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Influence of Stress and Cytokinic Profiles on Cognitive Performance in Older Adults

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by

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LIST OF ACRONYMS

ACTH	adrenocorticotropic hormone
AD	Alzheimer's disease
APC	antigen-presenting cells
AVLT	Auditory Verbal Learning Test
CES-D	Centers for Epidemiological Studies Depression-Scale
COWA	Controlled Oral Word Association
CRH	corticotropin-releasing hormone
GC	glucocorticoid
GR	glucocorticoid receptors
HIV	human immunodeficiency virus
HPA	hypothalamic-pituitary-adrenal
IFN	Interferon
IL	Interleukin
IRB	Institutional Review Board
LTP	long-term potentiation
M	mean
MMSE	Mini-Mental State Examination
MR	mineralocorticoid receptors
PSS	Perceived Stress Scale
SD	standard deviation
SE	standard error
SST	serum-separating tubes
Th1	type 1 T helper cells

Th2	type 2 T helper cells
TMT-A	Trail Making Test Form A
TMT-B	Trail Making Test Form B
TNF	Tumor Necrosis Factor
USF	University of South Florida
WAIS	Wechsler Adult Intelligence Scale
WCST	Wisconsin Card Sorting Test

ABSTRACT

With aging, changes in the immune system, makes cognitive performance, and the prevalence of stressors can lead to poorer overall functioning. Within the immune system, a balance should exist between cytokines regulating Th1 and Th2 immune responses; however, age-related declines in the endocrine and immune systems can disrupt this equilibrium. Several studies report higher levels of Th1 associated cytokines in inflammatory conditions of the brain, whereas fewer studies remark on Th2 associated cytokines and cognitive functioning. Declining cognitive abilities are a common concern that accompanies advancing age and some research has suggested the prevalence and impact of stressors lead to poorer performance.

Participants included 92 older adults (M = 74.05 years) who completed tests of cognitive performance and stress measures, and 41 persons who had valid data on Th1 and Th2 cytokines. The results indicated that increasing age is significantly associated with several cognitive domains including executive functioning, speed of processing, and episodic memory. As hypothesized, moderation analysis revealed the relationship between Th1 and Th2 cytokinic profiles, as denoted by the IFN-γ/IL-4 ratio, is a significant moderator between stress and cognitive performance. Specifically, immune profiles skewed towards Th1 predict a significant amount of variation between high stress scores and low cognitive performance, whereas this was

not found for immune profiles skewed towards Th2. Overall, the current study suggests that a pro-inflammatory state permits stress to exert a negative influence on cognitive performance.

CHAPTER ONE

INTRODUCTION

Background

Evidence suggests a complex network of stress hormones and immune-related signaling proteins are linked to age-related changes in cognitive functioning. A better understanding of this aging network will influence preventative efforts to delay cognitive impairments and help researchers distinguish factors associated with normal aging versus pathological age-related cognitive disorders.

In normal aging, age-related differences in cognitive functioning present across a variety of measures, but significant inter-individual differences in performance also exist. One outcome that has garnered considerable interest is episodic memory, or memory for information with temporal and contextual referents. The neural basis for this cognitive ability appears to be related to intact frontal and temporal lobe functioning, two brain structures that have demonstrated age-related variability. Executive functioning measures have also shown to be age sensitive and rely upon the integrity of frontal lobe functioning. The hippocampus, important for successful encoding, storing, and retrieval of memories, also shows age-related changes.

In addition to being important for cognitive abilities, the hippocampus is involved in modifying hypothalamic-pituitary-adrenal (HPA) axis activity. The HPA axis is the central mechanism governing the body's response to physical and psychological stressors. Stressors evoke a multitude of hormonal cascades, and under normal conditions the body is able to respond to these insults in a protective manner to ensure survival. However, advancing age and excessive stressful events may alter the brain structures involved in the HPA axis.

Alterations to the HPA axis can result in detrimental levels of stress hormones. Stress hormones include releasing and stimulating factors, secreted from the hypothalamus and pituitary gland, respectively, and cortisol, an important glucocorticoid (GC), released from the adrenal cortex. The stress hormones work in a feedback loop; in particular, cortisol will bind to receptors in the hippocampus and hypothalamus in efforts to quell the stress response. Excessive release of cortisol can damage hippocampal and hypothalamus neurons and prevent the HPA axis from correctly responding to future stressful events. Moreover, as a result of this maladaptive stress response, it is likely the damaged hippocampal neurons will negatively influence memory processing.

Several studies have reported relationships between higher stress levels and lower memory performance, yet not all studies show this relationship. Thus further variables need to be explored that explain the conflicting outcomes among studies addressing stress and memory performance. As research indicates, GCs can also alter signaling proteins

released by immune cells. Therefore, in this study, we explored the age-related changes in the endocrine and immune systems and predicted the combination of these two systems would explain variance seen in memory processing among the older population.

Immunosenescence, the decline of the aging immune system, leads to several changes in immune system functioning. One of the main components of the immune system is the thymus, which is important for the maturation of adaptive immune cells referred to as T cells. As part of the normal aging process, the thymus becomes involuted and consequently, T cell populations are reduced. This reduction in T cells leads to an imbalance in immune cell populations, production of signaling proteins, and alters the equilibrium of type 1 T helper cells (Th1) and type 2 T helper cells (Th2). Th1 and Th2 cells are defined by the activation, secretion, and resulting actions of signaling proteins called cytokines. For instance, Th1 cells, involved in cell-mediated immune responses, typically produce Interferon-gamma (IFN-γ), Interleukin (IL)-2, IL-12, and Tumor Necrosis Factor-alpha (TNF-α) to combat viral and bacteria antigens that invade host cells. Alternatively, Th2 cells release IL-4, IL-5, IL-9, IL-10, and IL-13 to confer resistance to parasitic organisms and allergens by activating the humoral antibody response. Th1 and Th2 responses should work in unison, with one response attempting to suppress the other to reach a relative equilibrium. Exceptions occur, as seen in early life development and disease states that can arise over the lifespan.

Research has shown early life development begins with infants exhibiting a predominant Th2 response. The Th2 response is necessary to manage the multitude of pathogen exposures during the first two years of life as well as to sensitize the host to parasitic and allergen exposures. Around age two, the immune system should shift to maintain a predominant Th1 response, important for various viral and bacterial invasions. At this point, the two systems will establish equilibrium between the signaling proteins to control inflammatory responses, with Th1 responses reflecting pro-inflammatory properties and Th2 associated cytokines suppressing inflammatory reactions (anti-inflammatory). However, disruptions in this purported equilibrium can result in a more active Th2 response, as seen in individuals with allergic diseases and human immunodeficiency virus (HIV). Moreover, literature suggests that chronic hormonal events due to stressful experiences may alter cytokinic profiles and similarly result in a predominant Th2 response in late-life. As the main premise of the current study, we investigated the relationship of stressful events and the Th1/Th2 balance. We anticipated that alterations in the Th1/Th2 balance due to dysregulated stress responses may explain differing outcomes seen in the late-life stress and memory processing literature.

Specific research questions and hypotheses

The current study examines the impact of stress and immune system functioning on cognitive performance in older adults. The specific research questions under investigation include:

Research question 1: What is the relationship between age and cognitive performance?

Research question 2: To what extent are age and cytokinic profiles related in older adults?

Research question 3: Is there a significant relationship between stress and cytokinic profiles?

Research question 4: Are there significant main effects and interactions between stress and predominant Th1/Th2 responses on cognition?

We hypothesized the current study would reveal variations in cognitive performance among cognitively healthy older adults. Additionally, we expected results would indicate age and stress are associated with cytokinic profiles skewed towards a Th2 response. This research is novel by investigating the combined effects of stress and cytokinic profiles on cognition. Several studies indicate negative effects of Th1 related cytokines, such as IFN-γ and TNF-α, on cognitive abilities, but far fewer studies have been published regarding Th2 related cytokines and cognition. Hence, we predicted a significant interaction between more stressful events and a predominant Th1 response would be associated with lower cognitive functioning.

Chapter summary

The preceding introductory chapter explained the major tenets explored in the current study including cognitive functioning, stressors, and

immune system functioning in older adults. In the following literature review, more details are provided on each of these topics.

First, cognitive variations in cognitively healthy older adults are presented. Second, we focus on the endocrine system as it relates to age-related and stress-related changes that occur in the HPA axis including conflicting studies addressing stress and cognition. This section includes theoretical framework regarding the body's efforts to adapt to stressful events via allostatic mechanisms. As a concept, allostasis is also important for understanding the dynamic relationships that occur within the immune system in regards to the balance needed in the cytokine environment. Thus, the next section introduces the interplay between immune cells and cytokines involved in the adaptive immune response followed by changes that occur in the immune system with normal aging. The immune section also reviews studies on cognitive performance and cytokines levels, and concludes with research explaining the consequences of aberrant stress-related hormones on cytokine allostasis. Lastly, the main objectives and implications of this research are considered.

CHAPTER TWO

REVIEW OF THE LITERATURE

In this chapter, literature relevant to the main questions posed in the dissertation is reviewed including cognitive, endocrine, and immune research in the older adult population.

Cognitive processing

A common concern among older adults is decline in cognitive performance and the existing literature suggests that these concerns are valid (see Craik & Salthouse, 2007; Hofer & Alwin, 2008 for reviews). Tests of episodic memory, executive functioning, working memory, and processing speed, show significant declines with advancing age, whereas measures of vocabulary and comprehension as measures of semantic memory show greater stability with normal aging. Thus, a key feature of the current research is the cognitive outcome examined in relation to the presence and magnitude of age-related differences or changes in functioning. In this section, we reviewed evidence for age differences in the cognitive outcomes that are the focus of the current study, namely episodic memory, semantic memory, executive functioning, working memory, and processing speed.

Episodic memory. Episodic memory is the recollection of personal history, particularly memorable events such as the birth of a new child or a family holiday. Episodic memory is typically tested by the recall or

recognition of lists of works, passages, or non-verbal figures (Backman, Small, & Wahlin, 2001). The majority of research examining episodic memory in old age has utilized cross-sectional comparisons between younger adults and older adults. Cross-sectional data suggested declines in episodic memory performance occur quite early in development. For example, Salthouse (2009) reported peak word recall performance occurred among individuals 25 to 30 years of age, whereas persons who were 55 to 60 years of age performed almost one standard deviation (SD) lower. However, the results of longitudinal studies suggest declines occur much later in life. Recently, Small and colleagues (2011) found very slight changes in episodic memory performance occurring between the ages of 55 and 75 and it was only after age 75 that precipitous changes were present.

Semantic memory. Semantic memory is memory for general knowledge, facts, and vocabulary learned during educational endeavors and over time (e.g., capitols of states; Backman et al., 2001). Semantic memory is considered fairly stable across the lifespan; however, evidence of a slight decline in verbal fluency tasks is shown after age 65 (Rönnlund, Nyberg, Bäckman, & Nilsson, 2005; Spaniol, Madden, & Voss, 2006). Recent longitudinal work demonstrated cognitively healthy older adults declined significantly less than older adults with preclinical Alzheimer's disease (AD) on two verbal fluency measures, Category Fluency and Letter Fluency. The authors also looked at differences between the rates of decline on these verbal fluency measures, and reported older adults experienced greater

declines on the Category Fluency test than the Letter Fluency tests (Clark et al., 2009).

Executive functioning. Executive functioning refers to complex thought and constitutes higher order mental processes that allow the human species to self-monitor, self-regulate, and self-evaluate behaviors (Luszcz & Lane, 2008). Tests used to measure executive functioning include the Wisconsin Card Sorting Test (WCST; Heaton, Chelune, Talley, Kay, & Curtiss, 1993) to measure task switching, perseveration, and reaction to feedback and Trail Making Tests (TMT; Reitan, 1958) to measure task switching, use of memory to make future decisions, and speed of processing. Research on executive functioning includes an analysis from 166 participants ranging in age from 20 to 89 which revealed older adults performed significantly worse on WCST than younger adults (Bugg, Zook, Delosh, Davalos, & Davis, 2006). A cross-sectional study by Salthouse, Atkinson, and Berish (2003) consisting of 261 participants grouped by age (18-39, 40-59, and 60-84) found differences by age group in WCST with the youngest age group having 6 more correct responses than the oldest age group and the oldest age group having 7.9 more perseverative errors than the youngest group, indicating a decline in task switching abilities. Additionally, cognitive tests scores (in seconds) on Trail Making Test A (TMT-A) and Trail Making Test B (TMT-B) from the Women's Health and Aging Study II revealed significant functional declines in test scores over a nine-year period. For TMT-A, the mean score at exam 6 was 14.2 seconds higher than exam 1 and for TMT-B, the mean score at exam 6 was 68.3 seconds higher than exam 1 (Carlson, Xue, Zhou,

& Fried, 2009). Additionally, a TMT-A and TMT-B normative data study of more than 900 persons between the ages of 18 and 89 found aging and education are significant predictors of performance after age 54 (Tombaugh, 2004).

Working memory. Passive short-term memory tasks require maintaining information for a limited amount of time (e.g. phone number) reveal little decline with age. However, more difficulties are seen when older adults must attend to and manipulate certain stimuli as required by working memory tasks (Backman, Small, Wahlin, & Larsson, 2000; Bopp & Verhaeghen, 2005). A meta-analysis of 123 studies comparing verbal memory abilities of younger (aged 16 to 29) and older adults (aged 60 to 77) found significantly larger age differences on working memory tests than short-term memory tests (Bopp & Verhaeghen, 2005). Subtests of the Wechsler Adult Intelligence Scale (WAIS)-III (Wechsler, 1987) are frequently used to measure short-term memory (Digit Span Forwards) and working memory (Digit Span Backwards), however, these two scores combined show the least amount of decline with aging as compared to other WAIS-III subtests such as measures of processing speed (Ardila, 2007).

Processing speed. Age-related changes are often seen on timed cognitive measures that require an individual to focus attention, discriminate between items, and/or to make quick judgments. Tests that provide an assessment of processing speed, such as Digit Symbol, a WAIS-III subtest, show a large amount of decline in scores in late-life (Ardila, 2007). Processing speed is also an influencing factor in other cognitive abilities such

as executive functioning and episodic memory tests. For instance, authors found speed of processing explains 70% of the variance in episodic memory tasks in a meta-analysis (Verhaeghen & Salthouse, 1997).

Summary. Although the work described above generally points to age-related losses in many cognitive ability domains, the magnitude of these differences varies considerably depending upon the cognitive ability under investigation. Moreover, more recent research has focused on individual differences in cognitive performance in order to understand why some persons maintain cognitive abilities longer than others (R. Wilson et al., 2002). Interest in slowing or reversing cognitive decline is growing and research suggests several factors such as physical, dietary, and psychological factors may be related to the cognitive health of older adults (Small, Hughes, Hultsch, & Dixon, 2007; Small, Rawson, Eisel, & McEvoy, 2012). In the next section, we focus on one particular individual difference variable, stress, and describe its potential to modify age-related differences in cognitive performance.

Endocrine system

The hypothalamus, pituitary gland, adrenal cortex, thyroid gland, ovaries and testes are the major components of the endocrine system. The hypothalamus, often called the relay station, receives incoming messages about the environment and based on incoming stimuli; the hypothalamus is able to control hormonal secretions such as testosterone, estrogen, thyroxine and, relevant to the current study, corticosteroid hormones such as cortisol (Neave, 2008). Cortisol secretion occurs in response to the activation of the

sympathetic nervous system during physical and psychological stress inducing events. The activation of the sympathetic nervous system is an adaptive response necessary to direct energy towards organ systems to enhance the body's chances of survival. The hypothalamus orchestrates the ensuing events by activating the HPA axis (see Figure 1; Glaser & Kiecolt-Glaser, 2005; Herman & Cullinan, 1997; Johnson, Kamilaris, Chrousos, & Gold, 1992; McEwen, 2007).

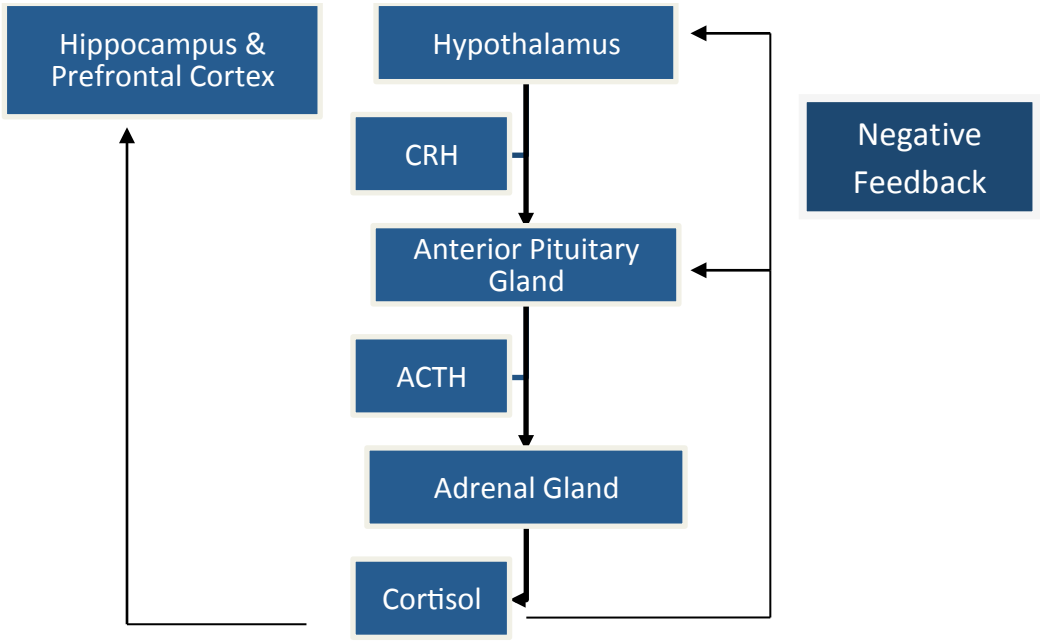

FIGURE 1. Hypothalamic-pituitary-adrenal axis structures and hormones.

Activation of the HPA axis in response to a stressor commences with the hypothalamus releasing corticotropin-releasing hormone (CRH). CRH release stimulates neuroendocrine neurons in the anterior pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH results in cortisol secretion from the adrenal glands (Herman & Cullinan, 1997; Herman et al., 2003; Lupien, McEwen, Gunnar, & Heim, 2009; McEwen, 2007). Lastly,

cortisol exerts a negative effect by binding to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) on hypothalamic neurons (Marin et al., 2011; Wolf, 2003). Cortisol is also able to bind to receptors in the prefrontal cortex and hippocampus. Under normal conditions, the combination of the hypothalamus, hippocampus, and prefrontal cortex provide inhibitory influences on the initiation and continued activation of the HPA axis (Herman & Cullinan, 1997; Marin et al., 2011). Termination of the stress response can however be disrupted with age-related and stress-related changes in the endocrine system (Juster, McEwen, & Lupien, 2010; McEwen, 2007).

Age-related changes in stress hormones. Studies investigating the dynamics of the HPA axis and age indicate changes in stress hormones and explore genetic expression of receptors as an underlying mechanism. Typically, cortisol's rhythms, referred to as the diurnal cycle, result in an increase of cortisol 30 minutes after awakening and decline during the day, with small increases seen after meal times (Karatsoreos & McEwen, 2011; O'Donnell, Badrick, Kumari, & Steptoe, 2008).

Compared to younger adults, more irregularities in cortisol's natural rhythms in older adults exist (Chahal & Drake, 2007). For instance, an analysis of 48 adults over the age of 65 indicated 48% of the sample exhibited the typical cycle, whereas inconsistent cycles were evident in 50% of the sample (Ice, Katz-Stein, Himes, & Kane, 2004). As a possible explanation of inconsistent cycles, rodent models have shown age-related variations in the mRNA expression of mineralocorticoid and glucocorticoid

receptors for corticosterone (the cortisol equivalent of man) in the hippocampus and higher levels of ACTH secretion (Dalm et al., 2005). Thus, these changes in cortisol receptors interfere with the feedback cycle and the resulting release of cortisol from the adrenal glands (Marin et al., 2011).

Stress-related changes in stress hormones. Divergent results are seen in studies investigating the timing and type of a stressor in relation to stress hormone measurements. A meta-analysis found significantly higher levels of cortisol in the morning, afternoon/evening, and daily output in persons currently experiencing chronic stress, whereas past stressors were significantly related to lower morning levels and higher afternoon/evening cortisol levels (Miller, Chen, & Zhou, 2007). Additionally, older adults who reference loneliness, sadness, feeling threatened and overwhelmed in daily diaries had significantly higher awakening cortisol levels the next day. However, these same feelings were unrelated to same-day morning cortisol measurements (Adam, Hawkley, Kudielka, & Cacioppo, 2006).

Chronic stressors common in older adults include bereavement and caregiving. Older female adults anticipating the loss of their husband during end-of-life care do not show significant differences in cortisol levels from controls whereas those who lost their husband during the six-month study period had significantly higher cortisol levels than controls (Irwin, Daniels, Risch, Bloom, & Weiner, 1988). Caregivers of patients with AD over the age of 70 had significantly higher levels of plasma ACTH than caregivers under the age of 70; however, no differences in cortisol levels were seen between the two groups (Irwin et al., 1997).

Previous literature has investigated coping styles, social support resources and ability to adapt to stress as possible factors to explain complicated findings between stressors and stress hormones in the older population. A London-based epidemiological study of 542 participants with a median age of 61 looked at the relationship between cortisol levels and coping behaviors. The authors found non-significant relationships between awakening cortisol levels and persons who seek out social support and engage in problem-solving behaviors, however, lower cortisol levels over the day were found to be significant in persons possessing these coping styles (O'Donnell et al., 2008). This finding suggests the normal morning increase in cortisol is less altered by positive behaviors to address stressful experiences than cortisol secretion throughout the day.

In regards to adaptability, McEwen (2003) reports variability in older adult's ability to adjust to everyday stressors due to individual differences experienced over the lifespan. Additionally, McEwen (2003) notes a portion of this change in adaptability can be explained by the brain's ability to respond to stress, such that higher levels of GCs can permit neurotoxic modifications to the HPA axis structures (Lupien et al., 2009; Marin et al., 2011). In human and animal models, higher levels of GCs are associated with smaller hippocampal volume and slower axonal transport in the prefrontal cortex (Lupien et al., 2009). As a consequence, the hippocampus is less able to regulate the HPA axis, resulting in further release of GCs and more damaging effects to the hippocampus. The prefrontal cortex and hippocampus are essential for encoding, storing, and retrieving memories, thus their

vulnerability to neurotoxic effects of GCs, can lead to changes in cognitive functioning (Borcel et al., 2008; Marin et al., 2011; Sandi & Touyarot, 2006; Sapolsky, Krey, & McEwen, 1985). Moreover, changes in adaptability to stressors with advancing age is supported by the Glucocorticoid Cascade Hypothesis (Sapolsky, Krey, & McEwen, 1986). This hypothesis notes elevated levels of GCs can not only perpetuate a dysregulated stress response but also impair cognitive functioning (Bauer, 2005).

In summary, the endocrine system is not resistant to age-related and stress-related changes. Several studies have shown variations in cortisol levels in both human and animal models. Biological and psychological factors explaining the variations seen between the younger and adult populations include changes in cortisol receptors in the brain, coping styles, nature of stressors, and neurotoxic effects of higher levels of GCs on the structures of the HPA axis. Two of the structures involved in regulation of cortisol secretion, the hippocampus and prefrontal cortex, are highly involved in memory processing and it would be expected that stressful events, by promoting an increase in HPA activity, can impact cognition (Bauer, 2005). However, as is explored in the next section, discrepancies exist in past literature examining the relationship between stress and cognition in the older population.

Stress and cognition. The prefrontal cortex and hippocampus are important in terminating a stress response as well as facilitating cognitive abilities. Research with older participants have shown persons with higher cortisol levels have poorer performance on a broad assortment of tests

including executive functioning, language, processing speed, verbal memory and learning, and visual memory (Lee et al., 2007). Older adults with higher cortisol levels also have demonstrated lower episodic memory scores (Wright, Kunz-Ebrecht, Iliffe, Foese, & Steptoe, 2005). A three-year study of cortisol levels and cognitive functioning in a sample of cognitively healthy older adults revealed a steeper decline in delayed paragraph scores in persons with higher cortisol levels whereas persons with lower levels of cortisol showed a slight improvement over time (Li et al., 2006).

However, some studies have not found a relation between stress and cognition. For instance, cortisol levels were not associated with memory impairment as measured by the Mini-Mental State Examination (MMSE), nor associated with a decline in MMSE scores after an average of 1.9 years in older adults aged 55-80 (Kalmijn et al., 1998). A study investigating diurnal patterns of cortisol in older adults found no differences in cognitive tests measuring working memory, declarative memory, and verbal fluency between persons with typical and inconsistent diurnal cycles of cortisol (Fiocco, Wan, Weekes, Pim, & Lupien, 2006). Additionally, a study comparing negative life events and cognition found persons who experienced an injury or illness of a friend in the last 12 months had better scores on tests of episodic memory, speed of processing, and attention (Rosnick, Small, McEvoy, Borenstein, & Mortimer, 2007).

The contradictory results may be due to the design of the study or as previously considered, biological or psychological factors such as timing and type of threat, social resources or changes in adaptability. Another possible

reason for these discrepancies may be an age-related disruption in homeostatic or allostatic mechanisms designed to maintain equilibrium within our bodily systems as theorized by the General Adaptation Syndrome.

The General Adaptation Syndrome, introduced by Hans Seyle, has undergone more contemporary revisions because conventional stressors evoking identical physiological responses are non-existent (Selye, 1946, 1950). Seyle's original explanation of the General Adaptation Syndrome remarked on the body's return to homeostasis after the initial reaction to a stressor; however, the term homeostasis reflects only a certain set of vital bodily mechanisms that fluctuate little from their set points. For instance, set points for homeostatic functions such as body temperature and glucose levels are not presented with normal age-related imbalances because these functions are essential for survival. The term homeostasis thus fails to capture the multitude of events that occur during a stress response. Sterling and Eyer (1988) introduced the term allostasis to represent mechanisms that do not directly sustain life, yet make it possible for other systems that do so. In this context, surrounding events accompanying activation of the HPA axis may also contribute to age and stress-related changes and explain differences seen in elderly cognition.

Previous literature suggests age-related changes in the immune system can influence neuroendocrine coordination (John & Buckingham, 2003). Cytokines, immune-signaling proteins, are an example of a bodily mechanism that needs to maintain an allostatic environment and can affect stress hormone production (McEwen, 2003). Thus, in this study we hope to

further elucidate the intricate nature of the interplay between the neurological, endocrine, and immune system among older adults.

Immune system

The human body has two main lines of defense against invading pathogens: the innate or natural response, and the adaptive or acquired response. The cells of the innate immune system are activated first and defend against pathogens quickly and in a non-specific manner (Medzhitov & Janeway, 1998). Antigen-presenting cells (APCs) of the innate response will engulf invading pathogens and present them to naïve T cells involved in the adaptive immune response (Agrawal, Agrawal, & Gupta, 2007; Medzhitov & Janeway, 1998). This presentation of pathogens can initiate cells of the adaptive immune system to respond in a more deliberate method to a specific pathogen via the expression of receptors that match the pathogen's shape (Segerstrom & Miller, 2004). Depending on the type of immune response needed, the adaptive immune system will elicit a cell-mediated immune response or a humoral immune response. The cell-mediated response is responsible for defending the body against intracellular foreign antigens, whereas the humoral immune response defends against extracellular foreign antigens. Both of these adaptive immune responses rely on a subtype of T cells called helper T cells (Th; Hoebe, Janssen, & Beutler, 2004). After the APCs display a foreign antigen to naïve T cells, immune modulators, termed cytokines, will be released and cause the differentiation of the naïve T cells into either type 1 helper T cells (Th1) or type 2 helper T cells (Th2), involved in cell-mediated and humoral immune responses,

respectively (Dong & Flavell, 2001; Mosman & Coffman, 1989; Segerstrom & Miller, 2004).

Cytokines are signaling proteins that allow communication between immune cells. There are several different types of cytokine messengers, most of them belonging to the interleukin (IL) family. The release of cytokines from one cell influences the cascade of immune-related events to follow (Conti et al., 2008). For instance, within hours of antigen recognition, IL-2 release will encourage proliferation of naïve T cells (Abbas, Lichtman, & Pillai, 2012). Next, and contingent on the type of pathogen, IL-12 or IL-4 will be released by APCs. IL-12 will prompt the naïve T cells to differentiate into Th1 cells to initiate a type 1 cell-mediated response, whereas IL-4 will cause the naïve T cells to differentiate into Th2 cells to initiate a type 2 humoral response (Agrawal, Agrawal, Tay, & Gupta, 2008; Dong & Flavell, 2001; Elenkov & Chrousos, 2002).

The principal cytokines involved in the cell-mediated response include IFN-γ, IL-2, IL-12, and TNF-α (Dong & Flavell, 2001; Elenkov & Chrousos, 2002; Mosman & Coffman, 1989). In addition to causing differentiation into Th1 cells, IL-12 elicits TNF-α release. TNF-α increases the inflammatory response by activating natural killer cells that induce apoptosis in infected cells. Like TNF-α, IFN-γ is also able to activate additional types of immune cells (macrophages and cytotoxic T cells) to destroy any cells infected with the foreign antigen (Abbas et al., 2012). Moreover, IFN-γ can promote naïve T cell differentiation into Th1 cells and inhibit activation of cells involved in the humoral response (Mosman & Coffman, 1989). IFN-γ is often used to

represent Th1 activation and is the primary Th1 cytokine used in the current study.

The humoral response is mediated by a different set of Th2 associated cytokines with the objective of inducing antibody release from B cells (Abbas et al., 2012; Segerstrom & Miller, 2004). After IL-4 promotes the differentiation of naïve T cells into Th2 cells, the activated Th2 cells will release additional IL-4 that will bind to receptors on B cells (Elenkov & Chrousos, 2002; Mosman & Coffman, 1989). B cells will subsequently replicate and differentiate into either memory B cells or effector B cells. Memory B cells will serve as a reserve to discourage future attacks of the foreign antigen on the host. Effector B cells, also called plasma cells, on the other hand will immediately begin producing antibodies. These antibodies circulate through the bloodstream and tag foreign molecules for destruction by phagocytosis. The attachment of the circulating antibodies to antigens also hinders the antigen's ability to infiltrate cells (Abbas et al., 2012).

Additional cytokines involved in the humoral response include IL-5, IL-9, IL-10, and IL-13 (Dong & Flavell, 2001; Elenkov, 2008; Mosman & Coffman, 1989). IL-5 and IL-13 encourage B cell proliferation. IL-5 and IL-9 are important for activating additional immune cells. In response to IL-5, eosinophils release toxic mediators to kill foreign antigens whereas mast cells, most known for their release of histamine during inflammation and allergy exposure, are activated by the release of IL-9. IL-10 has similar mechanisms to the Th1 cytokine IFN-γ by serving as an opposing force. IL-10 is able to inhibit IL-12 release, thus preventing naïve T cell differentiation

into Th1 cells (Abbas et al., 2012). Like IFN-γ, IL-4 is often a primary marker of Th2 activation and is analyzed in the current study.

The opposing forces between cytokines involved in the cell-mediated and humoral immune responses ensure the immune response to a foreign antigen is appropriate and prevents an overactive inflammatory reaction. By suppressing and initiating cell activity, cytokine regulation permits the immune system to maintain equilibrium under normal conditions (Elenkov, 2008; Quan & Herkenham, 2002). While the Th1 and Th2 responses should be counteractive, changes can occur and lead to certain disease states. For instance, autoimmune diseases such as rheumatoid arthritis and diabetes mellitus type 1 favor a Th1 profile whereas immune deficiencies such as HIV, asthma, and allergic disorders favor a Th2 profile (Elenkov & Chrousos, 1999). Moreover, interactions between cytokines and GCs during a stress response and an aging immune system can alter the Th1/Th2 cytokine balance. As with all bodily systems, maintaining a homeostatic or allostatic equilibrium is important for good health and disruptions of the Th1/Th2 cytokine balance need to be minimized (Elenkov, 2008; Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002; McEwen, 2003).

Immune system with age. An imbalance of Th1/Th2 cytokines may be a result of immunosenescence, the age-related decline in the immune system (McElhaney & Effros, 2009; Solana, Pawelec, & Tarazona, 2006). A multitude of changes in the immune system have been described that occur with age, the most notable being changes in T cell populations. Maturation of T cells occurs in the thymus, and with age the thymus reduces in size.

This phenomenon, called thymic involution, results in a decrease in the number of cytotoxic and helper T cells that are able to mature (Dorshkind, Montecino-Rodriguez, & Signer, 2009; Haynes, Eaton, Burns, Rincon, & Swain, 2004; McElhaney & Effros, 2009). As a consequence, older adults have less naïve and active T cells to fight against new pathogens (Czesnikiewicz-Guzik et al., 2008; Solana et al., 2006). Additionally, research has shown higher numbers of memory T cells in older adults than younger adults (Linton & Dorshkind, 2004); however, memory T cells generated in older age are less effective in guarding against future infections than those memory T cells generated in childhood (Czesnikiewicz-Guzik et al., 2008; Haynes et al., 2004).

In regards to B cells with age, animal studies indicate population decreases in their bone marrow origin. Studies of human subjects do not always show changes in B cells in the bone marrow; however, after B cells migrate to the spleen, where their maturation takes place, decreases in B cells are frequently noted. One possible explanation for the inconsistent findings in B cell populations is B cells may lose the ability to exit the bone marrow. Hence, unchanged populations may be seen in bone marrow; however, a decrease in the number of B cells in the spleen will lead to a reduction of maturing B cells, memory B cells, and activated B cells in the periphery (Dorshkind et al., 2009; Linton & Dorshkind, 2004). These age-related changes, in turn, result in less specific antibody production and diversity (Frasca & Blomberg, 2009; Frasca, Riley, & Blomberg, 2005).

Most studies have not found a decrease in the number of APCs; however, APCs are less capable of capturing pathogens for presentation to the adaptive immune cells (Agrawal et al., 2007). Due to the importance of APCs as modulators of naïve T cell differentiation into Th1 and Th2 subsets, functional changes in APC's can also contribute to imbalances in the cytokine environment.

In recognition that Th1 cytokines are most often considered pro-inflammatory whereas Th2 cytokines demonstrate anti-inflammatory properties, it is expected that older subjects experiencing inflammatory diseases would demonstrate higher levels of Th1 cytokines. For instance, in a study comparing controls, mild AD, moderate-severe AD, and vascular dementia, increased levels of IL-2 were only found in the persons with moderate-severe AD and vascular dementia (Huberman, Sredni, Stern, Kott, & Shalit, 1995). A second study investigating cytokine levels in persons with AD compared to elderly controls found higher levels of IL-2 and IFN-γ in persons with severe AD (Huberman et al., 1994). Additionally, IL-10 has been shown to have protective mechanisms in models of traumatic brain injury and experimental autoimmune encephalomyelitis, whereas IL-12 knockout mice were found to have decreased neuronal injury (Tansey & Wyss-Coray, 2008). In contrast, among healthy subjects, reports indicate that age is accompanied by a shift towards a primary Th2 response. For instance, in aging mouse models, decreased IL-2 production has been found (Haynes et al., 2004; Kubo & Cinader, 1990), whereas increased levels of IL-4 and IL-10 are seen (Agrawal et al., 2007; Kubo & Cinader, 1990). The

shift towards a Th2 response that is speculated to occur with age may not only be beneficial in the peripheral immune system, but may also contribute to anti-inflammatory properties in the brain.

In summary, age-related changes in the immune system, including changes in T cells, B cells, and APCs result in a disruption of the allostatic cytokine environment. In healthy aging mouse and human subjects, literature notes a decrease in Th1 associated cytokines coupled with an increase in Th2 cytokines, indicative that age may be associated with a more predominant Th2 cytokinic profile. Due to past research reporting higher levels of pro-inflammatory cytokines (Th1 associated cytokines IFN-γ and IL-2) in inflammatory conditions of the brain, such as AD, the shift towards an anti-inflammatory Th2 cytokinic profile may be a protective mechanism for cognitive functioning. Hence, while changes in immune cell populations and function with age may lead to less protection against newly acquired infection, the consequential disturbance in the Th1/Th2 balance may provide anti-inflammatory benefits needed in an aging body and brain. However, the underlying mechanisms explaining a shift towards the Th2 response with age need further explanation. Previous literature has remarked on the interactions that occur between GCs and cytokines. Therefore, in this study we hypothesized chronic stressors may influence changes in cytokinic profiles with age, and in turn, explain differences seen in cognitive performance. In the next section, we first review interactions of stress hormones and cytokines. Next, we discuss the entry of peripheral cytokines into the brain,

followed by studies investigating the effects of pro-inflammatory and anti-inflammatory cytokines on cognition.

Cytokines, stress, and the brain. Age-related changes of immune cells are not the only known mechanism to disrupt the allostatic cytokine environment. The body's attempt to deal with stressful experiences disrupts the allostatic balance of stress hormones, and in turn, stress hormones can result in alterations of the Th1/Th2 balance. For instance, GCs have been shown to lower IL-12 secretion by APCs. A decrease in IL-12 subsequently results in a decrease in IFN-γ and an increase in IL-4, thus promoting activation of cells involved in the Th2 response (Elenkov, 2008). In a series of studies by Smith and colleagues (2007) in both animal and human models, IL-10 was shown to induce CRH and ACTH production in the brain. They also reported IL-10 is able to reduce secretion of GCs by the adrenal glands by interfering with enzymes associated with GC synthesis. Moreover, IL-10 deficient mice undergoing both physiological and psychological stressful conditions had higher levels of GCs before the experimental conditions and significantly higher levels of GCs than controls after the stressful conditions (Smith, Tu, & Hughes, 2007). In CD4 T cells from rat models, GCs increase production of IL-4, IL-10, and IL-13, yet decrease IFN-γ and TNF-α synthesis (Ramirez, Fowell, Puklavec, Simmonds, & Mason, 1996). These studies suggest bidirectional effects of stress hormones and cytokines, such that stress hormones are able to reduce Th1 cytokines, increase Th2 cytokines, and Th2 cytokines are able to reduce stress hormone synthesis in the periphery. GCs have long been used to suppress overactive immune

responses, however, more recent research indicates GCs are selectively inhibiting Th1 responses, resulting in a shift towards a Th2 response (Glaser et al., 2001; Haddad, Saadé, & Safieh-Garabedian, 2002; Sapolsky, Romero, & Munck, 2000).

Evidence has shown cytokines are able to migrate from local sites in the periphery and exert effects on long-distance organs such as the brain, including structures comprising the HPA axis (Aubert & Renault, 2008; Groër, Meagher, & Kendall-Tackett, 2010). Researchers previously hypothesized the blood-brain barrier protected the brain from any peripheral insults due to inflammation. However, this line of thought has more recently been replaced with the advent of more advanced research methods. Literature comments on three main methods that permit diametric communication of cytokines between the brain and the periphery: (1) circumventricular organs; (2) vagus nerve, and (3) cytokine receptors on epithelial cells lining the barrier (Groër et al., 2010; Quan & Herkenham, 2002; Sternberg, 2001; Wilson, Finch, & Cohen, 2002).

Research directly investigating the effect of peripheral cytokine production on the brain is lacking due to methodological limitations. Thus, the precise mechanistic actions of Th1 versus Th2 cytokines on the brain are still not well understood and research is especially lacking in regards to Th2 related cytokines. However, the presence of cytokines within the brain is evident by reports of cytokine receptors, including IL-10 and TNF-α receptors in the hypothalamus, hippocampus, and pituitary glands (Conti et al., 2008). In addition, symptoms of sickness behavior, such as changes in body

temperature and sleep, clearly indicate peripheral cytokines interact with HPA axis structures (Quan & Herkenham, 2002).

Past reviews indicate Th1 related cytokines promote inflammation in the brain, whereas Th2 related cytokines reduce inflammation in the brain (Wyss-Coray & Rogers, 2012). Additionally, pro-inflammatory cytokines have been found to impair HPA axis functionality by interfering with GC feedback mechanisms (John & Buckingham, 2003). Hence, studies investigating the association between cytokines and cognition typically indicate poorer cognition is related to Th1 cytokines. Injection of IL-2 in the periphery of aged mice resulted in neurodegeneration of hippocampal neurons and significantly poorer performance on a passive-avoidance task than controls (Nemni et al., 1992). In humans, cancer patients receiving IL-2 injections as therapy demonstrate poorer performance on spatial memory and planning tasks but not reaction time (Capuron, Ravaud, & Dantzer, 2001). IL-2 has also been shown reduce long term potentiation (LTP) induction in the hippocampus (Rothwell, Luheshi, & Toulmond, 1996; Tancredi, Zona, Velotti, Eusebi, & Santoni, 1990). The Sydney Memory and Aging study found participants with non-amnestic multiple domain MCI had higher levels of TNF-α and IL-12 than controls and other forms of MCI. No differences were seen in IL-10 levels (Trollor et al., 2010). In a longitudinal study investigating knockout TNF-α mice, TNF-α-/- resulted in poorer performance on spatial retention and special learning tasks than controls at 3 months of age. However, at 12 months of age, the reverse was found; TNF-α-/- exhibited better performance on the tasks with age. TNF-α has been shown

to assist neurogenesis and synaptic plasticity, thus TNF-α may be more beneficial at younger ages (Baune et al., 2008; McAfoose & Baune, 2009). In contrast, IL-4 is decreased in the hippocampus of aged mice compared to young controls, and IL-4 is able to restore some deficits seen LTP in older mouse models (Nolan et al., 2005).

The series of studies reviewed reveal the interplay between GCs and cytokines result in anti-inflammatory mechanisms. Although this shift from a pro-inflammatory to an anti-inflammatory state may produce undesirable outcomes for older adults fighting against new infections, this shift may offer protection against neuronal insults. As noted, peripheral cytokines are able to enter into the brain and influence HPA structures. In particular, the hippocampus, important for memory processing, has shown degeneration and impaired LTP induction in the presence of Th1 cytokines such as IL-2 whereas IL-4, a Th2 cytokine is able to reverse LTP impairments.

Chapter summary

The literature described above reviewed changes in the cytokine environment due to advancing age and stress hormones and the impact of these changes on cognition. Results from cognitive measures in older adults reveal different outcomes depending on the cognitive domain under investigation. For instance, older adults frequently show poorer performance on tests of episodic memory, executive functioning, working memory, and processing speed as compared to stable performance on semantic memory measures. However, variation in cognitive tests is still found among the

older population. Additional factors to explain differences in cognition need to be explored in interest of slowing or reversing cognitive decline with age.

In this review, we investigated stress as an additional factor to explain variation of cognitive performance in older adults. Physiological and psychological stressors activate brain structures involved in the HPA axis and result in cortisol release. Under normal conditions, cortisol promotes negative feedback on the hypothalamus and hippocampus to return the body to equilibrium after stress induction. However, both age and chronic stress have been associated with irregular diurnal patterns of cortisol. In instances of increased cortisol levels, detrimental changes in cortisol receptors and HPA structures in the brain may result. Due to the importance of two HPA structures, the prefrontal cortex and hippocampus, for cognitive processing, it would be expected that age and stress related changes of stress hormones would result in poorer cognitive functioning. Conversely, not all studies investigating chronic stress in older adults find poorer cognition. To possibly explain this discrepancy, we next investigated previous literature of the aging immune system.

Similar to the endocrine system, allostatic equilibrium needs to be maintained within the immune system. Age-related changes, such as thymic involution, can result in changes in immune cell populations and proper functioning. In turn, a decrease of functional properties of immune cells, as seen in APCs, can disrupt the balance of cytokines. Cytokines are important cell signaling molecules, and their actions define pro-inflammatory (Th1) and anti-inflammatory (Th2) immune responses. In a normal state, a relative

balance should exist between Th1 and Th2 associated cytokines. In contrast, autoimmune diseases are coupled with a shift towards a predominant Th1 response, whereas HIV and allergic disorders result in a shift towards a Th2 response. Additionally, many researchers suggest a shift towards a predominant Th2 response occurs with age. While age-related changes in immune cells may explain some alterations in the Th1/Th2 balance, additional evidence indicates GCs may also play a role.

Research identifies complex interactions occur between GCs and cytokines. Briefly, stress hormones exert anti-inflammatory properties by decreasing Th1 cytokines production and increasing Th2 cytokines. Reversely, Th2 cytokines are also able to reduce stress hormone synthesis from the adrenal glands. As a result of the interplay of GCs and cytokines, a predominant Th2 cytokinic profile is likely to be established. Although this shift towards an anti-inflammatory state may be unfavorable to fighting off newly encountered pathogens in the aging host, it may, in contrast bring about favorable outcomes for brain functioning.

Pro-inflammatory cytokines in the brain are most often found to negatively impact cognitive functioning. While less research has been published on anti-inflammatory cytokines and the brain, evidence has shown protective and restorative actions of cognitive processes. Research on how peripheral immune responses impact the central nervous system is also lacking in the older adult population, however, entry of cytokines into the brain has been supported. Therefore, in this study, we anticipated that we would find a relationship between cytokines measured in the serum of older

adults and cognition. Specifically, we expected a Th1 cytokinic profile would be significantly associated with poorer cognitive performance, whereas a Th2 cytokinic profile would reveal normal cognitive functioning. Moreover, we expected that cytokinic profiles would moderate the relationship between stress and cognition.

It is anticipated the current research will provide many contributions to existing cognitive, endocrine, and immune system literature in the older adult population. First, the older adults participating in this study did not have a diagnosis of cognitive disorders, thus, the cognitive data provides information on cognitively healthy adults in several different domains. Second, we explored the relationship between cognition and stress. Third, this research determined the relationship between age and cytokinic profiles, as well if chronic stress is related to a shift towards a Th2 response. Lastly, previous studies investigating cognitive functioning, stress, and cytokinic profiles in one study are lacking, especially in the older adult population.

Specific research questions and hypotheses

Research question 1: To examine the relationship between age and cognitive performance. It was hypothesized that older age would be related to poorer memory, executive functioning, and processing speed measures.

Research question 2: To determine the relationship between age and cytokinic profiles. It was predicted that older age would be correlated with a predominant Th2 response.

Research question 3: To determine the relationship between chronic stress and cytokinic profiles. We expected to find more stress-related symptoms related to a predominant Th2 response.

Research question 4: To examine the interactions of stress and cytokinic profiles as predictors of cognitive functioning in older adults. It was proposed the interaction of a predominant Th1 cytokinic profile and stress symptoms would predict lower cognitive functioning, whereas the combination of a Th2 cytokinic profile and stress symptoms would exhibit normal cognitive scores (Figure 2).

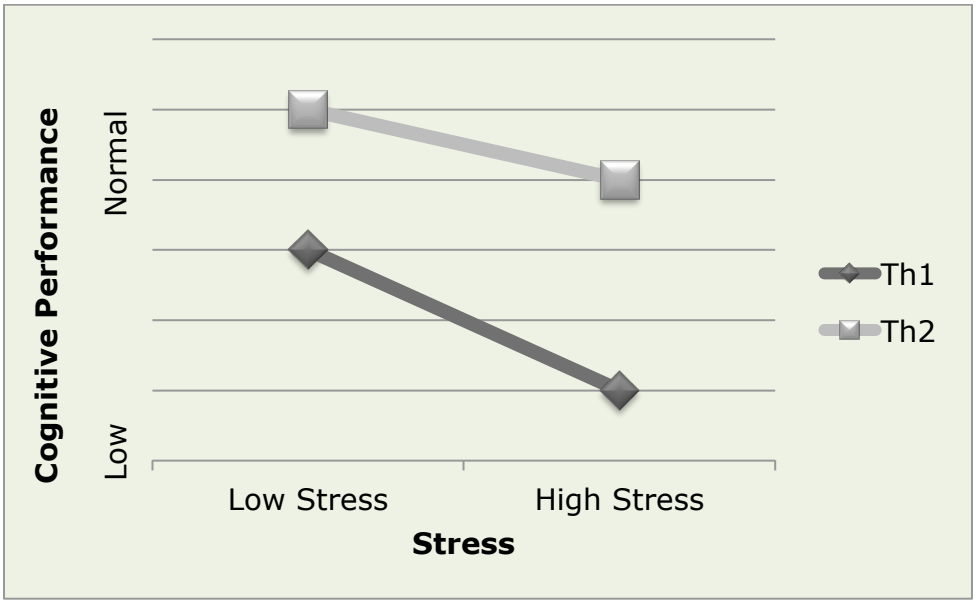

FIGURE 2. Hypothesized main effects and interaction terms.

CHAPTER THREE

METHODS

Participants

Participants consisted of older adults living in Tampa, Florida and recruited through media advertisements for the University of South Florida (USF) Neuroscience Collaborative Seed Grant titled *Nutraceutical Effects on Delay and Trace Eyeblink Conditioning in Humans*. This study was approved by the Western Institutional Review Board (IRB; Olympia, WA). To be enrolled in the study, participants had to be between the ages of 65 and 85, be willing and able to come to the USF campus for cognitive testing and blood draws, and able to give written informed consent. Characteristics of persons that were excluded from this study included persons who scored below a 24 on the MMSE (Folstein, Folstein, & McHugh, 1975), had significantly impaired hearing or vision that was not corrected, and/or exhibited more than five depressive symptoms as measured by the Centers for Epidemiological Studies Depression-Scale (CES-D; Radloff, 1977).

Measures

In the current study, we examined several different classes of measures including basic demographic information and well-being, measures of acute and chronic stress, cognitive performance outcomes, and biological

measures of cytokines during individual sessions. These measures are described below.

Demographic characteristics and well-being. Participants answered questionnaires on basic demographic information including age, race, education, marital status, chronic conditions, and depression. The CES-D form reflects ten depressive symptoms that a person may have experienced in the past week.

Acute and chronic stress measures.

Perceived Stress Scale (PSS). This scale measures acute stress by having the participant denote thoughts and feelings during the past four weeks on 10 items (Cohen, Kamarck, & Mermelstein, 1983). The participant used a Likert-scale to answer the responses (0=never to 4=very often). Responses were added together for a total score. Higher scores indicate more perceived stress.

Life-events and social stress scale. The life events and social stress scale is divided into five main subscales (Turner & Turner, 2005). The first subscale refers to life events that may have occurred to the participant during the past year. Participants answered yes or no to the 34 questions. The number of yes responses was added together for the "life events" score. The second subscale contains 51 questions regarding any ongoing stressful events that may have been true for them at that point in time. Participants had three choices for the second section: 0=not true, 1= somewhat true, and 2=very true. Responses were added together for the "chronic events" score. The third subscale refers to 11 events that may have occurred in

childhood. Participants answered yes or no for this section. Yes responses were added together to determine the "childhood stress" score. The fourth subscale lists lifetime adversities that may have occurred at any point in their life. The number of lifetime adversities that persons experienced was added together for the "lifetime adversity" score. These four sections were also added together to calculate a "total stress score."

Lastly, this scale proposes questions that are indicative of sources of social support among spouses, relatives, and friends. Participants answered using a 4 point Likert scale (1=very much like my experience to 4=not at all like my experience). A total score was calculated by adding the individual answers together and dividing by the number of social support resources available. For example, some participants did not have a spouse, thus their social support score was divided by two instead of three. The social support average was used as a covariate in the moderation analysis.

Cognitive performance measures. The domains of cognitive performance measured include Episodic Memory (Rey Auditory Verbal Learning Test), Processing Speed (Identical Pictures Test, Number Comparison Test), Verbal Fluency (Vocabulary, Controlled Oral Word Association Test, and Category Fluency Test), Working Memory (Digit Span Test, Digit Symbol Part II), and Executive Functioning (Digit Symbol Part I, Category Fluency, Trail Making Test-A, and Trail Making Test-B).

Rey Auditory Verbal Learning Test (AVLT) Immediate and Delayed Recall (Lezak, 1985). The total number of words recalled for each trial was recorded. A list of 15 words was read and participants recalled as many

words as possible for an initial 5 trials. Subsequently, a second list of 15 words was read and participants were asked to recall as many words as possible from this list. Next, the participants were asked to recall as many words as they could from the first list (Trial 6). After 30 minutes, participants were again asked to recall as many words as they could from the first list (AVLT Delay). For data analysis, two outcomes were examined: the average was calculated for the first six trials to denote the AVLT Immediate score, and the AVLT-Delayed recall score.

Educational Testing Service – Identical Pictures Test (Ekstrom, French, Harman, & Dermen, 1976). A speed of processing measure that tests how quickly a participant can choose the correct match to a given object. There are two parts of this test and the participant had 90 seconds to complete each part. Each correct response received 1 point with a maximum score of 48 for each part. The two parts were then averaged together for the Identical Pictures total score.

Educational Testing Service – Number Comparison Test (Ekstrom et al., 1976). This timed two part task is a measure of speed of processing that asks participants to quickly determine if two numbers are the same or different. Each correct response received 1 point with a maximum of 48 for each part. The two parts were then averaged together for the Number Comparison total score.

Educational Testing Service – Vocabulary Test (Ekstrom et al., 1976). As a measure of semantic memory, participants were asked to choose the

word or phrase that had the same meaning as the given word. Each correct answer is worth 1 point with a maximum number of 54.

Trail Making Test (TMT) - A & B (Reitan & Wolfson, 1993). These two tests measure executive functioning and processing speed. TMT-A required participants to connect 25 circles labeled 1-25 in numerical order. TMT-B required participants to alternate between letters and numbers (1-A-2-B-3-C, etc). Participants were asked to work as quickly as possible without lifting their pencil. Time and number of errors were recorded. Time to complete each of the components was used as the outcome in the current analysis. Positive estimates indicate longer latency to complete the task.

Wechsler Adult Intelligence Scale (WAIS) – Digit Symbol Test (Wechsler, 1987). Part one of this timed executive functioning and processing speed task requires participants fill in boxes with a symbol that matches a set of numbers. The number of correct answers was recorded. The second part of this task measured working memory and required participants to recall which symbols matched each number.

Wechsler Adult Intelligence Scale (WAIS) – Digit Span Test (Wechsler, 1987). This measure has two parts – Digit Span Forward to measure short-term memory and Digit Span Backward to measure working memory. For the first part, participants were asked to repeat the digits in the order they were presented. For the second part, participants were asked to repeat the digits in the reverse order in which they were presented. Each correct response received 1 point. Separate scores for forward and backward digit span were used in analysis, as well as a total maximum score of 30 points.

Controlled Oral Word Association Test (COWA; Reitan & Wolfson, 1993). Participants were asked to name as many words (excluding proper nouns) that begin with a certain letter for three trials. Participants were allowed 60 seconds for each letter. Correct responses that were not repeated for each letter were averaged together to constitute the raw score.

Category Fluency Test (Reitan & Wolfson, 1993). Participants named as many types of animals or vegetables as they could in 60 seconds. Correct responses that were not repeated were counted to designate the raw score on this measure.

Cytokine assays. Blood draws were completed by qualified staff at the USF Health Byrd Alzheimer's Institute. Serum samples were collected in serum-separating tubes (SST), prepped according to standard protocols, divided into three aliquots, and frozen at -80°C. Serum cytokines were measured by a Luminex 100 (Austin, TX) instrument using multiplex kits from Bio-Rad (Life Sciences Research Group, Hercules, CA). Bio-Plex Human Cytokine Assay included premixed coupled magnetic beads, detection antibodies, standards, reagents, and diluents for detecting IFN-γ and IL-4. Protocols from Bio-Rad were followed for cytokine assays. Concentration levels were calculated using Masterplex QT™ (Version 2.0.0.76; Hitachi Solutions, Ltd., MiraiBio Group, San Francisco, CA).

To standardize the values, individual data points were reconfigured to reflect the same set of standards and blanks. The standards were chosen based on the best root mean square error fit. Manual optimization was then utilized to improve the fit for the standard curve and individual data points.

Serum samples were run in triplicate. Coefficients of variations were examined for each case to ensure a replicate group of wells did not exceed 20%. In cases of the coefficient of variation exceeding 20%, outliers of the replicate group were excluded from calculating the mean concentration levels. Bead counts for individual data points were also examined and were excluded if the count was under 50. If the concentration value was below the limit of detection, half of the value between the lowest standard and 0 was used. For IFN-γ, this value was .875, for IL-4 this value was .11.

Log10 transformation was utilized to correct the positive skewness of cytokine distributions. Cytokine concentration values were then standardized to z-scores based on within assay plate means and standard deviations. Concentration values of IFN-γ, a Th1 cytokine, and IL-4, a Th2 cytokine served as the outcomes. Due to the interplay between cytokines, ratios of cytokines may provide a better indication of allostatic functions as opposed to looking at cytokines in isolation (Quan & Herkenham, 2002). Therefore we also explored IFN-γ/IL-4 ratio in this study.

Data analysis

For the first three research questions of this study, we conducted separate correlation analysis to examine (1) the relationships between age and cognition, (2) the relationship between age and cytokinic profiles, and (3) the relationship between stress and cytokinic profiles.

For the fourth research question of this study, we investigated the influence of stress and cytokines on cognition using moderation analysis (MODPROBE Procedure for Probing Interactions; Hayes & Matthes, 2009). In

this analysis, the stress measures served as the predictor variable and the Th1 and Th2 cytokines acted as the moderator variable. The outcome variables included the cognitive domains. Main effects were tested for the influence of stress on cognition, the influence of predominant Th1 cytokinic profile on cognition, and the influence of a predominant Th2 cytokinic profile on cognition. Significant interactions between stress and cytokines were probed using a simple slopes procedure. Using this method, the relationship between the stress scores and the cognitive outcome were examined at three different levels of the cytokine variable. Specifically, the levels of the cytokine variables were selected to be 1 SD below the mean, at the mean, or 1 SD above the mean. The slopes of the lines presented in Figures 4 through 8 are suggestive of the different immune profiles as indicated by the IFN-γ/IL-4 ratio. For instance, a ratio of IFN-γ/IL-4 1 SD below the mean represents Th1/Th2 ratios skewed towards a predominant Th2 response. A ratio of IFN-γ /IL-4 at the mean represents a balanced Th1/Th2 ratio and a ratio of IFN-γ/IL-4 1 SD above the mean represents ratios skewed towards a predominant Th1 response. For all moderation analyses, age, gender, education (years), number of chronic conditions, and social support averages were centered and entered as covariates.

Power analysis. Sample size considerations were based on statistical power analyses. The goal was to examine measurements from 90 older adults. To examine cognitive measures and age, we met this goal and had 92 participants in the study. This sample size allowed correlations of .25 to be detected with .80 power at a two-tailed alpha level of .05. However,

cytokine analysis was completed for 40 participants and this resulted in a power of .80 to detect correlations of .48 or above. Thus, enough power existed to detect medium size effects.

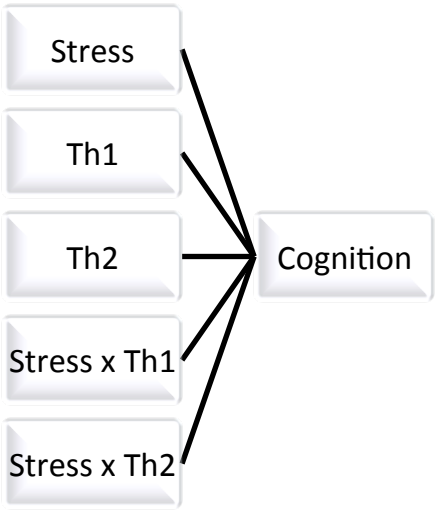

FIGURE 3**.** Moderation model for research question 4.

CHAPTER FOUR

RESULTS

We begin by describing the characteristics of the participants and describe the sample sizes included for the overall analyses, as well as those that focus on the cytokines. Next, the results for each of the research questions are described and are organized by each of the specific questions.

Sample descriptives

Ninety-two participants were included in the current study and they were approximately 74 years of age (M = 74.08, SD = 5.37, range = 65.14 – 85.37). Ninety-seven percent of the participants were white and 63.4% were female. Most of the participants were married (54.8%), 21.5% were widowed, 19.4% were divorced, and 4.3% were never married. Years of education ranged from 8 to 21 years (M = 15.42, SD = 2.65). Ninety-two participants had completed the stress questionnaires and cognitive data at the time of data analysis. Cytokine analysis was conducted on 65 serum samples. However, one assay of 13 samples was excluded due to uncertain results. An additional 11 samples were excluded from analysis for low bead counts or high coefficient of variation percentages, leaving a total of 41 valid samples available for analysis. Thus, for research question one, 92 participants will be included in analysis, whereas 41 participants will be included for the remaining research questions.

Specific research questions analyses

Research question 1. What is the relationship between age and cognitive performance?

TABLE 1. Correlations of age and cognitive measures (n=92).

Cognitive Measures	Age
AVLT Immediate	-.35†
AVLT Delay	-.28†
Identical Pictures	-.29†
Number Comparison	-.14
Vocabulary	-.00
Trail Making Test – A (TMT-A)	.11
Trail Making Test – B (TMT-B)	.25*
Digit Symbol – Part 1	-.14
Digit Symbol – Part 2	-.31†
Digit Span Forward	.00
Digit Span Backwards	-.05
Digit Span Total	-.02
COWA	-.04
Category Fluency	-.06
MMSE	-.21*

* - Correlation significant at or below the .05 level.
† - Correlation significant at or below the .01 level.

The relationship between years of age and cognitive performance for the cognitive outcome measures are shown in Table 1. As expected, statistically significant relationships were present including poorer

performance on the AVLT Immediate, AVLT Delay, Identical Pictures, TMT-B, Digit Symbol Part 2, and MMSE scores.

Research question 2. To what extent are age and cytokinic profiles related in older adults?

Correlations were used to evaluate the relationship between age and the levels of IFN-γ, IL-4, and the ratio between these two cytokines. Contrary to predictions, none of the measures were significantly associated with age (IFN-γ: $r = -.03$, $p = .86$; IL-4: $r = -.23$, $p = .15$; IFN-γ/IL-4 ratio: $r = .00$, $p = .99$).

Research question 3. Is there a significant relationship between chronic stress and cytokinic profiles?

TABLE 2. Correlations of stress measures and cytokines (n=41).

Stress Measures	IL-4	IFN-γ	IFN-γ/IL-4
PSS	.33*	.21	-.18
Chronic Events	-.18	-.33*	-.07
Life Events	-.00	.08	.12
Childhood Stress	-.07	.08	.21
Lifetime Adversities	-.01	-.04	.07
Total Stress Score	-.14	-.26	-.01

* - Correlation significant at or below the .05 level.
† - Correlation significant at or below the .01 level.

Table 2 displays the correlations between stress measures and the three cytokine values. Of the relationships examined, higher scores on the PSS were significantly correlated with higher levels of IL-4 ($r = .33$, $p = .04$) and higher scores on the chronic events subscale were significantly

associated with lower IFN-γ scores (r = -.33, p = .04). No significant correlations between stress questionnaires and the IFN-γ/IL-4 ratio were found. Upon examination of the correlation analysis of cytokine values and cognitive performance, Digit Symbol Part 2 was associated with IFN-γ/IL-4 ratio (r = .31, p = .05).

Research question 4. Do significant interactions between stress and cytokinic profiles predict cognitive performance in older adults?

Table 3 displays the results of the analyses for IL-4, stress, and cognition. Only one main effect for stress was present, with higher levels of PSS being related to lower performance on the Category Fluency outcome. Main effects for cytokine were observed for Digit Span Backwards and Digit Span Total, with higher levels of IL-4 being related to better performance.

Significant moderation effects were observed for Identical Pictures, TMT-B and COWA scores. For Identical Pictures, higher scores on the chronic events subscale was associated with poorer Identical Pictures scores when IL-4 was 1 SD above the mean (b = -.45, SE = .17, p = .01) but not when IL-4 was at or 1 SD below the mean (b = -.21, SE = .11, p = .07; b = .04, SE = .11, p = .74, respectively). Likewise, higher total stress scores were significantly associated with poorer Identical Pictures scores when IL-4 was 1 SD above the mean (b = .31, SE = .15, p = .04), but again, not when IL-4 was at or 1 SD below the mean (b = -.15, SE = .10, p = .14; b = .01, SE = .09, p = .87, respectively).

The same pattern was found for TMT-B for the life events subscale when examining IL-4 as a moderator. Higher scores on the life events

subscale were significantly associated with poorer scores on the TMT-B when IL-4 was 1 SD above the mean (b = 24.62, SE = 11.10, p = .03), but not when IL-4 was at or 1 SD below the mean (b = 13.88, SE = 8.61, p = .12; b = 3.13, SE = 9.22, p = .74, respectively). The interaction was significant for the childhood stress subscale and COWA; however, the results of the simple slopes analysis revealed none of the comparisons were statistically significant on their own.

TABLE 3. Summary of statistically significant main effects and interactions from moderation analyses for IL-4 (n=41).

	Main Effect Stress		Main Effect Cytokine		Interaction	
	b	*SE*	*b*	*SE*	*b*	*SE*
Identical Pictures						
Chronic Events	-.21	.11	-.35	.64	-.25	.10*
Total Stress Score	-.15	.10	-.16	.66	-.17	.08*
Trail Making Test - B						
Life Events	13.88	8.61	4.70	8.19	10.88	5.45*
Digit Span Backwards						
Life Events	-.44	.44	.85	.42*	-.35	.28
Childhood Stress	-.39	.49	.90	.42*	-.64	.48
Digit Span Total						
Life Events	-.33	.71	1.40	.68*	-.42	.45
Childhood Stress	-.50	.79	1.44	.68*	-.70	.77
COWA						
Childhood Stress	.16	.58	.40	.50	-1.3	.57*
Category Fluency						
PSS	-.43	.20*	-.37	.80	-.11	.20

All estimates are independent of age, gender, education, number of chronic conditions, and social support resources. *$p \leq .05$, †$p \leq .01$

TABLE 4. Summary of statistically significant main effects and interactions from moderation analyses for IFN-γ (n=41).

	Main Effect Stress		Main Effect Cytokine		Interaction	
	b	*SE*	*b*	*SE*	*b*	*SE*
Trail Making Test - A						
Chronic Events	.32	.27	1.18	1.60	-.65	.19†
Lifetime Adversities	.35	.96	.02	1.8	-2.30	1.12*
Total Stress Score	.17	.24	1.01	1.61	-.52	.17†
COWA						
Childhood Stress	.01	.60	.50	.52	-1.64	.78*

All estimates are independent of age, gender, education, number of chronic conditions, and social support resources. *p ≤ .05, †p ≤ .01

The results of the analysis with IFN-γ are shown in Table 4. For this cytokine, none of the main effects of stress measure or cytokine were statistically significant. However, moderator effects of IFN-γ were found for TMT-A and COWA. Higher scores on the chronic events subscale were significantly associated with poorer scores on TMT-A when IFN-γ is 1 SD below the mean (b = .96, SE = .28, p = .001), but not when IFN-γ is at or 1 SD above the mean (b = .32, SE = .27, p = .25; b = -.33, SE = .37, p =.39, respectively). Similarly, higher total stress scores were significantly associated with TMT-A when IFN-γ is 1 SD below the mean (b= .69, SE = 3.04, p = .004), but not at or 1 SD above the mean (b =.17, SE = .70, p = .49; b = -.35, SE = -1.01, p = .32, respectively). Although the interaction was significant for the lifetime adversities subscale and TMT-A, the results of the simple slopes analysis revealed that none of the comparisons were

statistically significant on their own. The interaction was also significant for the childhood stress subscale and COWA; however, the results again revealed no significant comparisons when examining the simple slope analysis.

The results of the analyses for the IFN-γ/IL-4 ratio are shown in Table 5. In terms of main effects, higher IFN-γ/IL-4, ratios were related to better scores on Digit Symbol Part 2. Higher levels of IFN-γ/IL-4 ratios indicate cytokinic profiles skewed towards a Th1 response. For Category Fluency, higher stress as measured by the PSS was related to poorer cognitive outcomes.

Of greater interest, the moderator analysis revealed several statistically significant interactions with Number Comparison, TMT-A, and Digit Symbol Part 1. Higher number of lifetime adversities was significantly associated with lower scores on Number Comparison (Figure 3) when IFN-γ/IL-4 ratio was at the mean and 1 SD above the mean ($b = -.80$, $SE = .41$, $p = .058$; $b = -2.55$, $SE = .82$, $p = .004$, respectively), but not 1 SD below the mean ($b = .94$, $SE = .68$, $p = .18$). Higher total stress scores were also significantly associated with poorer scores on Number Comparison (Figure 4) when the ratio was 1 SD above the mean ($b = -.40$, $SE = .15$, $p = .01$), but not at or below ($b = -.19$, $SE = .10$, $p = .07$; $b = .03$, $SE = .13$, $p = .80$, respectively).

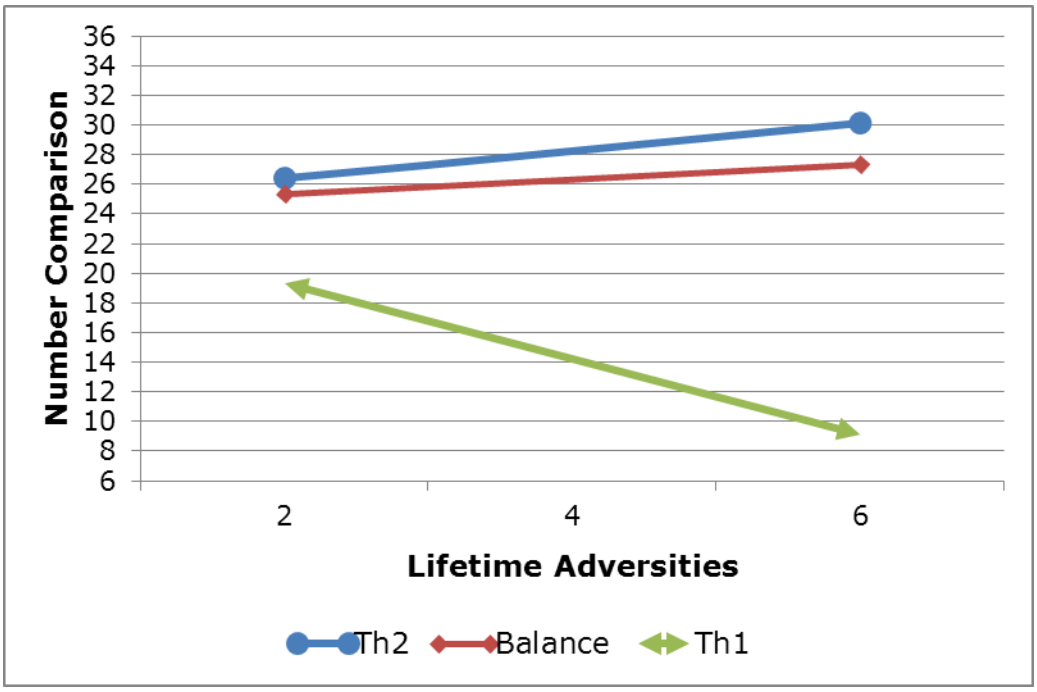

FIGURE 4. Relationship between the lifetime adversity subscale and Number Comparison at levels of the IFN-γ/IL-4 ratio.

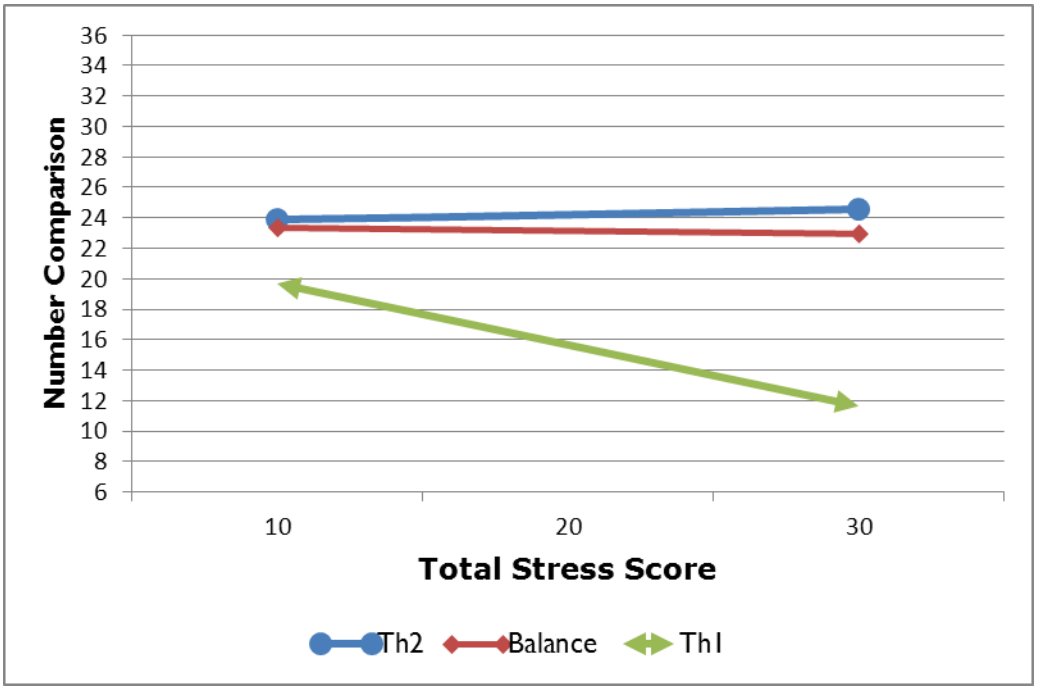

FIGURE 5. Relationship between total stress scores and Number Comparison at levels of IFN-γ/IL-4 ratio.

Higher number of chronic events was significantly associated with poorer scores on TMT-A (Figure 5) when IFN-γ/IL-4 ratio was at the mean or 1 SD above the mean (b = .78, SE = .21, p = .001; b = 1.58, SE = .33, p = .000, respectively), but not 1 SD below the mean (b = -.02, SE = .27, p = .95). Likewise, higher total stress scores were significantly associated with poorer TMT-A (Figure 6) scores when IFN-γ/IL-4 ratio was at the mean or 1 SD above the mean (b = .62, SE = .19, p = .002; b = 1.35, SE = .29, p = .000, respectively), but not 1 SD below the mean (b = -.10, SE = .24, p = .67). Although the interaction was significant for the lifetime adversities subscale and TMT-A for IFN-γ/IL-4 ratio, the results of the simple slopes analysis revealed that none of the comparisons were statistically significant on their own.

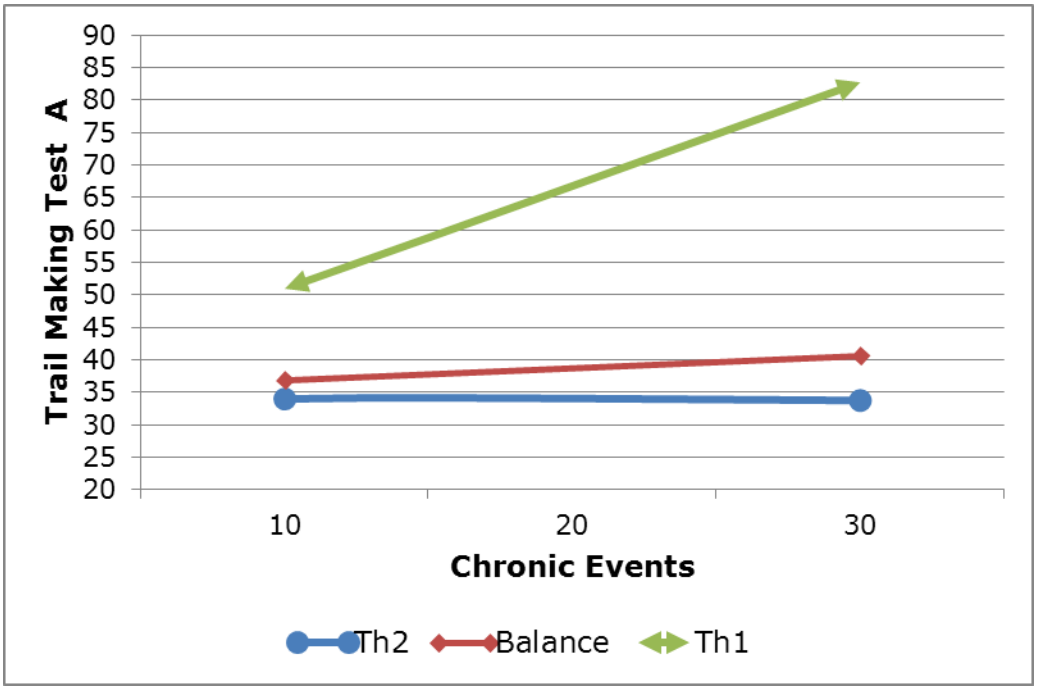

FIGURE 6. Relationship between the chronic events subscale and Trail Making Test A at levels of the IFN-γ/IL-4 ratio. Note: Higher scores (time) indicate poorer performance.

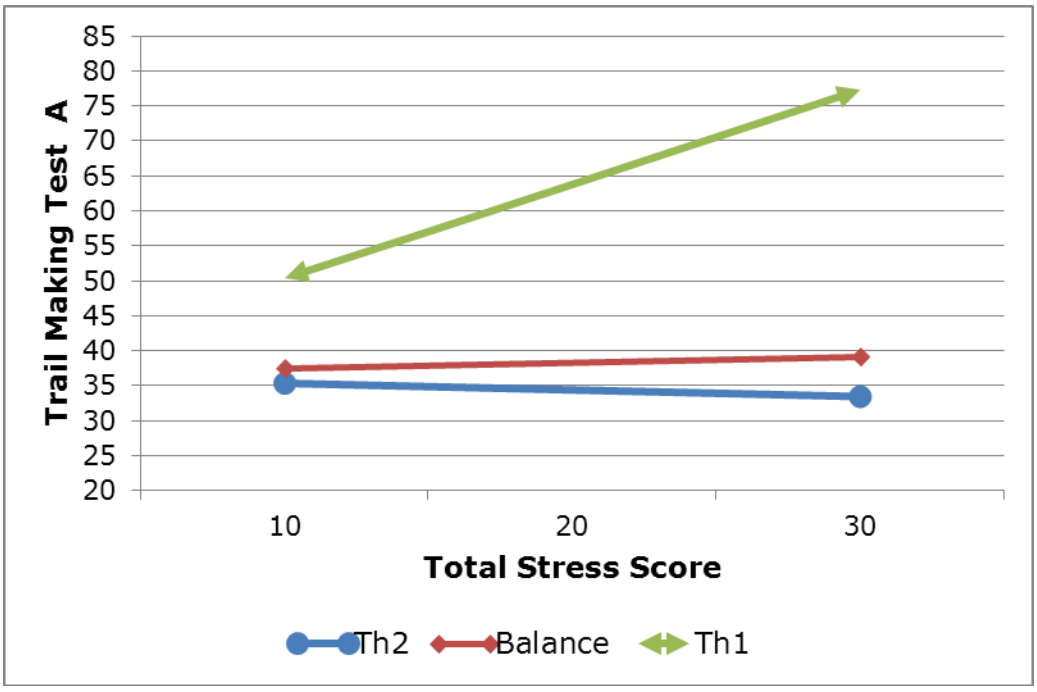

FIGURE 7. Relationship between the total stress score and Trail Making Test A at levels of the IFN-γ/IL-4 ratio. Note: Higher scores (time) indicate poorer performance.

Further examination of the results revealed a significant interaction for PSS, indicating higher PSS scores are related to lower performance on Digit Symbol Part 1 (Figure 7) when IFN-γ/IL-4 ratio is 1 SD above the mean (b = -1.53, SE = .75, p = .05), but not at or below the mean (b = -.50, SE = .40, p = .23; b = .53, SE = .54, p = .34, respectively).

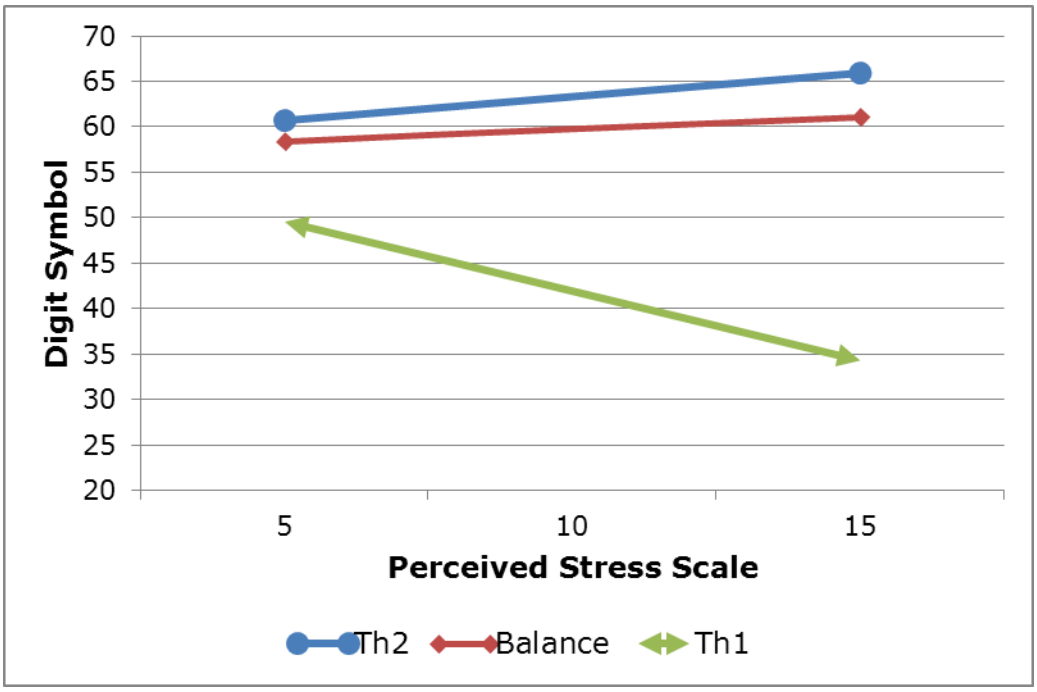

FIGURE 8. Relationship between PSS scores and Digit Symbol Part 1 at levels of the IFN-γ/IL-4 ratio.

TABLE 5. Summary of statistically significant main effects and interactions from moderation analyses for IFN-γ/IL-4 ratio (n=41).

	Main Effect Stress		Main Effect Cytokine		Interaction	
	b	*SE*	*b*	*SE*	*b*	*SE*
Number Comparison						
Lifetime Adversities	.50	.56	-.49	.72	-1.57	.57†
Total Stress Score	-.02	.11	.18	.71	-.19	.09*
Trail Making Test - A						
Chronic Events	.19	.24	2.02	1.33	.72	.19†
Lifetime Adversities	-1.68	1.34	2.90	1.74	2.74	1.37*
Total Stress Score	.08	.21	1.02	1.36	.65	.17†
Digit Symbol Part 1						
PSS	.27	.46	-3.08	1.82	-.92	.46*
Digit Symbol Part 2						
Life Events	-.56	.75	1.53	.71*	-.40	.57
Total Stress Score	-.04	.09	1.12	.56*	-.08	.07
Category Fluency						
PSS	-.53	.22†	1.03	.86	.11	.22

All estimates are independent of age, gender, education, number of chronic conditions, and social support resources. *$p \leq .05$, †$p \leq .01$

CHAPTER FIVE

DISCUSSION

The results of the current study provided varying levels of support for each of the four research questions that were specified here. For the first research question, the results indicated statistically significant age differences were present for several domains of cognitive performance, including speed of processing, executive functioning, and episodic memory. This pattern of results is consistent with the literature indicating that these measures are particularly age sensitive (Craik & Salthouse, 2007; Hofer & Alwin, 2008).

Poorer episodic memory, memory for personal history, was associated with increasing age when examining AVLT Immediate and AVLT Delayed scores. Similar to previous studies (Craik & Salthouse, 2007; Small et al., 2012), young-old (65 to 75) participants recalled more words than the older (75 to 85) participants for both immediate and delayed recall. Older participants also spent significantly more time to complete TMT-B, a measure of executive functioning and speed of processing. TMT-B requires use of memory processes to make future decisions as quickly as possible (Reitan, 1958). A second speed of processing test, Identical Pictures, was also significantly associated with increasing age. Younger participants were able to correctly answer more items than older participants in a 90 second time

frame. Lastly, Digit Symbol Test – Part 2, a measure of working memory, was also significantly associated with increasing age. This test requires participants to recall which symbols were paired with nine numbers on the previous part. Past research indicates older adults experience decline in short-term and working memory (Backman et al., 2000; Bopp & Verhaeghen, 2005). On the other hand, previous research has found that semantic memory remains stable with increasing age, and this was found true in the current study also (Rönnlund et al., 2005; Spaniol et al., 2006).

The second research question explored the relationship between age and cytokinic profiles. The results for this research question were not in line with previous expectation. Previous investigators have purported that a shift occurs from a predominant Th1 cytokinic profile to a predominant Th2 cytokinic profile with age (Agrawal et al., 2007; Haynes et al., 2004; Kubo & Cinader, 1990). To investigate this hypothesis we assayed serum samples for IFN-γ, a Th1 related cytokine, and IL-4, a Th2 related cytokine. Neither IFN-γ nor IL-4 was significantly associated with age. Additionally, a significant association was not found between age and the IFN-γ/IL-4 ratio. Examining a cytokinic ratio between Th1 and Th2 associated cytokines provides a better representation of the balance and interplay between the two immune profiles (Quan & Herkenham, 2002). Ratio values above 1 are indicative of cytokinic profiles skewed towards a Th1 profile, whereas ratio values under 1 are indicative of skewness towards a Th2 profile.

The third research question examined the correlation between measures of stress and cytokinic profiles. Like age, earlier research had

suggested that higher levels of stress can also influence a shift in cytokinic profiles (Elenkov, 2008; Ramirez et al., 1996). Abnormal levels of stress hormones are proposed to exert anti-inflammatory properties by decreasing Th1 associated cytokines and increasing Th2 related cytokines, thus disrupting the allostatic equilibrium that should exist between cytokine profiles (Glaser et al., 2001; Haddad et al., 2002; Sapolsky, 2000). For two of the stress measures this relationship was found. Higher stress scores on the PSS were correlated with higher levels of IL-4. The PSS includes stressful experiences that may have occurred during the past month. Additionally, higher stress scores on the chronic events subscale were associated with lower IFN-γ levels. The chronic events subscale includes items that may or may not be true for the participant at this time. Thus, recent or ongoing stressors reflect the hypothesized directionality between cytokines and stressors, whereas past stressors and cytokine levels were not associated in the current correlation analysis.

Finally, our main research question investigated the ability of cytokinic profiles to moderate the relationship between stress and cognitive performance. Some research has found increased levels of stress hormones are detrimental to structures of the brain important for cognition, but this finding is not consistent (Fiocco et al., 2006; Kalmijn et al., 1998; Wright et al., 2005). Therefore, we proposed the inconsistent findings in the stress and cognitive literature may be due to a third factor, namely a disruption in the allostatic balance (Sterling & Eyer, 1988) between pro- and anti-inflammatory signaling molecules. Past studies have indicated negative

effects on the brain associated with Th1 related cytokines and protective and restorative actions to brain structures associated with Th2 related cytokines (Baune et al., 2008; Capuron et al., 2001; Nemni et al., 1992; Wyss-Coray & Rogers, 2012). Thus, in the current study, we hypothesized the interactions between predominant Th1 cytokinic profiles and stress would predict lower cognitive functioning, whereas the interaction between Th2 cytokinic profiles and stress would exhibit normal cognitive functioning. The ratio of Th1 to Th2, in this study the IFN-γ/IL-4 ratio, provides the best metric to represent Th1 versus Th2 cytokinic profile (Quan & Herkenham, 2002). Our results indicated the IFN-γ/IL-4 ratio is a significant moderator for stress and Number Comparison, TMT-A, and Digit Symbol Part 1, which are primarily measures of processing speed and executive functioning.

The results indicated IFN-γ/IL-4 ratios at or 1 SD above the mean (comparable to allostatic equilibrium or comparable to Th1 cytokinic profile, respectively) were significantly related to higher stress and lower cognitive scores, whereas 1 SD below the mean (comparable to Th2 cytokinic profile) was not a significant moderator. The results of this analysis confirm our predictions among persons who have a cytokinic profile that is directed towards the Th1 phenotype and stress exhibit a deleterious effect on cognitive functioning, whereas persons who are more in line with a Th2 phenotype, the relationship between stress and cognitive performance was not statistically significant. While the interactions were in the hypothesized directions, the main effects for IFN-γ/IL-4 and Digit Symbol Part 2 were not.

Viewed broadly, the results of hypothesis four suggest that the cytokine ratios may be indicative of a biological vulnerability, upon which the negative influence of stressful life events on cognitive performance are observed. That is, among older adults who are not biologically vulnerable, as evidenced by allostasis of the Th1 and Th2 cytokines, as well as those that are predominantly Th2, the influence of stressors on cognitive performance is not observed. However, among older adults with a predominant Th1 response, the negative effect of stressors on cognitive functioning are observed.

Limitations and future research

Although the results of the current study are informative, several limitations must be recognized. Foremost is the small sample size available for the cytokinic analyses. Due to technical difficulties with the Luminex instrument, we had to use a second instrument to run some of the cytokine assays. In doing so, it was discovered that the two instruments have different software associated with extracting data for analysis. Thus, the data from one software program (Bio-rad Manager 4.0) was imported into a second software program (Masterplex QT). To correct for any between assay plate variance, cytokine concentration values were standardized based on the particular mean and SD per instrument.

Second, the presence of a small sample of participants made it difficult to exert stringent control over the number of statistical comparisons that were conducted. By conducting multiple moderation analyses without format correction, we increased the probability of committing Type I error. Larger

sample sizes and appropriate control of multiple comparisons using the Holm-Bonferroni correction method, for example, would allow us to have greater confidence in the results that were observed here. Nonetheless, it should be noted that many of the regression analyses accounted for significant amounts of variances, suggesting that these effects may not be spurious.

Finally, past studies have implied correlations between Th2 cytokinic profiles and age. Future studies investigating this research should include younger participants as well as older participants to better document changes in immune profiles across the life span. Comparing the current sample to a younger sample may have revealed significant correlations between cytokines and age. Additionally, this cross-sectional study was limited to cytokine analysis of one blood sample per participant. A longitudinal study investigating cytokines, stress, and cognition would provide information about individual variation over time.

Conclusion

In this study, we tested why stress can negatively affect cognitive functioning in some older adults but not others by investigating immune signaling proteins. Ideally, cytokines should maintain an allostatic balance; however, changes in our immune system with age may result in a shift towards a more active Th2 immune profile. Stress hormones also exert effects on cytokines, and can result in a shift towards a predominant Th2 response. Moreover, while further research is needed, there is some evidence that peripheral inflammatory markers are able to cross the blood-

brain barrier. These findings along with literature suggesting pro-inflammatory cytokines are detrimental to cognitive processing, whereas anti-inflammatory cytokines are protective, led us to the main aim of this project. We proposed that variance between stressful experiences and cognitive functioning could be moderated by immune profiles and this was indeed found. Moderation analysis revealed that the interaction of predominant Th1 cytokinic profile and higher stress symptoms predicted lower cognitive functioning, whereas the interaction between a predominant Th2 cytokinic profile and stress did not predict lower cognitive functioning. Accordingly, we conclude that the combination of stressors and an immune profile skewed towards a Th1 response results in older adults being more vulnerable to poorer cognitive performance on measures of executive functioning and speed of processing.

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