol elevation would be associated with lower cognitive performance. Thus far, there have been three past studies that have investigated the relationship between hair cortisol and cognition, in which findings have been mixed. For instance, Saleem et al., (2013) found higher hair cortisol to be predictive of less exercise-related memory improvement in patients with coronary artery disease. In contrast, another study showed lower hair cortisol to be associated with lower episodic and working memory performance in a sample of rigorously screened healthy older adults (Pulopulos et al., 2014). In a sample of young and middle-aged adults, mostly female nurses, hair cortisol was not significantly associated with either episodic memory or fluid intelligence (McLennan et al., 2016). Only one of these studies used a large sample size of n=246 (McLennan et al., 2016). Also, the three samples were composed of individuals with distinct characteristics, which makes it difficult to generalize the

findings. Overall, no firm conclusion can be reached at this point, and future studies should examine this relationship in a larger sample of cognitively normal older adults to confirm whether or not the current results replicate.

In terms of recent stress, no significant associations were found between recent stress and any of the cognitive measures. Similar to the interpretation with brain structure in Chapter 3, it is possible that the effect of recent stress may not be immediately observable at a behavioral level. Likewise, past studies that have examined recent stress in older adults mostly showed null results with both memory (Peavy et al., 2009; Rosnick et al., 2007; but see Peavy et al., 2007) and fluid intelligence (Rosnick et al., 2007). However, longitudinal investigation examining how recent stress relates to changes in cognitive function over time may reveal stronger relationships.

Chapter 5: Post-hoc Analyses: Age, Gender and Stress Timing

5.1 Post-hoc Analyses: Age

5.1.1 Rationale

Previous studies have demonstrated that aging is associated with some changes in HPA axis activity, such as reduced cortisol awakening response and flattened diurnal rhythm (Heaney, Phillips, & Carroll, 2010, 2012; Veldhuis, Sharma, & Roelfsema, 2013). Also, there is a possibility that individuals would experience more lifetime stress as they get older. Furthermore, past studies involving older adults have shown somewhat inconsistent findings as described in Chapter 1, whereas the past studies in young adults have shown somewhat more consistent negative associations of behavioral stress with hippocampus (Andersen et al., 2008; Driessen et al., 2000; Ganzel et al., 2008; Gorka et al., 2014; Papagni et al., 2011), memory (Navalta, Polcari, Webster, Boghossian, & Teicher, 2006; Nixon, Nishith, & Resick, 2004; Stein, Kennedy, & Twamley, 2002), and fluid intelligence (Evans & Schamberg, 2009; Klein & Boals, 2001; Stein et al., 2002; Wilding, Andrews, & Hejdenberg, 2007). Since one possible reason for null results may be that an age effect is accounting for a large portion of the variance, the current study examined whether age moderates the association between plasma cortisol/lifetime stress and brain structure/cognition as a post-hoc analysis.

5.1.2 Methods

Statistical Analysis

A series of robust regression analyses were conducted to examine the main effects of cortisol (or stress) and age, and the interactive effect of cortisol (or stress) and age on regional volumes and thickness. In the regression analyses, covariates were entered in the first step, cortisol (or stress) and age entered in the second step, and the cortisol (or stress) × age interaction was entered in the last step.

5.1.3 Results

Brain Structure

The plasma cortisol x age interaction was not significant for hippocampal volume (β=.028, p=.719, 95%CI:-.126-.182), amygdala volume (β=-.038, p=.638, 95%CI:-.196-.120), medial PFC thickness (β=.082, p=.355, 95%CI:-.093-.257), or primary visual cortical thickness (β=.084, p=.334, 95%CI:-.087-.256). In addition, the plasma cortisol x age interaction was not significant for the hippocampal head (β=-.021, p=.785, 95%CI:-.172-.130), hippocampal body (β=.066, p=.416, 95%CI:-.095-.228), or hippocampal tail (β=.105, p=.263, 95%CI:-.080-.289) volumes. Furthermore, the lifetime stress x age interaction was not significant for hippocampal volume (β=.035, p=.735, 95%CI:-.170-.240), amygdala volume (β=-.065, p=.542, 95%CI:-.278-.147), medial PFC thickness (β=.081, p=.454, 95%CI:-.133-.294), or primary visual cortical thickness (β=.114, p=.321, 95%CI:-.113-.341). Lastly, the lifetime stress x age interaction was not significant for the hippocampal head (β=.096, p=.361, 95%CI:-.112-.304), hippocampal body (β=-.164, p=.133, 95%CI:-.379-.051), or hippocampal tail (β=.183, p=.121, 95%CI:-.049-.415) volumes.

Cognition

The plasma cortisol x age interaction was not significant for memory (β=.012, p=.862, 95%CI:-.123-.147), fluid intelligence (β=-.047, p=.452, 95%CI:-.170-.076), or crystallized

intelligence (β=.053, p=.416, 95%CI:-.076-.183). In addition, the lifetime stress x age interaction was not significant for memory (β=.056, p=.570, 95%CI:-.139-.251), fluid intelligence (β=.069, p=.317, 95%CI:-.067-.205), or crystallized intelligence (β=.070, p=.474, 95%CI:-.124-.264).

5.2 Post-hoc Analyses: Gender

5.2.1 Rationale

Past studies have reported larger cortisol awakening response in middle-aged and older adult women compared to men (Kunz-Ebrecht et al., 2004; Pruessner et al., 1997; Wright & Steptoe, 2005). Furthermore, a meta-analysis study revealed a larger cortisol response to pharmaceutical or psychosocial challenge in elderly women compared to men, suggesting a possibility of less efficient inhibition of cortisol response in elderly women (Otte et al., 2005). Also, a past study has demonstrated a greater tendency for women to worry more than men, suggesting a possible gender difference in stress perception (McCann, Stewin, & Short, 1991). Therefore, the current study examined whether gender moderates the association between plasma cortisol/lifetime stress and brain structure/cognition.

5.2.2 Methods

Statistical Analysis

The same statistical methods were used as in post-hoc analyses with age.

5.2.3 Results

Brain Structure

The plasma cortisol x gender interaction was not significant for hippocampal volume (β=-.063, p=.470, 95%CI:-.234-.108), amygdala volume (β=-.125, p=.163, 95%CI:-.301-.051), medial PFC thickness (β=.116, p=.240, 95%CI:-.078-.310), or primary visual cortical thickness

(β=-.014, p=.885, 95%CI:-.206-.178). In addition, the plasma cortisol x gender interaction was not significant for the hippocampal head (β=-.021, p=.807, 95%CI:-.189-.147), hippocampal body (β=-.090, p=.323, 95%CI:-.269-.089), or hippocampal tail (β=-.074, p=.475, 95%CI:-.279-.131) volumes. Furthermore, the lifetime stress x gender interaction was not significant for hippocampal volume (β=.082, p=.508, 95%CI:-.164-.329), amygdala volume (β=-.086, p=.509, 95%CI:-.342-.171), medial PFC thickness (β=-.025, p=.849, 95%CI:-.285-.235), or primary visual cortical thickness (β=.013, p=.927, 95%CI:-.264-.290). Also, the lifetime stress x gender interaction was not significant for the hippocampal head (β=-.003, p=.984, 95%CI:-.259-.254) or hippocampal tail (β=.022, p=.880, 95%CI:-.263-.306) volumes. However, there was a significant lifetime stress × gender interaction on hippocampal body volume (β=.266, p=.045, 95%CI:.006-.525). Specifically, a non-significant, positive trend was observed between lifetime stress and hippocampal body volume in male participants (β=.422, p=.061, 95%CI:-.020-.865) whereas this association was not significant in female participants (β=-.023, p=.867, 95%CI:-.296-.250).

Cognition

The plasma cortisol x gender interaction was not significant for memory (β=-.054, p=.532, 95%CI:-.222-.115), fluid intelligence (β=-.070, p=.378, 95%CI:-.225-.086), or crystallized intelligence (β=-.029, p=.726, 95%CI:-.190-.133). In addition, the lifetime stress x gender interaction was not significant for memory (β=.137, p=.247, 95%CI:-.097-.372), fluid intelligence (β=.097, p=.244, 95%CI:-.067-.261), or crystallized intelligence (β=.072, p=.539, 95%CI:-.159-.302).

5.3 Post-hoc Analyses: Early Life vs. Late Life Stress

5.3.1 Rationale

There is a conceptualization that early adversities may have differential effects on brain structure and cognition compared to negative events in adulthood, possibly due to heightened susceptibility to environmental influences during the developmental period (Tottenham & Sheridan, 2010). Indeed, Gerritsen et al., (2015) found the associations between stress and amygdala to be going in an opposite direction depending on whether the stress had occurred early or late in life. Therefore, the current study examined whether there were significant differences among groups of individuals with or without stress at two different stages in life.

5.3.2 Methods

For the lifetime stress measures, 15 out of 31 questions of the LSC-R had information as to whether the event occurred before or after age 18. Participants were divided into three groups: (1) a no-stress group in which individuals experienced zero adverse event either before or after age 18 (Brain: n=31; Cognition: n=31); (2) an early life stress group in which individuals experienced one or more adverse events before age 18 (Brain: n=10; Cognition: n=10); (3) a late life stress group in which individuals experienced one or more adverse events after age 18 (Brain: n=26; Cognition: n=28). Individuals who experienced both early and late life stress were excluded for the post-hoc analyses because the goal was to differentiate the effects of early verses late life stress (Brain: n=22; Cognition: n=23). Analysis of covariance (ANCOVA) was performed, with stress group as a between-subject factor.

5.3.3 Results

Brain Structure

There was a non-significant trend for an effect of stress group for hippocampal volume ($F(2,59)=2.644$, $p=.079$). The no-stress group had significantly smaller hippocampal volumes than the early life stress group ($t=-2.285$, $p=.026$). There were no significant differences in

hippocampal volume between the no-stress and late life stress group (t=-.971, p=.335), or between the early life and late life stress groups (t=1.528, p=.132). There were no significant effects of stress group for either amygdala volume (F(2,59)=.678, p=.511) or medial PFC thickness (F(2,59)=.423, p=.657). However, there was a non-significant trend for an effect for primary visual cortical thickness (F(2,59)=2.944, p=.060). The late life stress group had significantly thicker primary visual cortex compared to both the early life stress (t=-2.048, p=.047) and the no stress (t=-2.033, p=.047) groups. There were no significant differences in primary visual cortical thickness between the no-stress and early life stress group (t=.610, p=.542).

Cognition

There was a significant effect of stress group for memory (F(2,62)=4.796, p=.012). The no-stress group showed significantly lower memory performance than the late life stress group (t=-3.065, p=.003). There was not a significant difference in memory between the no-stress and early life stress groups (t=-1.390, p=.170), or between the early life and late life stress groups (t=-.789, p=.433). In addition, there was a non-significant trend for an effect of stress group for fluid intelligence (F(2,62)=2.540, p=.087). The no-stress group had significantly lower fluid intelligence than the early life stress group (t=-2.240, p=.029). There were no significant differences in fluid intelligence between the no-stress and late life stress group (t=-0.960, p=.339), or between the early life and late life stress groups (t=1.511, p=.135). Lastly, there were no significant effects of stress group for crystallized intelligence (F(2,62)=.802, p=.453).

5.4 Post-hoc Analyses: Correlation Between Behavioral Stress and Cortisol

5.4.1 Rationale

It is difficult to distinguish whether the results from plasma cortisol are comparable to the results from lifetime stress since the current results were mostly null. Despite predominant conceptualization that higher stress is linked to greater elevation of cortisol output, a meta-analysis by Miller et al., (2007) suggested that the relationship between chronic stress and HPA axis activity is likely to depend on multiple factors, such as time interval between stress and cortisol measures and the time of the day in which the cortisol measures are taken. Therefore, there is a possibility that the findings with behavioral stress may be different from the findings with cortisol. Unfortunately, there is a dearth of studies that examined both behavioral stress and cortisol in relation to brain structure or cognition within the same sample. The current study examined the correlations among various behavioral stress (i.e., lifetime and recent stress) and cortisol measures (i.e., plasma and hair cortisol) as a post-hoc analysis, which may provide a better insight into the current results.

5.4.2 Methods

A subset of 72 individuals had both lifetime stress and plasma cortisol measures. The mean time interval between the two measures was 7.4 years. In addition, 32 individuals had lifetime and recent (i.e., 3-months and 1-year) stress measures collected on the same day. Among these 32 individuals, 27 of them also had hair cortisol measures collected on the same day. There were only 8 individuals with both plasma and hair cortisol measures, so the correlation between the two cortisol measures was not examined. Similarly, only 8 individuals had both plasma cortisol and recent stress measures, so the correlation between these two measures was also not examined. Zero-order correlations were examined to determine the associations among behavioral stress and cortisol measures.

5.4.3 Results

There was a non-significant trend for a negative association between lifetime stress and morning plasma cortisol measures (r=-.202, p=.089). In addition, hair cortisol (n=27) was significantly and positively correlated with lifetime stress (r=.472, p=.013), but it was not significantly correlated with either 3-months (r=.004, p=.986) or 1-year (r=.204, p=.306) reports of stress. Moreover, lifetime stress (n=32) was significantly correlated with 3-month (r=.371, p=.036) and 1-year (r=.446, p=.011) reports of stress.

5.5 Post-hoc Analyses: Discussion

There was not a significant moderating influence of age for any of the brain structures or cognitive domains. However, the current sample did not include any young adults. Thus, a significant moderating effect of age may be observed with the inclusion of younger samples. Furthermore, there were no significant moderating effects of gender on any of the brain structures or cognitive domains, except for the hippocampal body in the lifetime-MRI sample. Unlike previous studies that showed larger cortisol awakening response in women compared to men (Kunz-Ebrecht et al., 2004; Wright & Steptoe, 2005), the current study did not observe significant difference in morning plasma cortisol between men and women (t=-1.344, p=.181). It is possible that the gender effect may not be as pronounced with morning cortisol as with awakening cortisol, thereby contributing to non-significant findings. Also, the current study did not observe significant difference in lifetime stress between men and women (t=.127, p=.899). It is unclear as to why gender moderated the association between lifetime stress and hippocampal body volume. However, this significant interaction was not consistently found in other brain regions. Overall, even though neither age nor gender moderated the relationship, other lifestyle

or health factors could still influence the relationship between stress/cortisol and brain structure/cognition.

In terms of early life versus late life stress, one pattern that was generally more consistently observed in the current sample was that the no-stress group showed a poorer outcome, including smaller hippocampal volume and lower memory and fluid intelligence performance. The relationship between stress and outcome health variables, including brain and cognition, may not be linear but U-shaped, and mild stress may actually be beneficial (McEwen et al., 2015). In Liu's review (2015), the author brings up the idea that early moderate stress may lead to greater resilience to future stressors, possibly because individuals can attain the skills and experience that they would need to handle future adversities. Although most studies examining moderate stress (as opposed to absent versus severe stress) were done in non-human infants (see review Liu, 2015), the author also mentions the possibility that moderate stress experienced in adulthood may also "inoculate" individuals from future stress. However, a different pattern was observed for primary visual cortex in that the late life stress group displayed significantly greater thickness compared to the other two groups. The pattern is consistent in the sense that the no-stress group displayed significantly thinner primary visual cortex compared to late life stress group. However, it is unclear as to why there is a significant difference between early versus life stress groups for the primary visual cortex while other regions did not show the same pattern. In general, there is a lack of studies examining the relationship between stress and primary visual cortex, especially in adulthood. Thus, it is difficult to predict how late life stress might bring about positive effects on primary visual cortex at this point.

In general, the current results in terms of associations between behavioral stress and cortisol seem to be in agreement with the idea that cumulative stress may be associated with a

disrupted diurnal cortisol rhythm (Miller et al., 2007), as there was a non-significant trend for higher lifetime stress to be associated with lower morning plasma cortisol despite an approximately 7 years of time interval. However, it is surprising that hair cortisol measures were not significantly correlated with relatively more recent stress measures. According to a review by Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, (2013), 7 out of 13 studies also reported no significant associations between recent stress measures and hair cortisol. Also, a study by Steudte et al., (2011) found a significant positive association between lifetime traumas and hair cortisol in a sample of young adults with or without PTSD (N=27). This is consistent with the current finding of a positive association between lifetime stress and hair cortisol. Based on the evidence thus far, even though hair cortisol represents cortisol production in the last few months, the cortisol production itself may not only rely on stressful events that had happened in the last few months but also may reflect more of an integrative profile of the HPA axis, which incorporates stress effects that had occurred throughout the lifespan. Overall, the relationship between behavioral stress and the HPA axis dysregulation cannot be simply characterized as greater behavioral stress relates to greater cortisol output, but instead includes disruption of the diurnal cortisol rhythm. More work is needed to tease apart how each HPA axis marker relates to behavioral stress longitudinally, and observe how such changes are linked to brain structure and cognition.

Chapter 6: General Discussion and Conclusions

6.1 General Discussion

The goal of the current study was to assess for differential associations of stress and cortisol with brain structures with high versus low expression of receptors for glucocorticoid, and cognitive functions that are reliant upon these regions in cognitively normal older adults. Also, the current study investigated the moderating role of stress-system genes on these associations.

The predominant stress model in the current literature is that cumulative stress and HPA axis dysregulation will bring about detrimental effects on brain structure and cognition. However, the results from current study generally failed to support this predominant conceptualization. Instead, the current study observed largely null effects with the exception of few significant associations and non-significant trends. Overall, there was no consistent evidence that cumulative stress and cortisol are differentially associated with brain structures and cognitive functions that are related to high versus low MR/GR expression.

The current study examined a few moderators, including genetic scores, age and gender, to determine whether the presence of moderators influenced the associations of stress and cortisol with brain structure and cognition. However, examination of genetic effects failed to clarify the associations, with only one single significant interactive effect with plasma cortisol on fluid intelligence. A much larger sample size may be required to reveal any significant moderating influence of genetics. Furthermore, neither age nor gender clarified the associations.

Yet, a possibility of other factors (e.g., coping strategies) playing a moderating role in these associations remains open for investigation.

On the other hand, the current study suggested some relevant factors that may be fruitful to consider in future studies. For example, examination of hippocampal subdivisions provided some supporting evidence for the possibility of differential cortisol effects along the longitudinal axis of the hippocampus. However, the observed positive association between cortisol and the hippocampal head is not in alignment with the general view of higher cortisol leading to negative outcomes. Yet, it is consistent with the newer conceptualization that the HPA axis dysregulation may be characterized by blunted cortisol awakening response (CAR). Also, the current study found a trend for higher lifetime stress to be associated with lower morning cortisol, again in agreement with the newer conceptualization of blunted CAR as marker of HPA axis dysregulation.

Furthermore, as a post-hoc analysis, the current study examined the influence of stress at different time points in the lifespan (no stress vs. early stress vs. late stress). The results in general suggested the importance of identifying the timing of stress and the possibility of mild stress having beneficial effects compared to having no stress. In addition, the exploratory analyses with hair cortisol suggested the relevance of examining associations of hair cortisol with brain structure in larger samples since the effect sizes were moderate in some cases.

While I offered some speculation regarding the current findings in prior discussion sections, the overall pattern is not consistent and may even reflect spurious findings. In fact, when the p-values were corrected for multiple comparisons using a false discovery rate correction, none of the associations remained significant. Based on the current null findings, along with considerable amount of null effects in the literature, it is possible that stress effects

may not be as robust unless experienced in a substantial magnitude and/or duration. Relatively consistent findings of stress and corticosterone effects in non-human animals, which have been the foundation of the conceptualization of stress effects in aging, may be less applicable when examining healthy human participants. Specifically, these animal studies might have utilized stress procedures that generate abnormally high level of stress intensity or elevation of stress hormones, but such magnitude may not translate to the level of stress that healthy people typically experience in their daily lives. However, the conceptualization derived from animal work may still be useful in describing mechanisms for patients who are suffering from PTSD or Cushing syndrome.

In conclusion, the current study showed no consistent patterns of either stress or cortisol effects on brain structure and cognition in cognitively normal older adults. However, there may still be more subtle and complex effects of stress and cortisol perturbations that are not at the levels related to disease states. The current study provides hints of relevant directions and interpretations for smaller stress effects from which future studies may build upon. Lastly, addressing limitations in current study as suggested in the following paragraphs may reveal more complex role of stress in the brain and cognition.

6.2 Limitations and Future Studies

One of the strengths of the current study is that it explored other brain regions beyond the hippocampus whereas the current literature mainly focuses on the hippocampus, particularly in relation to cortisol. Also, the study expanded previous findings on the total hippocampus by examining its subdivisions along the longitudinal axis. Furthermore, to my knowledge, the current study is the first study to investigate the effects of stress-system genes in relation to brain

structure and cognition in cognitively normal older adults. Examining the sum of genetic variance provides greater statistical power compared to when examined each genotype separately (e.g., Nikolova, Ferrell, Manuck, & Hariri, 2011; Pagliaccio et al., 2014). Lastly, the current study was the first study to explore the relationship between hair cortisol and brain structure.

However, the current study is not without limitations. In terms of lifetime stress measures, the current sample experienced mostly a relatively low number of stressful events, hindering the examination of a fuller spectrum of stress. Also, no specific data were available as to when the stressful events occurred which prevented the current study from investigating specific stages across the lifespan. In addition, no questionnaire was administered to specifically measure childhood adversity, which may have clarified for the current study as to whether or not stress during a sensitive developmental period influenced the results. Furthermore, behavioral stress was measured retrospectively, thus there is a possibility of recall bias. For example, individuals may not recall negative events that had occurred earlier in their lifetime. As for the genotype data, the current study focused on genes that are related to MR and GR. However, future studies could also incorporate genes that are related to other parts of the stress system, such as corticotrophin-releasing hormone, or even genes that are associated with stress-related disorders, to examine their relationship with brain structure and cognition.

Moreover, there are some limitations with the cortisol measures as well. First, the dataset did not have information on participants' waking time. Since the cortisol circadian rhythm tends to shift earlier as people age (Veldhuis et al., 2013), there is a possibility that a significant time had passed between the time when the cortisol had reached its peak after awakening and the time when the morning cortisol was measured at 8 AM. In addition, only a single morning cortisol time point was measured rather than multiple measures throughout the day. Thus, the current

study cannot examine the relationships with diurnal rhythm or cortisol at other times of the day. Furthermore, examining the ratio of two different types of cortisol indicators may be a better predictor of brain structure and cognition. For example, Pulopulos et al., (2014) demonstrated that the saliva/hair cortisol ratio associates more strongly with working memory and verbal memory than when these cortisol measures were examined separately. However, the current study did not have sufficient sample size for the hair cortisol measure, and the time interval between plasma cortisol measures and hair cortisol measures were too far apart to create a reliable cortisol ratio measure. Future studies could obtain multiple cortisol measures throughout the day and obtain multiple types of circulating cortisol levels (e.g., in saliva and hair) and determine how such measures relate to brain structure and cognition.

In addition, although cortisol is the mostly commonly used physiological marker for assessing HPA axis activity, there are other biomarkers of stress available for research. For example, HPA axis involves release of corticotropin-releasing factor (CRF) and adrenocorticotropin (ACTH) hormones, in addition to a release of cortisol (Chrousos & Gold, 1992). Also, salivary alpha-amylase (sAA) is a commonly used indicator of sympathetic nervous system activity in acute stress studies (Rohleder & Nater, 2009). Thus, obtaining these physiological measures, in addition to cortisol measures, may provide a more robust estimate of the overall function of the stress system, and future studies may investigate how such measures relate to brain structure and cognitive function.

Another limitation of the current study was that only two tasks were used to assess fluid intelligence (i.e., Trailmaking A and B) in order to maximize the sample size available from the ADRC. However, using multiple tests across sub-domains would allow one to assess different aspects of fluid intelligence, which would be important especially since some studies found

different results within the same sample depending on which cognitive domains were assessed (Majer et al., 2010; Stein et al., 2002). Also, using multiple tests would bring about more robust estimates of the fluid intelligence domain. Therefore, assessing multiple measures of fluid intelligence and examining how these measures relate to brain structure and cognition would be an important future direction.

Furthermore, individuals with depression were not excluded in order to increase sample size. Depression has been characterized by dysregulation of the HPA axis (Pfohl, Sherman, Schlechte, & Winokur, 1985) and lower hippocampal volume (e.g., Bremner et al., 2000; Campbell, Marriott, Nahmias, & MacQueen, 2004). Thus, the relationship between stress and brain structure may have been confounded by depression, although the current study statistically controlled for the presence/history of depression. Also, the current study attempted to increase the power by creating a composite score for genotype; however, this method may still not have been sufficient to detect small effect sizes. In addition, the sample sizes for exploratory analyses were underpowered. Overall, future studies would benefit greatly by using larger sample sizes, especially in the research related to hair cortisol since this area is still at an early stage.

Moreover, the current study did not explore the moderating role of appraisal and coping. A past study demonstrated that stress coping enhanced hippocampal neurogenesis in non-human primates (Lyons et al., 2010). It is possible that one reason for the null findings may be due to difference in individuals' coping strategies. Although investigating the role of coping was beyond the scope of this study, incorporating the moderating role of coping may elucidate the relationship between stress/cortisol and brain structure/cognition.

Furthermore, individual differences in personality may also moderate the associations of stress and cortisol with brain structure and cognition. For example, a past study found

neuroticism to be negatively associated with episodic memory (Wilson et al., 2003). Another study found negative associations of age with cortisol response at awakening and hippocampal volume only in individuals with low self-esteem (Pruessner et al., 2005). Therefore, future studies could investigate whether personality plays any moderating role for the effects of stress and cortisol on brain structure and cognition.

Lastly, the current study was designed as a cross-sectional study. However, one major problem with stress research is that there is a dearth of longitudinal studies. Various conceptual ideas, including neuronal changes from hypertrophy to atrophy overtime and differential stress effects during sensitive (e.g., developmental phase) vs. less sensitive periods (e.g., adulthood), will likely be elucidated with longitudinal studies. Conducting longitudinal studies in stress research is difficult since stress events occur unexpectedly, and it is difficult to locate these individuals and follow them for a long time. However, longitudinal study is essential in order to go beyond the prevailing conceptualization that stress leads to negative outcomes to truly understand the complex relationship among stress exposure, HPA axis functioning, brain structure and cognition.

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