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Hypothalamic-Pituitary-Adrenal axis and cognitive function in healthy older people: genetic, situational and individual factors

Eje Hipotalámico-Hipofisario-Adrenal y función cognitiva en personas mayores sanas: factores genéticos, situacionales e individuales

PhD Thesis

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THESIS OUTLINE

The world population is getting older, and this age group faces physical and mental health challenges, among which age-related cognitive decline stands out. However, there is great heterogeneity in the way people age; some older adults maintain good health and cognitive functioning until advanced ages ("healthy aging"), whereas others experience a significant decline ("pathological aging") (World Health Organization [WHO], 2018). Therefore, it is important to identify vulnerability and protective health factors in order to understand ways to improve cognitive function in this age group.

Among these factors, the Hypothalamic-Pituitary-Adrenal (HPA) axis functioning, which intervenes in the stress response, plays an important role. A dysregulation of the HPA-axis has been described in chronic stress (Miller, Chen, & Zhou, 2007), leading to neurological damage and cognitive impairment during aging (McEwen, 2008). However, many issues still remain unclear, and, therefore it is necessary to increase the knowledge about factors that could explain individual differences in age-related cognitive changes.

Therefore, this thesis aims to address the relationship between the HPA-axis and cognitive functioning during aging, taking into account genetic (Apolipoprotein E, ApoE, polymorphism), situational (loneliness), and individual factors (personality traits), which are described in the chapter one. The ApoE genotype is a major risk factor for late-onset Alzheimer's disease (AD) (Verghese, Castellano, & Holtzman, 2011), which is associated with a dysregulation of the HPA-axis functioning (Ouanes & Popp, 2019). Similarly, loneliness and personality traits

have also been related to HPA-axis functioning (Steptoe, Owen, Kunz-Ebrecht, & Brydon, 2004 and Soliemanifar, Soleymanifar, & Afrisham, 2018, respectively) and increased risk of dementia (Lara et al., 2019 and Terracciano et al., 2014, respectively). However, the role of the ApoE alleles, loneliness, and personality in cognitive function in non-demented older people is unclear (Lancaster, Tabet, & Rusted, 2016, Boss, Kang, & Branson, 2015 and Curtis, Windsor, & Soubelet, 2015, respectively).

Chapter two includes the main goals and hypotheses of this thesis and which empirical chapter addresses each of these goals.

Chapter three examines the role of the three allelic variations of the ApoE genotype in the association between the HPA-axis functioning during neuropsychological assessment and cognitive performance in healthy older adults.

Chapter four analyzes the association between loneliness and cognitive function in healthy older people, as well as the mediating role of the dysregulation of the HPA-axis in this association, as proposed by some authors (Boss et al., 2015; Ong, Uchino, & Wethington, 2015; Cacioppo, & Hawkley, 2009; Cacioppo, Capitanio, & Cacioppo, 2014). In addition, sex differences in these relationships are also examined.

Chapters five, six and seven present the analyses of the relationship between the big five personality traits and the HPA-axis functioning and cognitive change/decline in older men and women. In addition, it explores whether a dysregulation of the HPA-axis functioning or cognitive reserve mediates the relationship between personality and cognitive change/decline. Finally, the

association between personality and other objective and subjective health indexes are explored, as well as sex differences in these relationships.

Chapter eight contains a general discussion and the main findings of the aforementioned chapters, the limitations and strengths of this thesis, and directions for future research. Finally, chapter nine presents the main conclusions of this thesis.

ABBREVIATIONS

ACTH = Adrenocorticotropic Hormone

AD = Alzheimer's disease

ApoE = Apolipoprotein E

AUCg = Area Under the Curve with respect to the Ground

AUCi = Area Under the Curve with respect to the Increase

BMI = Body Mass Index

CAR = Cortisol Awakening Response

CRH = Corticotropin Releasing Hormone

CRQ = Cognitive Reserve Questionnaire

CVD = Cardiovascular Disease

DBP = Diastolic Blood Pressure

DCS = Diurnal Cortisol Slope

DS = Digit Span

GDS = Geriatric Depression Scale

GRs = Glucocorticoid Receptors

HbA1c = Glycated Hemoglobin

HDL- Cholesterol = High-Density Lipoprotein Cholesterol

HPA-axis = Hypothalamic-Pituitary-Adrenal axis

LDL-Cholesterol = Low-Density Lipoprotein Cholesterol

LNS = Letter-Number Sequencing

MMSE = Mini-Mental State Examination

MRs = Mineralcorticoid Receptors

NEO- FFI = NEO Five-Factor Inventory

R-UCLA = Revised University of California Los Angeles Loneliness Scale

RAVLT = Rey Auditory Verbal Learning Test

SBP = Systolic Blood Pressure

SES = Socioeconomic Status

SF-36 = Short Form Health Survey 36

TMT = Trail-Making Test

WC = Waist Circumference

WHO = World Health Organization

WHR =Waist-Hip Ratio

WM = Working Memory

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CHAPTER 1
GENERAL INTRODUCTION

1.1. AGING, HEALTH AND COGNITIVE FUNCTION

The pace of the population's aging around the world is increasing dramatically, and projections indicate that the number of people over 60 years old will reach two billion in 2050. This age group faces physical and mental health challenges, among which age-related cognitive changes stand out. However, there is great diversity in the way people age. Whereas some older adults maintain good health and cognitive functioning until advanced ages ("healthy/normal aging"), others experience a significant decline in their physical and cognitive capacities (World Health Organization [WHO], 2018), and in the most dramatical situations, develop dementia ("pathological aging"). In fact, the WHO has recognized dementia as a public health priority because it affects around 50 million people worldwide and is projected to reach 152 million in 2050, and it is one of the major causes of disability and dependency among older people (WHO, 2019). Therefore, it is important to identify vulnerability and protective health factors in order to understand ways to improve cognitive function in this age group.

Among these factors, Hypothalamic-Pituitary-Adrenal (HPA) axis functioning, which intervenes in the stress response, and specifically, cortisol, its final product, are of special relevance. HPA-axis dysregulation (i.e. with increased cortisol levels) has been described in chronic stress (Miller et al., 2007) and aging populations (Luz et al., 2003). Because the hippocampus and prefrontal cortex, brain structures that play an important role in memory and executive function, are rich in glucocorticoid receptors,

elevated cortisol levels would exert neurotoxic effects on these structures, leading to cognitive impairment (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007).

In this context, our group, the Laboratory of Social Cognitive Neuroscience, has an important research trajectory (MNEME Project) in the study of HPA-axis functioning, in both basal conditions and stressful situations, and its association with cognitive function in both young and older men and women, cross-sectionally, and more recently, longitudinally. In this line, we have analyzed the HPA- axis functioning by measuring reactivity to and recovery from an acute standardized laboratory stressor (for a review see: Pulopulos, Hidalgo, Puig-Perez, Salvador, 2018), as well as basal measures such as the cortisol awakening response (CAR) (Almela, Meij, Hidalgo, Villada, & Salvador, 2012; Hidalgo, Almela, Pulopulos, & Salvador, 2016; Pulopulos, Hidalgo, Puig-Perez, Salvador, 2016; Pulopulos, Puig-Perez, Hidalgo, Villada, & Salvador, 2016) and the diurnal cortisol slope (DCS) (Hidalgo et al., 2016) in saliva samples and in hair cortisol (Pulopulos et al., 2014). We demonstrated a modulator effect of sex and age in the stress response. Men showed higher cortisol responses than women in both older and young people. In addition, older women showed worse recovery of their cortisol levels (Almela et al., 2011; Hidalgo et al., 2012). Regarding the association between stress and cognitive function, our results showed that acute stress affects retroactive interference in older adults (Hidalgo et al., 2012), especially in women (Almela et al., 2011). Our results also support the idea that older people are less sensitive to acute effects of cortisol on working memory (Pulopulos et al., 2015) and on retrieval (Hidalgo et al., 2015; Pulopulos et al., 2013). Due to the importance of

individual differences in stress responses (Salvador, 1993; Salvador & Costa, 2009), our research has also focused on some personality dimensions that exert protector (optimism) or harmful (neuroticism and anxiety) effects on health. We reported that optimism was related to stress recovery, but not to reactivity (Puig-Perez et al., 2015), whereas neuroticism was not associated with either of them (Puig-Perez, Villada, Pulopulos, Hidalgo, Salvador, 2016b). By contrast, neuroticism was related to CAR in women (Puig-Perez, Almela, Pulopulos, Hidalgo, & Salvador, 2016a).

The present thesis aims to advance the knowledge about other factors that may affect the relationship between the HPA-axis and cognitive functioning during aging. This thesis includes the bedtime cortisol level index, not previously analyzed by our research group. Bedtime cortisol seems to be an important index to consider because previous research observed that higher bedtime cortisol levels were related to worse cognitive function (Geerlings et al., 2015; Li et al., 2006; Tene et al., 2018), and smaller total brain volume (Geerlings et al., 2015).

This thesis also includes the Apolipoprotein E (ApoE) genotype as a possible moderator in the relationship between HPA-axis functioning and cognition. The ApoE-ε4 allele is a major risk factor for late-onset Alzheimer's disease (AD), whereas the ApoE-ε2 allele seems to be protective (Bertram, Lill, & Tanzi, 2010; Verghese et al., 2010). Although several studies have explored the role of these alleles in cognitive function in non-demented older people, this question remains unclear (Lancaster et al., 2016). Moreover, the ApoE genotype may also be associated with HPA-axis functioning because increased cortisol levels are observed in AD, which may induce

or exacerbate cerebral AD pathology by increasing the amyloid-β brain burden, tau pathology, and oxidative stress, leading to neurodegeneration (Ouanes & Popp, 2019). Therefore, this evidence suggests that the ApoE gene may affect the association between the HPA-axis and cognitive function.

Growing evidence also suggests that loneliness is associated with an increased risk of cognitive decline and dementia (Cacioppo & Hawkley, 2009; Lara et al., 2019), although studies in non-demented older people are inconsistent (Boss et al., 2015). Moreover, loneliness has been suggested to be a psychological experience that contributes to biological stress (Steptoe et al., 2004). Therefore, several studies have proposed HPA-axis functioning as one of the biological mechanisms underlying the association between loneliness and cognition (see reviews: Boss et al., 2015; Cacioppo & Hawkley, 2009; Cacioppo, 2014; Ong et al., 2015). Furthermore, despite evidence showing that women report greater loneliness, which seems to affects women's health more than men's (Christiansen, Larsen, & Lasgaard, 2016), sex differences have hardly been studied. Therefore, analyzing the mediating role of HPA-axis functioning in the association between loneliness and cognition, as well as sex differences in healthy older people, could help to clarify the inconsistent findings reported in the literature.

The big five personality traits are also known to influence several health outcomes (Friedman, Kern, & Reynolds, 2010; Jerram & Coleman, 1999; Weston, Hill, & Jackson, 2015), including dementia (Terracciano et al., 2014). However, the association between personality traits and cognitive function in nonclinical populations of older adults remains unclear (Curtis et al., 2015). Personality is known to play a role

in stress reactivity (Soliemanifar et al., 2018), and therefore it may influence the HPA-axis functioning. Therefore, in this thesis we have analyzed the association between personality and HPA-axis functioning. Moreover, this thesis aimed to explore the mediating role of HPA-axis functioning in the association between personality and cognitive decline in a four-year follow-up study. Furthermore, this thesis also aimed to explore the mediating role of cognitive reserve in the association between personality traits and cognitive decline, as proposed in some studies (Chapman et al., 2012; Sharp, Reynolds, Pedersen, & Gatz, 2010; Soubelet & Salthouse, 2010). Sex differences were also taken into account as a possible moderator of these associations.

Finally, this thesis also aimed to analyze the relationship between personality traits and other objective health outcomes (i.e. adiposity, glycated hemoglobin [HbA1c], cholesterol, and pulse pressure). These health outcomes, which are risk factors for metabolic syndrome, have also been related to HPA-axis dysregulation (Björntorp, & Rosmond, 2000) and cognitive impairment and dementia (Frisardi et al., 2010). In addition, the relationship between personality and subjective health was also addressed, as well as the moderating role of sex, which may explain some of the inconsistent results (Jerram & Coleman, 1999; Sutin et al. 2010b; Sutin & Terracciano, 2016B). Although the relationships between these health indexes and cognitive function have not been addressed in this thesis, it could help to indicate possible mediators in the association between personality and cognitive function that could be addressed in future research.

In summary, the aim of the thesis is to advance the knowledge about factors that may intervene in the relationship between the HPA-axis and cognitive function during aging, in order to explain individual differences in cognitive decline. Among these factors, we have proposed genetic (ApoE), situational (loneliness), and individual (personality) variables.

1.2. HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

The HPA-axis is a complex neuroendocrine system that plays a key role in the stress response and comprises three endocrine glands: the hypothalamus and the pituitary and adrenal glands. The activation of this axis occurs when, in response to a mental or physical stressor, neurons in the paraventricular nucleus of the hypothalamus secrete the corticotropin-releasing hormone (CRH). The CRH travels through the portal system to the pituitary gland, which responds to this stimulus with the release of the adrenocorticotropic hormone (ACTH) into the bloodstream. The ACTH stimulates the *zona fasciculata* layer of the adrenal cortex, which synthesizes and releases cortisol, the main glucocorticoid in humans, into the bloodstream (Jacobson, 2005). The main function of glucocorticoids is to prepare the organism to cope with a threat, and therefore glucocorticoids are essential for the survival of individuals. In these situations, glucocorticoids increase blood pressure and breathing and the supply of glucose and oxygen to neurons and heart and skeletal muscle fibers in order to respond appropriately to the stressful situation (Sapolsky, Romero, & Munck, 2000). The HPA-axis functioning is regulated by a negative feedback system to recover homeostasis, that is, to keep glucocorticoids at tolerable levels. Thus, the secretion of CRH and

ACTH can be suppressed if the glucocorticoid concentrations in blood are high (Keller-Wood, & Dallman, 1984).

In non-stressful situations, the HPA-axis follows a diurnal rhythm. Cortisol levels are high upon awakening and show a rapid 50-160% increase, peaking between 30 and 45 min post-awakening (conceptualized as the cortisol awakening response; CAR) (Clow, Thorn, Evans, & Hucklebridge, 2004; Fries, Dettenborn, & Kirschbaum, 2009), and followed by a steady decrease throughout the day, reaching the lowest levels in the evening (the difference between awakening and evening cortisol is conceptualized as the diurnal cortisol slope; DCS) (Adam et al., 2017). In the current thesis, these indexes (CAR and DCS), along with awakening and evening cortisol levels and the diurnal cortisol output (measured as the area under the curve with respect to the ground; AUCg), have been used to measure the HPA-axis functioning.

1.2.1. HPA-axis dysregulation: aging and cognitive impairment

A dysregulation of the HPA-axis has been reported in chronic stress, showing a higher diurnal cortisol output and a flattened diurnal slope with lower morning and higher evening cortisol levels (Miller et al., 2007). Similarly, this dysregulated HPA-axis pattern with a flattened diurnal profile has been associated with poorer mental and physical health (Adam et al., 2017; Clow et al., 2004; Fries et al., 2009) and with aging (Heaney, Phillips, & Carroll, 2010). Although there are important inter-individual differences in the HPA-axis functioning in older adults (Lupien et al., 1996), it has been proposed that a dysregulation of HPA-axis functioning is possibly due to the

cumulative exposure of the brain to cortisol throughout life. Based on the glucocorticoid cascade hypothesis, this would produce a down regulation of glucocorticoid receptors in the hippocampus, leading to a dysregulation of the feedback system and, therefore, to higher glucocorticoid levels (Sapolsky, Krey, & McEwen, 1986).

The hippocampus and prefrontal cortex are brain structures that play a role in HPA-axis regulation (Fries et al., 2009), but also in declarative memory, attention, working memory, and executive function. Because these brain areas are rich in glucocorticoid receptors, they are especially susceptible to structural damage in conditions where the release of glucocorticoids is abnormally elevated (Sapolsky et al., 2000). Therefore, an HPA-axis dysregulation and, particularly, high cortisol levels in the elderly, have been associated with an increased risk of dementia and AD (Ouanes, & Popp, 2019).

However, the association between the HPA-axis functioning and cognitive performance in non-demented older people is complex and poorly understood. Higher diurnal cortisol levels have been related to poorer cognitive performance, specifically on verbal memory and executive function (Li et al., 2006; Ouanes et al., 2017a), but other studies did not find these results (Ennis, Moffat, & Hertzog, 2016; Harris, Cox, Brett, Deary, & Maclullich, 2017; Singh-Manoux et al., 2014). Similarly, a flatter DCS has been associated with poorer cognition in some studies (Beluche, Carrière, Ritchie, & Ancelin, 2010; Gerritsen, Comijs, Deeg, Penninx, & Geerlings, 2011; Stawski et al., 2011), but others failed to observe this association (Ennis et al., 2016; Hidalgo et al.,

2016; O'Hara et al., 2007; Singh-Manoux et al., 2014). Likewise, higher awakening cortisol levels have been associated with better (Ennis et al., 2016; Singh-Manoux et al., 2014) and worse cognitive performance (Beluche et al., 2010; O'Hara et al., 2007) even in follow-up studies (Beluche et al., 2010). However, although less studied, higher evening cortisol levels have been associated more consistently with worse cognitive performance (Gerritsen et al., 2011; Li et al., 2006; Singh-Manoux et al., 2014; Stawski et al., 2011).

The complexity of the association between HPA-axis functioning and cognitive performance can also be explained by the effect of glucocorticoids through two types of receptors: type I (Mineralocorticoid Receptors, MRs) and type II (Glucocorticoid Receptors, GRs). MRs show 6 to 10 times higher affinity for glucocorticoids than GRs (de Kloet, Oitzl, & Joëls,1999; Joels, 2006). Under basal conditions, MR occupation is approximately 70-80%, whereas GR show a low occupation. However, in conditions of high glucocorticoid levels, such as stress situations, MR is saturated, whereas there is a GR occupation of approximately 70%. (Reul & de Kloet, 1985; De Kloet, Joëls, & Holsboer, 2005). Moreover, these receptors are distributed differently throughout the brain. The hippocampus, mainly involved in episodic memory, expresses both MRs and GRs, whereas the prefrontal cortex, involved in executive functions, only expresses GRs. (Patel et al., 2000; Joëls, Krugers, Lucassen, & Karst, 2009). Whereas MRs have been associated with enhancing effects on cognitive performance, GRs have been related to negative effects. Therefore, higher cortisol levels would lead to worse executive function performance in a linear way. By contrast, the effects of cortisol

levels on episodic memory have often been described as having an inverted-U shape pattern. Therefore, very low or high glucocorticoids levels would worsen cognitive performance, whereas medium levels would facilitate it. Because both GRs and MRs are expressed in the hippocampus, moderate levels of cortisol would only activate the MR with higher affinity, leading to memory enhancement effects. As cortisol levels increase, MRs would be saturated, and the activation of GRs would lead to detrimental effects on memory (Lupien et al., 2007; McEwen, 2007).

1.3. APOLIPOPROTEIN E

The ApoE genotype is the strongest genetic risk factor for late-onset AD (Bertram et al., 2010). The gene encoding ApoE, which is located on chromosome 19, has three common allelic variations: ApoE-ε2, ApoE-ε3, and ApoE-ε4. Every individual inherits from each of his/her parents one allele of ApoE, which generates six possible genotypes: ApoE-ε2ε2, ApoE-ε2ε3, ApoE-ε2ε4, ApoE-ε3ε3, ApoE-ε3ε4, and ApoE-ε4ε4. The most common variant throughout the population is the ApoE-ε3 allele, with an occurrence rate of approximately 80%. The ApoE-ε2 and ApoE-ε4 alleles are less common, with an occurrence rate of 8% and 14%, respectively (Small, Rosnick, Fratiglioni, & Bäckman, 2004).

ApoE plays a role in lipid and glucose metabolism, cerebral vascular function, synaptic plasticity, and neuroinflammation processes, as well as clearance and accumulation of the amyloid-β, one of the pathological components of AD (Liao, Yoon, & Kim, 2017; Verghese et al., 2011). Specifically, the amyloid-β accumulation

in the human brain follows the pattern of ApoE-ε4>ApoE-ε3>ApoE-ε2 (Castellano et al., 2011).

1.3.1. ApoE and cognitive function

Of the three allelic variations, the presence of the ε4 allele is the major risk factor for developing late-onset AD. Approximately, 50% of the individuals with AD possess at least one copy of the ApoE-ε4 allele, which is two to three times higher than in the general population (Parker et al., 2005). The presence of the ApoE-ε4 allele increases the probability of developing AD in a dose dependent manner, where individuals' heterozygous ApoE-ε4 (ApoE-ε2/ε4 or ApoE-ε3/ε4) are 3-4 times more likely to develop AD, compared to homozygous ApoE-ε4 (ApoE-ε4/ε4), where the risk is increased 10-12 fold (Farrer et al., 1997). Additionally, the ApoE-ε4 allele has been associated with a higher risk of developing Mild Cognitive Impairment (MCI) (Dixon et al., 2014), accelerated cognitive decline in normal aging (Bretsky, Guralnik, Launer, Albert, & Seeman, 2003; Caselli et al., 2004; 2011; Schiepers et al., 2012; Smith, 2002), and decreased longevity (Corder et al., 1996; Smith, 2002). By contrast, the ApoE-ε2 allele has been associated with survival in older people (Corder et al., 1996) and a reduced risk of developing AD (Verghese, 2011).

This evidence has generated interest in the role ApoE variants play in the cognitive function of healthy individuals, with heterogeneous results. Some studies observed worse cognitive performance in healthy individuals who were ApoE-ε4 carriers (e.g. Bondi, Salmon, Monsch, Galasko, & Butters, 1995; Deary et al., 2004;

Flory, Manuck, Ferrell, Ryan, & Muldoon, 2000; Honea, Vidoni, Harsha, & Burns, 2009; Kang, Logroscino, De Vivo, Hunter, & Grodstein, 2005; Small et al., 2004; Wilson, Bienias, Berry-Kravis, Evans, & Bennett, 2002). Conversely, other studies found better cognitive performance in ApoE-ε4 carriers (Alexander et al., 2007; Mondadori et al., 2006; Riley et al., 2000) or no association (Cohen, Small, Lalonde, Friz, & Sunderland, 2001; Jorm et al., 2007; Lineweaver, Bondi, Galasko, & Salmon, 2014; Small et al., 2000; Smith et al., 1998).

This heterogeneity in the results could be due to several factors, such as the different age range of the participants. Some studies suggested that the ApoE effect on cognition may vary across the life span. Two studies in young ApoE-ε4 carriers demonstrated beneficial effects on cognitive performance (Alexander et al., 2007; Mondadori et al., 2007). However, a meta-analysis including children, adolescents, and young adults found no effect of the ApoE-ε4 allele on cognitive performance (Ihle, Bunce, & Kliegel, 2012), whereas two meta-analyses in healthy older people reported that the ApoE-ε4 allele would have adverse effects on cognitive function (Small et al., 2004; Wisdom, Callahan & Hawkins, 2011). Nevertheless, a recent study on mid-adulthood supported comparable cognitive performance between ApoE-ε4 carriers and non-carriers, and the authors concluded that the cognitive performance profile of ApoE- ε4 carriers remains elusive (Lancaster et al., 2016).

Another factor that could lead to inconsistent findings would be the inclusion of undetected preclinical dementia cases in some studies. Therefore, some of the ApoE-ε4 related deficits may be attributable to an overrepresentation of preclinical cases of

AD, rather than reflecting the effect of this allele alone (Bondi, Salmon, Galasko, Thomas, & Thal, 1999).

Moreover, due to the association between ApoE and AD, and the evidence that declarative memory is the most affected cognitive function in this disease, most of the research on ApoE in healthy older people has only focused on declarative memory performance, with few studies investigating the association between ApoE and other cognitive functions, such as working memory, attention, and executive function (Reinvang, Winjevoll, Rootwelt, & Espeseth, 2010). This tendency may have led to an overrepresentation of the effect of ApoE on declarative memory, rather than on other cognitive functions.

Another important factor in explaining these inconsistent findings could be the way of grouping the various types of allelic variations. Most of the studies grouped participants as ApoE-ε4 carriers or ApoE-ε4 non-carriers (including the ApoE-ε2ε2, ApoE-ε2ε3, and ApoE-ε3ε3 genotypes). Few studies have explored the effect of the ApoE-ε4 allele independently from the ApoE-ε2 effects. The ApoE-ε2 allele has been related to better cognitive performance and lower rates of decline compared to ApoE-ε4 and ApoE-ε3 carriers (Deary et al, 2004; Kang et al., 2005; Helkala et al., 1995; 1996; Wilson et al., 2002). Therefore, not considering the ApoE-ε2 independently could lead to an overestimation of ApoE-ε3 performance and an underestimation of ApoE-ε4 performance.

1.3.2. ApoE and HPA-axis functioning

Increased cortisol levels are a well-established feature in AD dementia, and findings have suggested that increased cortisol may induce or exacerbate cerebral AD pathology by increasing amyloid-β brain burden, tau pathology, and oxidative stress, leading to neurodegeneration (Ouanes & Popp, 2019). This evidence, along with the fact that ApoE-ε4 is a risk factor for developing AD (Bertram et al., 2010), suggests that the ApoE gene may affect the association between the HPA-axis and cognitive function (Peavy et al., 2007). Furthermore, similar to preclinical brain changes related to AD, healthy older-adult ApoE-ε4 carriers showed structural and functional abnormalities of the hippocampus (Lu et al., 2011), a brain structure involved in both HPA-axis regulation and memory processes (Lupien et al., 2007).

Few studies have explored the relationships between ApoE genotype, cortisol levels, and cognitive performance in healthy older people, with inconsistent results. Some studies reported differences in the association between HPA-axis dysregulation and worse cognition according to the ApoE genotype, where the ApoE-ε4 allele carriers were especially vulnerable compared to the ApoE-ε4 allele non-carriers (Gerritsen et al., 2011; Lee et al., 2008; Singh-Manoux et al., 2014), but not others (Berteau-Pavy, Park & Raber, 2007; Fiocco, Poirier, Joober, Nair & Lupien, 2008; Lara et al., 2013; Li et al., 2006). Therefore, more research is needed to clarify the moderation role of the ApoE genotype in the association between cortisol levels and cognitive function, considering the ApoE-ε2 and the ApoE-ε4 alleles independently in healthy older adults.

1.4. LONELINESS

1.4.1. Loneliness and aging

Loneliness or perceived social isolation is understood as a subjective feeling of dis-satisfaction with social relationships (Young, 1982). It is an important concern among older people, and prevalence statistics indicate that approximately thirty percent of older adults report feelings of loneliness (Ong et al., 2015; Perissinotto, Cenzer, & Covinsky, 2012; Victor, Scambler, Bowling, & Bond, 2005). This age group is especially vulnerable due to life transitions in older adults that may lead to social disconnection, such as retirement, widowhood, the death of same aged friends, children leaving home, health problems, impaired mobility and hearing loss, and declining economic resources (Holt-Lunstad, 2017; Pinquart & Sorensen, 2001). In addition, being a woman has been associated with an increased risk of loneliness because they are more likely to be affected by widowhood and assume a care-giving and housemaker role that may keep them from establishing non-family social contacts (Pinquart & Sorensen, 2001). The importance of loneliness lies in the fact that it has been considered a major risk factor for physical and mental health problems in later life and mortality (Holt-Lunstad, Smith, Baker, Harris, & Stephenson, 2015; Ong et al., 2015), a situation that has also been recognized by the WHO (2013).

1.4.2. Loneliness and cognitive function

Loneliness has been positively associated with an increased risk of MCI and dementia (Cacioppo & Hawkley, 2009; Lara et al., 2019). In a systematic review

conducted by Boss et al. (2015) on loneliness and cognitive function in older adults, the authors concluded that greater loneliness was associated with lower cognitive functioning, specifically global cognitive function, intelligence quotient, processing speed, and immediate and delayed recall. However, some of these associations were weak and/or disappeared after controlling for psycho-sociodemographic variables. Moreover, studies included in this review reported mixed findings about the association between loneliness and the different cognitive domains assessed, which could be explained by methodological differences between studies, such as the cognitive status of participants and the questionnaires administered to assess loneliness. Furthermore, despite the evidence that women are at higher risk of loneliness than men (Pinquart & Sorensen, 2001), most of the studies did not explore the role of sex in the association between loneliness and cognitive function. Therefore, the relationship between cognitive function and loneliness (assessed with the 20-item revised UCLA [R-UCLA] Loneliness Scale) in healthy older people, as well as sex differences in this relationship, is a pending issue.

1.4.3. Loneliness and HPA- axis functioning

Because loneliness has been described as a risk factor for several physical and mental health problems in later life, including not only impaired cognitive function and dementia, but also depressive symptomatology, hypertension, heart disease and stroke, and diminished longevity (Cacioppo & Hawkley, 2009; Cacioppo et al., 2014; Holt-Lunstad et al., 2015; Ong et al., 2015), it is important to increase the knowledge about the neurobiological substrates of loneliness, especially in older adults. In a review,

Cacioppo et al. (2014) proposed age-related changes in neuroendocrine, cardiovascular, and inflammatory stress responses as candidates.

Furthermore, loneliness has been suggested to be a psychological experience that contributes to biological stress (Steptoe et al., 2004), and therefore several studies have explored its association with the HPA-axis functioning. Several studies have explored the association between loneliness and the diurnal cortisol rhythm in young (Cacioppo et al., 2000; Doane & Adam, 2010; Drake, Sladek, & Doane, 2016; Lai, Leung, Lee, Lam, & Berning, 2018; Pressman et al., 2005), middle-aged (Ebrecht et al., 2004; Grant, Hamer, & Steptoe, 2009; Steptoe et al., 2004), or older participants (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Cole et al., 2007; Schutter et al., 2017), with inconsistent findings. Of the three studies carried out in older people, two studies did not observe an association between loneliness and awakening cortisol levels or the DCS (Adam et al., 2006; Schutter et al., 2017), whereas another study found that lonelier individuals showed a blunted cortisol rhythm (Cole et al., 2007). In addition, none of these studies analyzed the association between loneliness and the diurnal cortisol output or evening cortisol levels, although other studies in young people observed a significant positive association (Cacioppo et al., 2000; Lai et al., 2018). Furthermore, although sex seems to be an important moderator in the association between loneliness and health outcomes (Christiansen, Larsen, & Lasgaard, 2016), none of these studies analyzed it. Therefore, more research is needed to clarify the association between loneliness and the diurnal cortisol rhythm in older adults, considering several cortisol indexes and taking sex differences into account.

Evidence shows that loneliness has been associated with an increased risk of cognitive decline and dementia (Cacioppo & Hawkley, 2009; Lara et al., 2019). Several studies have analyzed the relationship between loneliness and HPA-axis functioning, and HPA-axis dysregulation has been proposed as one of the biological pathways underlying the association between loneliness and cognitive function (Boss et al., 2015; Ong et al., 2015; Cacioppo & Hawkley, 2009; Cacioppo et al., 2014). However, the mediating role of the HPA-axis in the relationship between loneliness and cognitive function has not yet been explored and is a pending issue.

1.5. PERSONALITY

1.5.1. Personality, health, and aging

Personality traits are among the factors that are known to influence health, subjective well-being, and mortality risk during aging (Friedman et al. 2010; Jerram & Coleman, 1999; Weston et al., 2015). There is a general consensus that the big five personality traits (neuroticism, extraversion, openness, agreeableness, and conscientiousness) are largely representative of the most basic factors of adult personality (Costa & McCrae, 1992), and these findings have been replicated cross-culturally (Costa & McCrae, 2003). Personality traits measure individual differences in relatively enduring patterns of thoughts, feelings, and behaviors, although increased age is associated with changes in these traits. Whereas conscientiousness and agreeableness tend to increase in older ages, neuroticism and openness tend to decrease. On the contrary, some components of extraversion tend to increase whereas

others decrease (Roberts, Walton, & Viechtbauer, 2006). Moreover, it has been suggested that the link between personality traits and health may be cumulative over time, and therefore personality could have a greater influence on disease in old age (Weston et al., 2015).

Neuroticism is defined as the tendency to be emotionally unstable and experience negative emotions such as anger, anxiety, and depression (Costa & McCrae, 1992a; McCrae & John, 1992). Neuroticism has considerable importance in public health because it is a robust predictor of different mental and physical disorders, as well as quality of life and longevity (Lahey, 2009). Although neuroticism is heritable and relatively stable over time, it peaks in early adolescence and adulthood, with no sex differences at these ages. However, after this period, the heritability of neuroticism deceases with age in both sexes, but slightly more in men. In addition, women tend to score higher on neuroticism than men (Lahey, 2009). Therefore, sex seems to be an important factor to take into account when analyzing the relationship between neuroticism and health, where older women may be especially vulnerable to the adverse effects of neuroticism on health.

The influence of neuroticism on physical and mental health outcomes could be due to genetic influences because the same genes that influence neuroticism also influence many different mental disorders. In addition, individuals who are higher in neuroticism appear to be more likely to experience stressful events and have less social support, and they tend to respond to stressful events with more pronounced and less well-regulated emotional responses through less effective coping strategies. Furthermore, although not fully consistent, there is growing evidence suggesting that this personality

trait could moderate the magnitude of the physiological response to stressors, leading to greater sympathetic and HPA-axis reactivity and immune system alterations. Moreover, individuals who were high in neuroticism tend to engage in risky health behaviors, such as smoking and alcohol or drug abuse (Lahey, 2009).

Conscientiousness is defined by the tendency to be persistent, organized, and goal-directed, and show self-control and self-discipline (Costa, McCrae & Dye, 1991; McCrae & John, 1992). There is growing evidence that conscientiousness is a strong predictor of well-being, health, and longevity. Conscientiousness, despite being a trait, tends to increase in young adulthood, middle age, and into old age (Roberts et al., 2006). The influence of conscientiousness on health may be explained by the evidence that conscientious individuals tend to engage in healthier behaviors such as less smoking, moderate alcohol consumption, physical exercise, and eating healthier food, as well as maintaining healthier friendships and more stable marriages, and having a better education, meaningful careers, and higher incomes. All this evidence would explain the fact that conscientiousness is a predictor of health and lower mortality (Friedman & Kern, 2014).

Extraversion is defined by the tendency to be assertive and social, experience positive affect, and seek excitement (Costa & McCrae, 1992a; McCrae & John, 1992). Extraversion has also been related to better health, although to a lesser degree than neuroticism and conscientiousness (Weston et al., 2015). Extraversion has been linked to better positive affect and well-being and to a better perceived health status (Costa & McCrae, 1984; McCrae & Costa, 1991). However, the literature on the association between extraversion and health seems less consistent, possible because extraversion

has been related to better (diet and exercise), but also to negative (alcohol and smoking) health behaviors (Booth-Kewley & Vickers, 1994).

Openness is defined by the tendency to be creative, curious, sensitive to aesthetics, and open to new ideas and experiences (Costa & McCrae, 1992a; McCrae & John, 1992), whereas agreeableness is defined by the tendency to be altruistic, trusting, modest, and compliant (Costa et al., 1991; Costa & McCrae, 1992a). Although these two personality traits have been studied less, higher scores seem to be protective risk factors in older adults (Weston et al., 2015).

1.5.2. Personality and HPA-axis

The evidence that personality traits predict health outcomes and mortality risk has aroused interest in studying the biological pathways that could explain this association. Personality is known to play a role in ways of coping with psychological stress (Kaur, Chodagiri, & Reddi, 2013; Soliemanifar et al., 2018). Individuals who score higher on neuroticism tend to perceive more stressors and respond with intense emotional reactions, and so this personality trait correlates highly with chronic stress, negative feelings, and mental disorders such as anxiety and depression. By contrast, conscientiousness has been related to better coping and emotion regulation abilities (see reviews: Friedman & Kern, 2014; Lahey, 2009). Similarly, because extraversion is associated with warmth, assertiveness, and positive emotions (Costa & McCrae, 1992b), extraverted individuals may deal better with stress.

Thus, several studies have analyzed how personality influences the biological reactivity to stressful events, which involves the autonomic nervous system, the immune system, sex hormones, the sympathetic-adrenal-medulla system, and the HPA-axis (see review: Soliemanifar et al., 2018).

Evidence has suggested that personality moderates an age-related decline in prefrontal and medial temporal regions (Jackson, Balota, & Head, 2011), which are brain structures involved in HPA-axis regulation and, therefore, in the stress response (Fries et al., 2009). HPA-axis dysregulation has been associated with both physical and mental health outcomes (see review and meta-analysis: Adam et al., 2017; Clow et al., 2004; Fries et al., 2009) and chronic stress. Moreover, although HPA-axis dysregulation has also been reported in aging (Heaney et al., 2010), few studies have analyzed the association between the diurnal cortisol pattern and personality in older people (Gerritsen et al., 2009; Ouanes et al., 2017b; Puig-Perez et al., 2016a).

In older adults, higher neuroticism has been associated with higher evening cortisol levels (Gerritsen et al., 2009) and with lower overall morning cortisol concentrations and greater CAR only in women (Puig-Perez et al., 2016a), but not with the DCS or diurnal cortisol (Gerritsen et al., 2009; Ouanes et al., 2017b). Similarly, extraversion was related to increased diurnal cortisol in one study (Ouanes et al., 2017b), but not to morning cortisol levels (Puig-Perez et al., 2016a). Only Ouanes et al. (2017b) included the big five personality traits and found that less openness was associated with increased diurnal cortisol, but no association was observed with conscientiousness or agreeableness. Evidence suggests that sex is an important moderator in the relationship between personality and HPA-axis functioning (DeSoto & Salinas, 2015). However,

only one of these studies considered sex differences (Puig-Perez et al., 2016a). Therefore, more research is needed to clarify the association between the big five personality traits and HPA-axis functioning in older adults, considering different cortisol indexes and the moderating role of sex.

1.5.3. Personality and cognitive function

Personality traits have been associated with risk of dementia. Specifically, neuroticism has been associated with a higher risk of AD, whereas openness, agreeableness, and conscientiousness have been associated with a lower risk (Terracciano et al., 2014).

Several studies have examined the role of personality as a possible predictor of some of the variability in cognitive function in nonclinical populations of older adults, but the results are inconsistent, and the question remains unclear (Curtis et al., 2015). In a review analyzing the relationship between the big five personality traits and cognitive ability in older adults, Curtis et al. (2015) reported that openness and conscientiousness were the personality traits most consistently related to cognitive ability. Openness has been related to better performance on several cognitive abilities (Aiken-Morgan et al., 2012; Baker, & Bichsel, 2006; Booth, Schinka, Brown, Mortimer, & Borenstein, 2006; Chapman et al., 2012; Sharp et al., 2010), but not to cognitive decline (Chapman et al., 2012; Sharp et al., 2010). However, conscientiousness has not been generally related to any cognitive ability (Chapman et al., 2012; Ouanes et al., 2017b), but it has been related to reduced rates of cognitive

decline (Chapman et al., 2012; Wilson, Schneider, Arnold, Bienias, & Bennett, 2007b). In addition, extraversion seems to be related to better long-term memory in cross-sectional studies (Allen, Kaut, Baena, Lien, & Ruthruff, 2011; Baker & Bichsel, 2006), whereas agreeableness seems to be unrelated to cognitive function and/or cognitive decline (Booth et al., 2006; Chapman et al., 2012), but not in all studies (Aiken-Morgan et al., 2012; Baker & Bichsel, 2006). Moreover, the association between neuroticism and cognitive abilities also had mixed results, with some studies reporting no association (Baker & Bichsel, 2006; Ouanes et al., 2017b), whereas other studies found that higher neuroticism was related to worse verbal memory, executive function, and/or cognitive decline (Aiken-Morgan et al., 2012; Booth et al., 2006; Caselli et al., 2016; Chapman et al., 2012; Klaming, Veltman, & Comijs, 2017; Meier, Perrig-Chiello, & Perrig., 2002).

The heterogeneity in the results highlights the importance of taking into account the mediating/moderating mechanisms that could influence the associations between personality traits and cognitive ability in older adults. It has been hypothesized that personality may affect the risk of cognitive decline through different mechanisms such as the stress response, health behaviors, and cognitively stimulating activity (Curtis et al., 2015). It has been hypothesized that openness could be related to better cognitive ability because individuals scoring high on this personality trait have a greater predisposition to performing cognitively stimulating activities, which would contribute to greater cognitive reserve (Chapman et al., 2012; Sharp et al., 2010; Soubelet & Salthouse, 2010) and, therefore, to coping better with age-related changes and

pathology in the brain (Stern, 2009). Moreover, it has been suggested that, because conscientiousness is related to health behaviors, high scores on this personality trait would protect against age-related brain changes (Curtis et al., 2015; Wilson et al., 2007b). Regarding extraversion, it has been associated with lower arousal, which could provide beneficial effects on cognitive performance (Chamorro-Premuzic & Furnham, 2004), but highly extroverted individual could also be more easily distracted and, therefore, show impaired performance (Costa, Fozard, McCrae, & Bossé, 1976; Gold & Arbuckle, 1990). Moreover, highly extraverted individuals may compensate for the loss of social interactions and the decline in sensory functions related to aging, which are also related to cognitive performance (Curtis et al., 2015). Furthermore, extraverted individuals experience higher positive affect, which would enhance memory encoding and subsequent memory retrieval (Allen et al., 2011). Although there is no theoretical base for the association between agreeableness and cognitive function, it has been reported that is important to select which covariates could moderate/mediate these associations (Curtis et al., 2015). Finally, neuroticism could impair cognitive performance because individuals scoring high on this personality trait perceive and experience greater stress in daily life (Bolger & Schilling, 1991; Suls & Martin, 2005). The HPA-axis activation in chronic stress would lead to increased cortisol levels, which would exert neurotoxic effects on brain areas that play a role in cognitive function (Sapolsky, 1994) (Curtis et al., 2015). However, although neuroticism is the personality trait most strongly associated with stress reactivity, the other personality traits have also been associated with stress reactivity (Soliemanifar et al., 2018).

Therefore, the HPA-axis functioning is proposed as one of the biological mechanisms mediating the association between personality and cognitive function. To our knowledge, only one recent study explored this association, and the authors did not observe a mediating effect of personality in the association between cortisol (measured with the AUCg and the CAR) and different cognitive domains, including global cognition, memory, and verbal fluency (Ouanes et al., 2017b). However, because several studies have proposed HPA-axis functioning as one of the mechanisms underlying the association between personality traits and cognitive function, it is possible that other cortisol indexes could explain these associations. Furthermore, Ouanes et al. (2017b) did not consider the moderating effect of sex on these associations, and sex may be an important moderator that could help to clarify the associations between personality and cognitive function in older people.

1.5.4. Personality and other objective health outcomes

Personality also seem to be related to adverse health outcomes, such as risk of obesity, dysglycemia, dyslipidemia, and arterial hypertension (Sutin, Stephan, & Terracciano, 2019; Terracciano et al., 2014; Weston et al., 2015), whose prevalence increases with age (Hildrum, Mykletun, Hole, Midthjell, & Dahl, 2007). These health outcomes, which are factors in the metabolic syndrome, are also related to HPA-axis dysregulation (Björntorp, & Rosmond, 2000) and to cognitive impairment and dementia (Frisardi et al., 2010), and they are considered risk factors for cardiovascular disease (CVD). CVD is the number one cause of death globally, although it can be

prevented by addressing behavioral risk factors, such as smoking, an unhealthy diet, obesity, physical inactivity, and harmful use of alcohol (WHO, 2017). Therefore, improving knowledge about the association between personality traits and these CVD risk factors could help to prevent adverse health outcomes, including cognitive decline and dementia, and reduce mortality risk.

Several studies have explored the relationship between the big five personality traits and the objective health outcomes of obesity, dysglycemia, dyslipidemia, and arterial hypertension. However, previous literature shows inconsistent results that may be explained in part by important moderators such as sex and age (Sutin et al., 2010b; Sutin & Terracciano, 2016b).

Sutin & Terracciano (2016b), in a broad sample including participants from 18 to 91 years old, observed that high neuroticism was associated with higher body mass index (BMI) and risk of obesity, whereas conscientiousness and, to a lesser extent, extraversion and openness were related to lower BMI and less risk of obesity. In addition, they reported that sex moderated the association between neuroticism, conscientiousness, and extraversion. Specifically, the association between neuroticism and conscientiousness was stronger for women than for men, whereas extraversion was protective for women but unrelated for men. Moreover, they observed that age also moderated the association between personality and BMI, whereas the association between conscientiousness and BMI was stronger for older adults. Moreover, older participants who scored higher on agreeableness had a lower BMI, but this association was not observed in younger participants. By contrast, Mõttus et al. (2013), in a sample

that only included older adults, observed that higher conscientiousness was associated with a lower BMI, but the rest of the personality traits were not.

It is important to note that neither of these two studies analyzed the moderating role of sex in the relationship between personality and BMI in older adults, even though previous studies in other age ranges reported sex differences (Armon, Melamed, Shirom, Shapira, & Berliner, 2013; Brumett et al., 2006; Faith, Flint, Fairburn, Goodwin, & Allison, 2001; Sutin & Terracciano, 2016a; 2016b). Moreover, these two studies included BMI as a single measure of global adiposity. However, growing evidence suggests that BMI is not the most relevant measure of the effects of adiposity on health. Other measures, such as waist circumference (WC), or specifically waist-hip ratio (WHR), which is a better indicator of abdominal adiposity distribution than WC, seem to be better adiposity risk markers for health outcomes such as acute myocardial infarction (Yusuf et al., 2005).

Sutin et al. (2010a) also analyzed the association between the big five personality traits and cholesterol in a sample from 14 to 102 years old, and they reported that high conscientiousness and low openness were related to a healthier cholesterol profile (higher High-density lipoprotein [HDL] cholesterol and lower triglycerides), but not to Low-density lipoprotein (LDL) cholesterol. They also found that sex and age moderated these associations. Specifically, the association between conscientiousness and higher HDL-cholesterol was stronger in women than in men, whereas the association between conscientiousness and triglycerides held for older participants, but not for young participants. Chapman, Roberts, Lyness, & Duberstein (2013) observed

that in older adults, personality traits, specifically higher neuroticism and lower conscientiousness and agreeableness, were associated with illness burden morbidity, and they reported that cholesterol explained some of these effects.

Another study collected health measures through telephone interviews and reported that higher neuroticism and lower conscientiousness, extraversion, and openness were associated with a higher risk of developing high blood pressure, whereas lower conscientiousness was related to a higher risk of developing diabetes in older adults (Weston et al., 2015). In contrast, another study with participants from 30 to over 70 years old observed that higher extraversion, but not neuroticism, was related to higher blood pressure and glucose biomarkers (Ohseto et al., 2018).

A good proxy for hypertension is the pulse pressure index, which reflects arterial stiffness and is measured as systolic minus diastolic blood pressure, and is potentially a better measure of chronic effects of hypertension than blood pressure itself (Nation et al., 2012; Power, Tchetgen, Sparrow, Schwartz, & Weisskopf, 2013). Pulse pressure increases with age and has been associated with cardiovascular risk factors (Franklin, Khan, Wong, Larson, & Levy, 1999) and other health outcomes such as cognitive decline (McDade et al., 2016). HbA1c assesses average glucose control over the previous 2-3 months. Compared to fasting glucose, it has the advantage that it can be measured at any time of day, regardless of the fasting time or the content of the previous meal. In addition, HbA1c has been similarly associated with a risk of diabetes and more strongly associated with risk of cardiovascular disease and death from any cause, compared to fasting glucose (Selvin et al., 2010). To our knowledge, no previous study

has analyzed the relationship between pulse pressure and HbA1c and the five major personality traits in older people.

Therefore, more research in needed to explore the effect of the big five personality traits on the CVD risk factors BMI and WHC, cholesterol, Pulse pressure, and HbA1c in older adults, and, importantly, taking into account the moderating role of sex in these associations.

1.5.5. Personality and subjective health

Subjective health has also been associated with health outcomes and mortality (Idler & Benyamini, 1997), and this association also appears to be more pronounced with increasing age (Duberstein et al., 2003). The Short Form Health Survey (SF-36) questionnaire has been widely administered to assess subjective health (Ware, & Sherbourne, 1992), and several studies have analyzed its relationship with personality traits in older people. Higher neuroticism was related to worse subjective health in all studies (Chapman, Duberstein, Sörensen, & Lyness, 2006; Chapman, Duberstein, & Lyness, 2007; Duberstein et al., 2003; Jaconelli, Stephan, Canada, & Chapman, 2012; Jerram & Coleman, 1999; Löckenhoff, Sutin, Ferrucci, & Costa, 2008). Similarly, lower conscientiousness was also related to worse subjective health in most studies (Chapman et al., 2006; 2007; Jaconelli et al., 2012; Jerram & Coleman, 1999; Löckenhoff et al., 2008), but not in all of them (Jerram & Coleman, 1999; Duberstein et al., 2003). Likewise, most studies observed that higher extraversion was related to better subjective health (Chapman et al., 2006; Duberstein et al., 2003; Jaconelli et al.,

2012; Jerram & Coleman, 1999), but others did not (Chapman et al. 2007; Löckenhoff et al., 2008).

By contrast, higher openness was related to better (Duberstein et al., 2003; Jerram & Coleman, 1999) or worse (Chapman et al. 2007) subjective health, or it was not associated (Chapman et al., 2006; Jaconelli et al., 2012; Löckenhoff et al., 2008). Finally, most studies failed to observe an association between agreeableness and subjective health (Chapman et al., 2006; 2007; Duberstein et al., 2003; Jaconelli et al., 2012; Löckenhoff et al., 2008), except one study that observed a positive association (Jerram & Coleman, 1999). Jerram & Coleman (1999) also analyzed these associations separately for men and women. They observed differences within each sex group, suggesting that results for the whole sample were a poor reflection of the results for men and women separately. Moreover, subjective health can be classified in eight different subscales that can be grouped as physical and mental health, and some personality traits may be related to them in different ways, which could explain some of the inconsistent findings.

CHAPTER 2

OBJECTIVES AND HYPOTHESES

Based on the mixed results and the gaps in the literature presented in the introduction chapter, the current thesis will address the general and specific objectives and hypotheses presented below:

General objective 1. Determine the impact of the three allelic variations of the ApoE polymorphism (ApoE-ε2, ApoE-ε3, and ApoE-ε4) on different cognitive domains and the HPA-axis functioning assessed through cortisol levels during the neuropsychological assessment, in healthy older adults.

Specific objective 1.1: Study differences in cognitive performance (i.e. attention, executive function, and working and declarative verbal memory), according to the ApoE group.

Specific objective 1.2: Study differences in cortisol levels (mean and delta cortisol levels), according to the ApoE group.

Specific objective 1.3: Investigate the relationship between cortisol levels and cognitive performance separately for the ApoE groups, as well as the moderating role of the ApoE in these associations.

Based on previous literature, we hypothesized worse cognitive performance in the ApoE-ε4 group and better cognitive performance in the ApoE-ε2 group (see Wisdom et al., 2011). We also hypothesized higher cortisol levels in the ApoE-ε4 group, compared to the ApoE-ε2 and ApoE-ε3 groups (Peskind, Wilkinson, Petrie,

Schellenberg, & Raskind, 2001). Moreover, we hypothesized that both higher mean cortisol levels and an increase in cortisol levels during the neuropsychological assessment would be related to worse cognitive function, mainly on declarative memory, and in the ApoE-ε4 group (Gerritsen et al., 2011; Lee et al., 2008).

Data and results obtained to respond to these research objectives are presented in Chapter 3.

General objective 2. Analyze the mediation effect of the diurnal cortisol pattern, as a measure of HPA-axis functioning, in the relationship between loneliness and cognitive function, in healthy older people.

Specific objective 2.1: Study the relationship between loneliness and cognitive performance (i.e. global cognition, attention, executive function, and working and declarative verbal memory).

Specific objective 2.2: Study the relationship between loneliness and the diurnal cortisol pattern (awakening cortisol, DCS, AUCg, and bedtime cortisol).

Specific objective 2.3: Analyze the association between the diurnal cortisol pattern and cognitive performance.

Specific objective 2.4: Analyze the role of the diurnal cortisol pattern as a mediator in the relationship between loneliness and cognitive function.

Specific objective 2.5: Investigate the role of sex in the specific objectives mentioned above.

Due to the mixed findings on the relationship between loneliness and the DCS (Adam et al., 2006; Cole et al., 2007; Schutter et al., 2017) and the lack of studies on the relationship between loneliness and the diurnal AUCg in older people, we did not have specific hypotheses about the direction of these relationships. In addition, as Boss et al. (2015) reported in a review, we expected to find a weak but significant negative association between loneliness and performance on different cognitive domains. Moreover, we hypothesized a negative association between cognitive function and awakening cortisol (Beluche et al., 2010; O'Hara et al., 2007), bedtime cortisol (Li et al., 2006; Stawski et al., 2011), the DCS (Beluche et al., 2010; Stawski et al., 2011), and the diurnal cortisol output (Li et al., 2006; Ouanes et al., 2017a). We also hypothesized that this dysregulated HPA-axis pattern would mediate the relationship between loneliness and poorer cognitive performance. Finally, we expected these associations to be more pronounced in women than in men because some authors have suggested that loneliness affects women's health more than men's (Christiansen et al., 2016).

These objectives will be addressed in chapter four of this thesis.

General objective 3. Determine the impact of the big five personality traits (neuroticism, conscientiousness, extraversion, openness, and agreeableness), on HPA-axis functioning, assessed through the diurnal cortisol pattern, on other objective and subjective health indexes, and on change in cognitive performance, in older adults.

Specific objective 3.1: Study the relationship between personality traits and the diurnal cortisol pattern (awakening cortisol, CAR, DCS, and bedtime cortisol).

Specific objective 3.2: Study the relationship between personality traits and change in cognitive performance (i.e. attention, executive function, and working and declarative verbal memory).

Specific objective 3.3: Study the mediation effect of the DCS and cognitive reserve in the association between personality traits and the change in cognitive function.

Specific objective 3.4: Study the relationship between personality traits and other objective health measures (BMI, WHR, HbA1c, LDL and HDL-cholesterol, and pulse pressure).

Specific objective 3.5: Study the relationship between personality traits and subjective physical and mental health.

Specific objective 3.6: Investigate the role of sex in the specific objectives mentioned above.

We hypothesized that a dysregulation of the HPA-axis functioning (i.e., lower awakening cortisol, greater CAR, flatter DCS, and higher bedtime cortisol) would be associated with higher scores on neuroticism, a personality trait considered to be a risk factor, and lower scores on personality traits considered protective factors (i.e. conscientiousness, extraversion, openness, and agreeableness) for health (Weston et al., 2015). In addition, we expected the association between neuroticism and a dysregulation of the HPA-axis functioning to be more pronounced in older women than in older men (Puig-Perez et al., 2016a).

Regarding the association between personality and cognitive decline, we hypothesized an association between higher openness and conscientiousness and less cognitive decline. We also expected to find an association between higher extraversion and less cognitive decline on delayed recall verbal memory, and no association between agreeableness and cognitive change. Because mixed results have been observed in the association between neuroticism and cognitive change, we did not have a hypothesis for this association (see review: Curtis et al., 2015). We also hypothesized that higher neuroticism would be related to a flattened DCS, whereas higher openness would be related to greater cognitive reserve. Moreover, we expected an association between a flattened DCS and lower cognitive reserve and greater cognitive decline. Furthermore, we hypothesized a mediating effect of the DCS on the association between neuroticism and cognitive change, as well as a mediating effect of cognitive reserve on the association between openness and cognitive change.

Finally, we hypothesized that worse objective and subjective health would be associated mainly with higher neuroticism and lower conscientiousness, and to a lesser degree, with lower extraversion, openness, and agreeableness. In addition, we expected the associations between the personality traits and health to be more pronounced in older women than in older men, at least for neuroticism, as in previous studies with other age ranges (Armon et al., 2013; Brumett et al., 2006; Faith et al., 2001; Sutin & Terracciano, 2016a; 2016b).

Chapters 5, 6, and 7 aim to respond to these specific research objectives. Specifically, chapter 5 will address objectives 3.1 and 3.6, chapter 6 will address objectives 3.2, 3.3, and 3.6, and chapter 7 will address objectives 3.4, 3.5, and 3.6.

CHAPTER 3

THE RELATIONSHIP BETWEEN CORTISOL AND COGNITIVE FUNCTION IN HEALTHY OLDER PEOPLE: THE MODERATING ROLE OF APOLIPOPROTEINE POLYMORPHISM

The main results of this chapter have been published in: Montoliu, T., Hidalgo, V., Pulopulos, M. M., Ivorra, J. L., Martínez, M. J., & Salvador, A. (2018). The relationship between cortisol and cognitive function in healthy older people: The moderating role of Apolipoprotein E polymorphism. *Neurobiology of Learning and Memory, 155*, 297-305.

3.1. INTRODUCTION

The increase in life expectancy has aroused interest in the biological mechanisms underlying cognition and its decline during aging (Payton et al., 2005). The ApoE polymorphism has been proposed as a mediator of this age-related cognitive impairment (Helkala et al., 1996).

The ApoE gene has three common allelic variations (Bertram et al., 2010), being the ApoE-ε3 the most common (78%), and the ApoE-ε2 and ApoE-ε4 alleles are less frequent (8% and 14%, respectively) (Menzel, Kladetzky, &Assmann, 1983). While the ApoE-ε4 allele presents the main risk factor for late-onset AD (Bertram et al., 2010), the ApoE-ε2 allele has been associated with a reduced risk (Verghese et al., 2011). These findings have created interest in the role ApoE variants play in the cognitive functioning of healthy people. Although two meta-analyses reported that the ApoE-ε4 allele would have adverse effects on cognitive function in healthy elderly people (Small et al., 2004; Wisdom et al., 2011), a recent study on mid-adulthood supported comparable cognitive performance between ApoE-ε4 carriers and non-carriers (Lancaster et al., 2016). However, Lancaster et al. (2016) concluded that the cognitive performance profile of ApoE- ε4 carriers remains elusive. It is worth noting that there are some important differences among the empirical studies, such as the inclusion of preclinical dementia cases, the cognitive domains assessed, the age range of the participants, or the way of grouping the various types of allelic variations. Most of the studies group the participants as ApoE-ε4 carriers or non-carriers, without

considering the ApoE-ε2 allele independently, which could lead to an overestimation of ApoE-ε3 performance and an underestimation of ApoE-ε4 performance.

In addition to the effects of the ApoE, cognitive function is also affected by the HPA-axis function through the influence of its end product, cortisol. Several studies in healthy older people have reported an association between higher cortisol levels and worse memory performance at basal levels (Almela et al., 2012; Hidalgo et al., 2016) and in response to stress (Almela et al., 2011; Hidalgo et al., 2014). Furthermore, increased cortisol levels are a well-established feature of AD, although the mechanism responsible for HPA-axis hyperactivity is unknown (Gil-Bea et al., 2010; Peskind et al., 2001). This fact, along with the evidence that ApoE-ε4 is a risk factor for developing AD (Bertram et al., 2010), suggests that the ApoE gene may affect the association between the HPA-axis and cognitive function (Peavy et al., 2007), as animal studies have shown (de Kloet, Grootendorst, Karssen & Oitzl, 2002; Grootendorst et al., 2002; Grootendorst, Enthoven, Dalm, de Kloet & Oitzl, 2004). Moreover, similar to preclinical brain changes related to AD, structural and functional abnormalities of the hippocampus have been found in healthy ApoE-ε4 carriers (Lu et al., 2011).

Nonetheless, studies exploring the associations among the ApoE genotype, cognitive function, and the HPA-axis in non-demented older people are scarce and report mixed results. Whereas some studies found an association in the ApoE-ε4 allele between an HPA-axis alteration and cognitive decline (Gerritsen et al., 2011; Lee et al., 2008; Peavy et al., 2007; Singh-Manoux et al., 2014), others failed to find this association (Berteau-Pavy et al., 2007; Fiocco et al., 2008; Lara et al., 2013; Li et al.,

2006). These different findings could at least partly be explained by some methodological differences. Thus, participants with different age ranges (i.e. mean age over 55 (Fiocco et al., 2008), 60 (Fiocco et al., 2008; Lee et al., 2008; Singh-Manoux et al., 2014), 75 (Gerritsen et a., 2011; Li et al., 2006; Peavy et al., 2007), and 80 (Berteau-Pavy et al., 2007) years old) have been included in the studies. Another difference is the cognitive domain assessed, with declarative memory being studied the most (Fiocco et al., 2008; Gerritsen et al., 2011; Lee et al., 2008; Li et al., 2006; Peavy et al., 2007; Singh-Manoux et al., 2014), and only a few studies carried out on object recognition and spatial navigation (Berteau-Pavy et al. (2007) or attention and executive functioning (Lee et al., 2008; Li et al., 2006). In addition, most studies employed basal cortisol measures, and only two studies measured cortisol levels during the neuropsychological evaluation, with different results. Berteau-Pavy et al. (2007) found no association between ApoE alleles, cortisol levels, and cognition, whereas Lee et al. (2008) reported that, although higher cortisol was associated with lower cognitive performance, the slopes were steeper in the ApoE-ε4 group. However, these studies included different cognitive tests and inclusion criteria. More importantly, none of these studies considered the ApoE-ε2 allele. Therefore, more research is needed on the associations among the ApoE genotype, cognitive functioning, and cortisol levels during cognitive testing in healthy elderly people.

With all this in mind, we aimed to examine: (i) the differences in several cognitive domains (i.e. declarative and working memory, attention, and executive function) and cortisol levels during the neuropsychological assessment in different ApoE groups and (ii) the relationship between cortisol and cognitive performance,

taking into account the ApoE groups, in non-stressed, healthy older people from 55 to 77 years old. To do so, a neuropsychological battery was administered to assess a wide range of cognitive functions. Based on previous literature, we hypothesized that the ApoE-ε4 group would show worse cognitive performance, whereas the ApoE-ε2 group would show better performance (Wisdom et al., 2011). We also hypothesized that there would be higher cortisol levels in the ApoE-ε4 group, compared to the ApoE-ε2 and ApoE-ε3 groups (Peskind et el., 2001). Finally, we expected to find an association between higher mean cortisol levels, as well as an increase of cortisol levels during the session, and a worse cognitive function, especially on declarative memory, and this association would be more pronounced in the ApoE-ε4 group (Gerritsen et al., 2011; Lee etal.,2008).

3.2. METHODS

3.2.1. Participants

The final sample was composed of 84 participants of both sexes (40 men and 44 woman), ranging in age from 55 to 77 years (*M*= 65.18, *SD*= 4.631). Most of the participants had an educational level beyond high school (79.8%), and their subjective socioeconomic status (SES) was medium (*M*= 5.51, *SD*= 1.086) (subjective SES scale: Adler et al., 2000).

The total sample was categorized in three groups: ApoE-ε2 (n= 9; E2/E2=1 and E2/E3=8), ApoE-ε3 (E3/E3=59), and ApoE-ε4 (n=16; E4/E4=2 and E4/E3=14). Due to the small number of participants who were homozygous for the E2 and E4 alleles (E2/E2=1 and E4/E4=2), both the homozygous and heterozygous participants were

grouped in the broad category of ApoE-ε2 and ApoE-ε4, respectively. The ApoE group frequencies were 10.7 % for ApoE-ε2, 70.2 % for ApoE-ε3, and 19 % for ApoE-ε4. There were no significant differences among the ApoE-ε2, ApoE-ε3, and ApoE-ε4 groups on sex, age, BMI, SES, or educational level (all $p > 0.115$) (Table 3.1).

Table 3.1. Descriptive data for the total sample and for each ApoE group (ApoE-ε2, ApoE-ε3, and ApoE-ε4).

	N (%)	Women (%)	Age (*M*)	(*SD*)	SES (*M*)	(*SD*)	BMI (*M*)	(*SD*)	Educational level
Total	84 (100%)	52.4%	65.18	4.631	5.51	1.086	27.34	3.248	79.8%
ApoE-ε2	9 (10.7%)	55.6%	62.78	3.383	5.67	.707	27.81	2.693	77.8%
ApoE-ε3	59 (70.2%)	49.2%	65.27	4.634	5.62	1.040	27.35	3.405	81.4%
ApoE-ε4	16 (19%)	62.5%	66.19	4.996	5.00	1.317	27.09	3.050	75%
		p= .625	*p*=.203		*p*=.115		*p*=.881		*p*=.627

Note: SES = Subjective Socioeconomic Status; BMI = Body Mass Index; *M*= Mean; *SD* = Standard; ApoE= Apolipoprotein E**.** Deviation. Educational level is represented as the percentage of participants with an educational level beyond high school (%). Differences between ApoE status were analyzed with ANOVAs (age, SES, and BMI) and chi-square (sex and educational level) analyses. No differences in sex, age, SES, BMI, or educational level were found depending on the ApoE group (all $p \geq .115$).

All the women were postmenopausal and had their last menstrual period more than 3 years before the testing time. None of the participants scored less than 28 on the MEC (Spanish version of the Mini-Mental Status Examination; Lobo et al., 1999), indicating the absence of cognitive impairment.

Participants were Caucasian, and they were recruited from a study program at the University of Valencia for people over 55 years of age. Two hundred and twenty volunteers were interviewed by telephone in order to check whether they met the study prerequisites. Exclusion criteria were: smoking more than 10 cigarettes a day, alcohol or other drug abuse, visual or hearing problems, diabetes, neurological or psychiatric disease, using any medication directly related to emotional or cognitive functioning or

able to influence hormonal levels such as glucocorticoids, psychotropic substances, or sleep medications, having been under general anesthesia once or more than once in the past year, and the presence of a stressful life event during the past year.

One hundred twenty-eight individuals participated in the study, of whom 37 were eliminated because they did not meet the inclusion or exclusion criteria: 20 participants for using medication related to emotional or cognitive functioning or able to influence hormonal levels, such as glucocorticoids, psychotropic substances, or sleep medications; 7 participants for having diabetes; 4 participants due to severe and uncorrected visual or hearing problems; 4 participants for having a stressful life event during the past year; 1 for alcohol abuse; and 1 participant for having been under general anesthesia in the past year. Of the remaining 91 participants, the ApoE polymorphism of three participants could not be genotyped, and one participant was excluded for being ApoE-ε2/ε4. Finally, after dividing the sample according to the ApoE genotype (ApoE-ε2, ApoE-ε3 and ApoE-ε4), three participants in the ApoE-ε3 group were excluded from the analyses, two participants because their cortisol concentrations differed by more than 2.5 SD from the mean, and one participant because his age differed by more than 2.5 SD from the mean.

3.2.2. Procedure

Participants who met the criteria were asked to attend one session that took place from 10:00 to 12:00 hours in a laboratory at the Faculty of Psychology. Before the session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume

alcohol from the night before the first session. They were also instructed to drink only water, and not eat, smoke, take any stimulants (such as coffee, cola, caffeine, tea or chocolate), or brush their teeth at least 1 hour prior to the session. All participants provided written informed consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Committee of the University of Valencia. In this session, a neuropsychological battery was administered. Additionally, participants were asked to provide two saliva samples, from which the ApoE genotype and cortisol levels were extracted. The first saliva sample was collected at the beginning of the neuropsychological evaluation (pre-test cortisol), which took place 15 minutes after the participant's arrival. The second saliva sample was collected at the end of the neuropsychological evaluation (post-test cortisol), which took place 1 hour and 25 minutes after the beginning of the neuropsychological evaluation.

3.2.3. Neuropsychological tests

3.2.3.1. Declarative memory

Rey Auditory Verbal Learning Test (RAVLT). The Spanish version of the RAVLT (Miranda & Valencia, 1997) was used. This task consists of a target list (List A) of 15 neutral words repeated five times by the experimenter (trials I–V: Total Learning) that participants had to learn. Then, an interference list (List B) was presented only once, and participants had to repeat it. Participants were asked to recall the target list again immediately after the interference list (trial VI), and again after a delay of 20 min (trial VII). Three outcomes were used in subsequent analyses: (i) RAVLT Total Learning: total number of words recalled on the first five trials (trial I to

V); (ii) RAVLT Immediate Recall: percentage of total number of words recalled after the interference trial compared to the number of words recalled on trial V (trial VI/trial V x 100); and (iii) RAVLT Delayed Recall: percentage of total number of words recalled after the 20-min delay compared to the number of words recalled on the immediate recall trial (trial VII/trial VI x 100).

Rivermead Stories Subtest. The Story recall subtest from the Spanish version of the Rivermead Behavioral Memory Test (Wilson, Cockburn, &Baddeley, 1985) was used. The experimenter read aloud two short stories, and participants had to recall as many memory units or ‘‘ideas’’ as possible immediately after their oral presentation and after a 20-min delay. Participants’ answers were audio recorded and corrected by an expert, and the sum of the correctly recalled ‘‘ideas’’ from the two stories was calculated. From this test, two outcomes were used for the subsequent analysis: (i) Rivermead Immediate recall: total “ideas” recalled from the two stories immediately after the oral presentation and (ii) Rivermead Delayed recall: total “ideas” recalled from the two stories after 20 min, compared to the number of “ideas” recalled from the two stories immediately after the oral presentation (Delayed recall/ Immediate recall x100).

3.2.3.2. Working memory (WM)

Digit Span (DS). The Spanish version of the Wechsler Memory Scale III was administrated (Wechsler, 1997). The experimenter read aloud a series of numbers (from 0 to 9) of increasing length (from 2 to 9 digits) at a rate of one digit per second. The participant had to repeat the numbers, first in the same order (DS-Forward) and then in reverse order (DS-Backward). Each set length was tested twice. The test was finalized when the participant failed two consecutive trials of the same length. For each

correctly repeated digit set, one point was given. Two outcomes were obtained: (i) DS-Forward: total number of correctly recalled attempts in the same order and (ii) DS-Backward: total number of correctly recalled attempts in the reverse order. DS-Forward was used as a measure of the attention and memory span component of WM, whereas DS-Backward was used as a measure of the executive component of WM (Conklin, Curtis, Katsanis, & Iacono, 2000).

Letter-number sequencing (LNS). The Spanish version of the Wechsler Memory Scale III was administrated (Wechsler, 1997). The experimenter read aloud a series of mixed numbers (from 0 to 9) and letters (from A to Z) of increasing length (from 2 to 8 items). The participant had to repeat the series, ordering the numbers in ascending order and the letters in alphabetical order. Each set length was tested three times. The test was finalized when the participant failed three consecutive trials of the same length. One point was given for each correctly recalled attempt. One outcome was obtained: LNS (total number of correctly recalled attempts).

3.2.3.3. Executive Function

Trail-Making Test (TMT). The TMT (Reitan, 1992) consists of two trials, TMT-A and TMT-B, each composed of 25 circles distributed on a white sheet of paper. In TMT-A, the circles were numbered from 1 to 25, and the participant was asked to trace a line connecting the circles in numerical sequence as quickly as possible. TMT-B included numbers from 1 to 13 and letters from A to L, and the participant was instructed to alternate between numbers and letters in ascending sequence. The score obtained was the number of seconds required to finish each trial. Errors were pointed out instantly by the examiner and contributed to the score, due to the additional time

needed for corrections. Two outcomes were obtained: (i) TMT-A: total number of seconds required to finish the TMT-A, and (ii) TMT-B: total number of seconds required to finish the TMT-B. The TMT-A was used to assess attention and general psychomotor speed, whereas the TMT-B was used to evaluate attention-switching performance.

Stroop Color-Word Interference test. Golden's version of the Stroop Color-Word Interference Test (Golden, 1978) was administered. The test is composed of three trials. In each trial, participants had to name as many words as possible in 45 seconds. In the first trial, participants had to read the written word (W), which was red, blue, or green. In the second trial, participants had to name the printed color (C), red, blue, or green, of the XXX letters. In the third trial, participants had to name the color of the printed word (red, blue, or green), which was different from the written word (red, blue, or green) (WC), for example, the word green printed in red color. Afterwards, the WC' was calculated (WC' = (W x C) / (W + C)). Finally, the Stroop Interference outcome was obtained (Stroop Interference = WC – WC'), which is a measure of the ability to inhibit automatic responses.

3.2.4. Biochemical analyses

Participants provided two saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany), the first one at the beginning of the neuropsychological assessment, and the second one at the end of the session. Participants were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all salivary glands.

The samples were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was stored at -80ºC until the analyses of cortisol and ApoE genotype determination were performed.

3.2.4.1.Salivary Cortisol

The activity of the HPA-axis was measured by analyzing the salivary cortisol levels. Each sample was measured in duplicate, and each participant's samples were analyzed in the same trial. The salivary cortisol samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Spectria Cortisol RIA (cat. Nu 06119) from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/L, and the within- and inter-assay variation coefficients were all below 8%. Salivary cortisol levels were determined in the Central Research Unit of the Faculty of Medicine, University of Valencia (Spain).

3.2.4.2. ApoE genotype determination

To determine the ApoE genotype, the genomic deoxyribonucleic acid (DNA) was isolated from the saliva by using a standard commercial extraction method (KIT REALPURE "SSS"). The genotype of each ApoEpolymorphism was amplified by the polymerase chain reaction (PCR) using two primers: Forward (ACAGAATTCGCCCCGGCCTGGTACAC) and Reverse (TAAGCTTGGCACGCCTGTCCAAGGA). Subsequently, DNA was digested with the *Hhal* restriction enzyme. Next, DNA electrophoresis in 2% agarose gel was performed. Finally, after ethidium bromide staining, the DNA-banding patterns (ApoE-ε2, ApoE-ε3 and ApoE-ε4) were visualized under the ultraviolet lamp and recorded for

further analysis. The ApoE genotype was determined at the Department of Genetics of the University of Valencia (Spain)

3.2.5. Statistical Analysis

Participants' characteristics were described using percentages or means (standard deviation, SD) when appropriate, according to the ApoE groups (ApoE-ε2, ApoE-ε3, and ApoE-ε4).

To investigate whether there were differences between the ApoE groups in age, BMI, and SES, one-way analyses of variance (ANOVAs) were performed. Pearson's Chi-square test was used to assess differences in sex and education level.

Before statistical analyses were performed, cortisol data were checked for normal distribution and homogeneity of variance using Shapiro-Wilks and Levene's test. These analyses revealed significant deviations in cortisol values; therefore, cortisol data were logarithm 10 (Log10) transformed. The *mean cortisol* index was obtained averaging pre-test and post-test cortisol levels and the *delta cortisol* index by subtracting post-test cortisol minus pre-test cortisol levels.

To investigate whether there were differences in cognitive performance and cortisol levels among the ApoE groups, we performed one-way ANOVAs. As dependent variables, we used the following outcomes from the (i) RAVLT: RAVLT Total Learning, RAVLT Immediate recall, and RAVLT Delayed recall, (ii) Rivermead stories: Rivermead Immediate recall and Rivermead Delayed recall, (iii) DS: DS-Forward and DS-Backward, (iv) LNS, (v) TMT: TMT-A and TMT-B, (vi) Stroop

interference, and (vii) mean cortisol. *Post-hoc* comparisons were performed using Bonferroni adjustments for the *p* values.

In addition, to analyze the change in cortisol levels during the neuropsychological assessment an ANOVA for repeated measures analysis was performed including Time (pre-test and post-test cortisol) as a within-subject factor and ApoE group as an between-subject factor. These analyses were performed unadjusted and adjusted for the covariates age, sex, SES and BMI.

Next, to investigate whether an association exists between cortisol levels and the different cognitive domain outcomes, linear regressions were performed, unadjusted and adjusted for covariates, as previous studies showed that cortisol levels and/or cognition could be affected by sex (Almela et al., 2011), age (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Van Hooren et al., 2007), SES (Cohen, Doyle, & Baum, 2006), level of education (Sattler, Toro, Schönknecht & Schröder, 2012), and BMI (Champaneri et al., 2013; Cournot et al., 2006). Thus, separate analyses were performed for each cognitive domain as dependent variable. For unadjusted analyses, we included cortisol indexes (mean or delta) in step one. For adjusted analyses, we included sex, age, BMI, SES, and educational level as covariates in step one, following stepwise analysis. In step 2, cortisol indexes (mean or delta) were included. Finally, in order to investigate whether the relationships between the cortisol levels and the different cognitive domain outcomes were different depending on the ApoE group, these analyses were repeated with the sample divided into ApoE groups.

All *p* values were two-tailed, and the level of significance was taken as $p<0.05$. To perform these statistical analyses, version 22.0 of SPSS was used.

3. RESULTS

3.3.1. ApoE group differences in cognitive function

One-way ANOVAs revealed significant effects of the ApoE groups on Total Learning from the RAVLT test (F (2, 81) = 4.191, p = .019). Post hoc analysis revealed significant differences between the ApoE-ε2 and ApoE-ε4 groups (p = .015), showing that the ApoE-ε2 group obtained higher scores than the ApoE-ε4 group. A marginal difference was also observed, where the ApoE-ε2 group obtained higher scores than the ApoE-ε3 group (p = .065) (Figure 3.1). None of the other cognitive domain outcomes showed significant differences among the ApoE groups. For the rest of the tests that evaluated declarative memory, no significant differences were found between the ApoE groups on immediate (F (2, 81) =1.298, p =. 279) and delayed recall (F (2, 81) =2.552, p = .084) from the RAVLT test, or immediate (F (2, 80) = .034, p = .967) and delayed recall (F (2,80) = .791, p =.457) from the Rivermead test. Likewise, no significant differences were found between the ApoE groups on the tests that evaluated working memory, such as the DS-Forward (F (2, 81) = 1.113, p= .333), DS-Backward (F (2, 80) = .228, p = .796), and LNS (F (2, 81) = .694, p = .503). Finally, no significant differences were found between the ApoE groups on the tests that assessed executive function attention, such as TMT-A (F (2, 80) =1.212, p = .303), TMT-B (F (2, 80) = .329, p = .720), and Stroop interference (F (2, 78) = .360, p = .699).

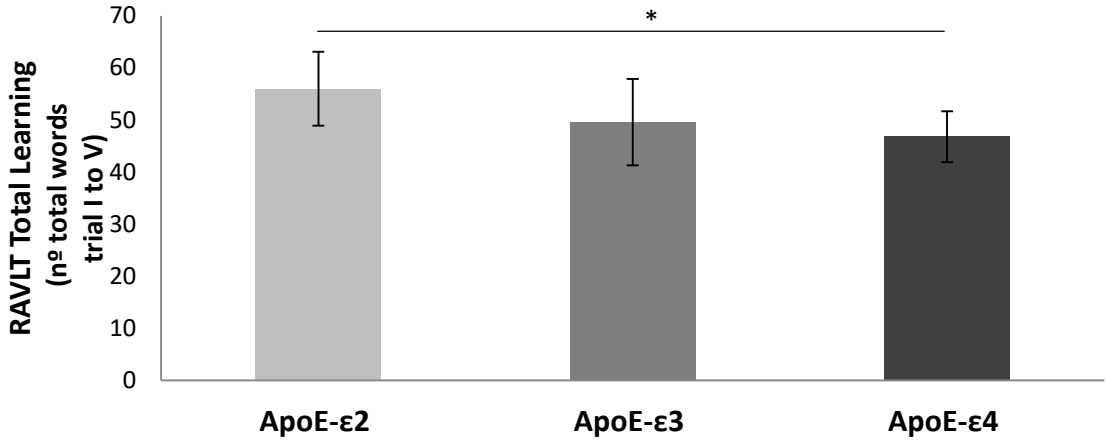

Figure 3.1. Mean performance on RAVLT Total Learning according to APOE group. One-way ANOVAs showed significant differences in declarative memory performance between ApoE groups (p=.019). Post hoc analysis showed better performance in the ApoE-ε2 group compared to ApoE-ε4 (*p=. 015). Although without reaching significance, a trend was also observed where the ApoE-ε2 group performed better than the ApoE-ε3 group (p=.065).

3.3.2. ApoE group differences in cortisol levels

The results showed no significant differences among the ApoE groups in mean cortisol levels (F (2, 80) = 1.351, p= .265) (Table 3.2).

Table 3.2.
Cortisol data (nmol/L) for the total sample and for each ApoE group (ApoE-ε2, ApoE-ε3, and ApoE-ε4).

	Total	ApoE-ε2	ApoE-ε3	ApoE-ε4
Cortisol *M (SEM)*	5.747 (.286)	6.437 (.651)	5.733 (.317)	5.406 (.887)

Note: M= Mean; SEM= Standard error of mean; ApoE = Apolipoprotein E.

The ANOVA for repeated measures showed an effect of Time (F (1, 80) = 5.483, *p* =.022), where cortisol levels decreased along the session. On the contrary, no interaction Time*ApoE group (F (2, 80) = .579, p=.563) was observed. These results were similar after controlling for age, sex, SES and BMI (Time: F (1, 74) = 4.396, *p* =.039; Time*ApoE group: F (2, 74) = 1.607, *p* =.207).

3.3.3. Relationship between cortisol levels and cognitive function

Unadjusted regression analyses showed, for the complete sample, a negative association between mean cortisol levels and the delayed recall on the RAVLT test (*p* = .009), and marginally with immediate recall on the Rivermead story test (*p* = .077). Besides, a negative association was observed between mean cortisol levels and time performing the TMT A test (*p* = .037). In addition, delta cortisol was positively associated with time performing the TMT B (*p* = .038) and, marginally, with delayed recall on the RAVLT test (*p* = .055).

After repeating these analyses considering the ApoE groups, only the ApoE-ε4 group showed a negative association between mean cortisol levels and delayed recall on the RAVLT (*p* = .021) and immediate recall on the Rivermead story test (*p* = .012). On the other hand, only the ApoE-ε3 group showed a negative association between mean cortisol levels and time performing the TMT A (*p* = .019). Also, in this group a positive association between delta cortisol and delayed recall on the RAVLT (*p* = .023) and time performing the TMT B (*p* = .039), as well as a negative association between delta cortisol and DS-Forward performance (*p* = .049) were found.

Adjusted regression analyses showed that, for the complete sample, higher mean cortisol levels were related to worse performance on delayed recall on the RAVLT test (p = .009) and immediate recall on the Rivermead story test (p = .026). In turn, a trend was observed where higher cortisol levels were associated with lower times performing the TMT A (p = .060) and, therefore, better performance. Regarding the delta index, an increase of cortisol levels during the session was related to a better performance on delayed recall on the RAVLT test (p = .028) and, marginally, to learning ability on the RAVLT test (p = .052).

After repeating these analyses considering the ApoE groups, only the ApoE-ε4 group showed a negative association between mean cortisol levels and performance on delayed recall on the RAVLT test (p = .015) and immediate recall on the Rivermead story test (p = .008). In addition, for the ApoE-ε3 allele it was observed a negative association between mean cortisol levels and time performing the TMT A (p = .024). Besides, only the ApoE-ε3 group showed a positive association between delta cortisol and performance on delayed recall on the RAVLT test (p = .007), as well as a trend with performance on learning ability on the RAVLT test (p = .093). None of the other associations were significant (all p> .110) (Table 3.3 and 3.4, see pages 87 and 88)

Finally, to investigate whether the significant relationships between mean or delta cortisol and the cognitive outcomes were statistically different for the three groups, we conducted regression models using PROCESS (v2.13.6). To do this, separately regression models with each cognitive measure tested as the dependent variable (i.e., RAVLT delayed recall, Rivermead Immediate recall or TMT A), the cortisol indexes (mean or delta) as the independent variables, the ApoE group (ApoE-

ε2, ApoE-ε3, and ApoE-ε4) as the moderator variable, and age, sex, BMI, SES and educational level as covariates were performed.

In order to decompose the significant interactions between ApoE and the regression estimates, we ran a second model with the two ApoE dummy varibles as moderator variables (ApoE-ε2 vs ε3 and ApoE-ε2 vs ε4; ApoE-ε3 vs ε2 and ApoE-ε3 vs ε4; ApoE-ε4 vs ε2 and ApoE-ε4 vs ε3, depending on the allele where the significant relationship had been observed), and age, sex, BMI, SES, and educational level as covariates. The regression model for RAVLT delayed recall revealed a main effect of mean cortisol (p=.013), a main effect of ApoE (p=.012), and a significant mean cortisol*ApoE interaction (p=.013). By decomposing this interaction for RAVLT delayed recall, we did not obtain a significant mean cortisol*ApoE-ε4 vs ε2 (p=.156) interaction, or a mean cortisol*ApoE-ε4 vs ε3 (p=.085) interaction, although a trend was observed in the latter. By contrast, we did not find a significant mean cortisol*ApoE interaction for Rivermead Immediate recall (p= .464) or the TMT A (p=.640), nor a significant delta cortisol*ApoE interaction for RAVLT delayed recall (p=.360) (Table 3.5, see page 89). It is important to note that, given our sample size and the different proportions of participants in each ApoE group, there may not be enough statistical power to detect some interactions. Therefore, these exploratory results should be interpreted with caution.

3.4. DISCUSSION

We aimed to examine the differences between the ApoE groups in cognitive performance and cortisol levels during the neuropsychological assessment in healthy

older people. Our second aim was to analyze the association between cortisol levels and cognitive performance, taking into account the three ApoE groups. To do so, we explored these possible relationships, first in the complete sample and then for each ApoE group.

Regarding our first aim, our results showed significant differences among the ApoE groups only on declarative memory, where, consistent with previous results, ApoE-ε2 allele carriers performed better than the other allele groups (Wisdom et al., 2011); specifically, Helkala et al. (1995; 1996) also observed better learning ability in the ApoE-ε2 group, but in a sample of older elderly people (mean age 74 years old). However, contrary to what was reported by Wisdom et al. (2011) in their meta-analysis, we found no differences in declarative memory between the ApoE-ε3 and ApoE-ε4 groups. It is worth noting that, more recently, Lancaster et al. (2016) did not report differences in cognitive function between ApoE-ε4 carriers and non-carriers in mid-adulthood (mean age between 35 and 60 years). In addition, in our study, all participants underwent very restrictive criteria (28 on the MEC), indicating no cognitive impairment, whereas it has been reported that cognitive deficits related to the ApoE-ε4 allele could be due to the inclusion of preclinical cases of AD (Batterham, Bunce, Cherbuin, & Christensen, 2012).

No differences were found among the ApoE groups in mean cortisol levels, coinciding with a lack of association between serum or salivary cortisol levels and the ApoE genotype in AD and healthy controls (Fiocco et al., 2008; Lara et al., 2013; Li et al., 2006). However, an association between higher CFS cortisol levels and the ApoE-ε4 allele was also found in AD patients (Gil-Bea et al., 2010; Peskind et al., 2001) and

healthy elderly people (Peskind et al., 2001). As cortisol secretion follows a diurnal rhythm, with a peak after awakening and a steady decline throughout the day (Edwards, Evans, Hucklebridge, & Clow, 2001), in our study cortisol levels decreased during the session. However, we did not observe an effect of ApoE group, suggesting that no differences between groups occur in cortisol secretion. However, it is important to note that this result may also be due to the relatively small sample size of the groups, and more research is needed.

Regarding our second aim, for the complete sample, higher mean cortisol levels were associated with worse declarative memory performance, which is consistent with previous studies (Lee et al., 2007; Li et al., 2006; MacLullichet al., 2005; Seeman, McEwen, Singer, Albert, & Rowe, 1997; Vedhara, Hyde, Gilchrist, Tytherleigh, & Plummer, 2000; Wright, Kunz-Ebrecht, Iliffe, Foese, & Steptoe, 2005). In addition, a trend was observed where higher mean cortisol levels were associated with less time performing the TMT-A and, therefore, better attention. Glucocorticoid receptors are widely distributed in the hippocampus and prefrontal cortex, which are involved in declarative memory and attention and executive function, respectively (Lupien et al., 2007). An inverted-U pattern has been observed in the association between cortisol and cognition, suggesting that very low or high glucocorticoids levels would worsen cognitive performance, while medium levels would facilitate it (de Kloet et al., 1999; Lupien et al., 2007). Therefore, our results suggest that the point at which cortisol levels begin to impair cognitive performance would be different for declarative memory and attention, justifying the fact that higher cortisol levels worsened declarative memory but improved attention. On the other hand, although higher mean cortisol levels

worsened declarative memory performance, it was also observed that a progressive increase along the session improved it. Hence, these results would also support the inverted-U pattern, and highlights the complex dynamics that exist in the relationship between cortisol levels and cognitive function.

When we analyze the groups categorized by the ApoE allele, the association between higher mean cortisol levels and worse declarative memory performance, both immediate and delayed recall, was only observed in the ApoE-ε4 group. In addition, we found a significant interaction between ApoE and mean cortisol levels for delayed recall, but not for immediate recall. Although few studies have explored cognitive function, the HPA-axis, and ApoE polymorphism together, previous results showed an association between the ApoE-ε4 allele, a faster decline in verbal fluency, and a flatter diurnal slope, that is, higher evening cortisol and lower morning cortisol (Gerritsen et al., 2011; Singh-Manoux et al., 2014). As mentioned above, two studies explored these associations by measuring cortisol levels during the cognitive assessment (Berteau-Pavy et al., 2007; Lee et al., 2008). Berteau-Pavy et al. (2007) found that in people from 62 to 92 years old, the ApoE-ε4 allele was associated with worse performance on the object recognition and spatial navigation test, but they did not find a relationship with cortisol levels. By contrast, Lee et al. (2008) observed that, whereas higher cortisol was associated with lower cognitive performance, the slopes were steeper in the ApoE-ε4 group. This study was carried out in participants with a similar age range (50-70 years), and it assessed similar cognitive domains to those assessed in our study. However, in Lee et al. (2008), subjects were not clinically assessed for dementia, and they were not excluded due to drug or medication use, which could have altered

cognition or diseases such as diabetes (19.3% of the sample) despite it has been observed a dysregulation in the HPA-axis function in responses to stress (Steptoe et al., 2014). In addition, these authors grouped the participants only as ApoE-ε4 allele carriers and non- carriers. Thus, in our study, we consider the three allelic variations (ApoE-ε2, ApoE-ε3 and ApoE-ε4), and cortisol was obtained while a wide range of cognitive functions were assessed. The findings support the idea that, in healthy non-demented older people from 55 to 77 years old, ApoE-ε4 carriers might be more vulnerable to potential detrimental effects of HPA-axis dysfunction on verbal memory performance.

On the contrary, only the ApoE-ε3 group showed an association between an increase of cortisol levels during the neuropsychological assessment and a better declarative memory performance, suggesting a more adaptive response to a cognitive challenge of the HPA-axis in this group. What is more, the association between higher mean cortisol levels and better TMT A performance, was only observed in ApoE-ε3 group. Therefore, our findings would also support lower vulnerability in ApoE-ε3 to the detrimental effects of cortisol on attention and eye-hand coordination. Recently, Piskunowicz et al. (2017) reported that ApoE-ε3 allele carriers performed better on TMT B than ApoE-ε2 and ApoE-ε4 allele carriers. Therefore, our results show that the ApoE genotype modulates attention and eye-hand coordination, and the ApoE-ε3 allele would confer an advantage compared to the other alleles. Finally, it is worth noting that we found no association between cortisol levels and cognition for the ApoE-ε2 allele, despite it has been related to an increased risk of developing post-traumatic stress disorder (Kim et al., 2013), susceptibility to stress-induced impairments in memory

(Freeman, Roca, Guggenheim, Kimbrell, & Griffin, 2005) and HPA-axis dysregulation (Johnson et al., 2015).

Some limitations should be considered. First, it is important to note the correlational nature of the results, and so we cannot claim causal relationships. In addition, due to the unequal distribution of the three ApoE alleles and the low frequency of the ApoE-ε2 and ApoE-ε4 alleles in the population, in our sample the number of participants in each ApoE group differed, and the sample size for the ApoE-ε2 and the ApoE-ε4 groups was small. Therefore, it is possible that, due to the small sample sizes, some of the hypothesized results were not observed. Thus, a larger sample size would be necessary to increase the statistical power. Nevertheless, it is important to highlight the effort that has been made in this study to separate and compare the three allelic variations and study their relationship with a wide range of cognitive domains and the HPA-axis. In addition, the strict exclusion criteria make it possible to obtain a healthy older sample and control the effect of confounding variables. Moreover, due to the sample size, sex differences were not taken into account, although several studies with healthy elderly people have shown that women outperform men on verbal memory (Zhang, Zhou, Wang, Zhang & Study, 2017; Munro et al., 2012). Furthermore, there is evidence that women are at higher risk of AD than men (Farrer et al, 1997), and so it seems necessary to study sex differences in the influence of the ApoE gene on cognitive function. Thus, future studies with larger samples are needed to shed light on this issue.

In summary, our results show that, in healthy older people, the ApoE-ε2 allele may have a protective effect on declarative memory, specifically learning ability and no association between cortisol levels and cognition was observed for this allelic group. In addition, we did not find differences in cortisol levels between the ApoE groups. Additionally, although the ApoE-ε4 allele did not show a negative effect on cognitive function compared to ApoE-ε3, high cortisol levels would be especially detrimental to declarative memory in this group, compared to the ApoE-ε2 and ApoE-ε3 groups. Thus, the ApoE-ε4 allele could add greater vulnerability to the adverse effects of HPA-axis dysregulation on declarative memory during aging. On the other hand, the ApoE-ε3 allele could be related to a more adaptive stress response, where higher cortisol levels would improve attention and declarative memory.

Table 3.3.
Regression analyses with mean cortisol as predictor and cognitive outcomes as dependent variables, unadjusted and adjusted for covariates. Values in bold represent significant or marginal p values.

		RAVLT Total Learning	RAVLT Immediate Recall	RAVLT Delayed Recall	Rivermead Immediate Recall	Rivermead Delayed Recall	DS-Forward	DS-Backward	LNS	TMT-A	TMT-B	Stroop Interfe-rence
Unadjusted analyses												
Total	AdjR2	.004	-.007	**.069**	**.026**	-.012	-.012	-.012	-.012	**.041**	-.011	-.012
	Beta	-.126	.069	**-.284**	**-.196**	-.006	.020	.006	-.010	**-.230**	.033	.024
	p	.256	.533	**.009**	**.077**	.956	.858	.958	.928	**.037**	.768	.830
ApoE-ε2	AdjR2	-.069	-.142	-.086	.164	-.125	-.081	-.053	.092	-.142	-.141	-.141
	Beta	-.254	-.020	.223	-.518	-.126	.233	.281	-.454	-.024	-.036	.044
	p	.510	.960	.564	.153	.747	.546	.464	.220	.951	.926	.910
ApoE-ε3	AdjR2	.028	-.017	.017	-.017	.005	.009	-.006	-.017	**.079**	-.017	-.014
	Beta	-.212	-.019	-.185	-.027	-.150	.163	.111	.026	**-.309**	-.028	-.070
	p	.110	.885	.165	.841	.265	.222	.409	.847	**.019**	.837	.611
ApoE-ε4	AdjR2	-.056	.034	**.277**	**.325**	.024	.073	.111	-.065	-.071	.068	.083
	Beta	-.119	.314	**-.570**	**-.609**	.298	-.368	-.413	-.075	-.003	.361	.379
	p	.661	.237	**.021**	**.012**	.263	.161	.112	.783	.990	.170	.147
Adjusted analyses for covariates												
Total	AdjR2	.003	-.005	**.072**	**.227**	-.013	.364	.163	.285	**.085**	.193	-.012
	Beta	-.125	.085	**-.289**	**-.224**	-.016	-.015	-.022	-.030	**-.206**	.062	.033
	p	.267	.452	**.009**	**.026**	.888	.867	.828	.751	**.060**	.544	.771
ApoE-ε2	AdjR2	-.160	-.153	-.121	-.047	-.071	-.111	-.127	.551	-.088	-.167	-.124
	Beta	-.073	.109	.197	-.320	-.287	.218	.185	-.394	.260	-.003	.192
	p	.863	.798	.639	.440	.491	.605	.662	.189	.535	.995	.649
ApoE-ε3	AdjR2	**.032**	-.018	.019	.102	.009	.390	.180	.376	**.074**	.056	-.015
	Beta	**-.221**	-.004	-.190	-.086	-.163	.091	.124	-.038	**-.302**	.015	-.063
	p	**.098**	.974	.156	.505	.229	.394	.314	.723	**.024**	.909	.653
ApoE-ε4	AdjR2	.161	.034	**.548**	**.605**	.261	.640	.280	-.065	.190	.068	.083
	Beta	-.032	.314	**-.491**	**-.518**	.221	.010	-.250	-.075	-.096	.361	.379
	p	.895	.237	**.015**	**.008**	.343	.956	.303	.783	.690	.170	.147

Note. RAVLT=Rey Auditory Verbal Learning Test; DS= Digit Span; LNS= Letter-number sequencing; TMT= Trail-Making Test.; ApoE = Apolipoprotein E.

Table 3.4.
Regression analyses with delta cortisol as predictor and cognitive outcomes as dependent variables, unadjusted and adjusted for covariates. Values in bold represent significant or marginal *p* values.

		RAVLT Total Learning	RAVLT Immediate Recall	RAVLT Delayed Recall	Rivermead Immediate Recall	Rivermead Delayed Recall	DS-Forward	DS-Backward	LNS	TMT-A	TMT-B	Stroop Interfe-rence
Unadjusted analyses												
Total	AdjR2	.013	-.009	**.033**	-.008	-.002	.014	-.010	.014	-.011	**.041**	-.013
	Beta	.158	.058	**.211**	-.065	-.100	-.162	.049	-.162	.038	**.230**	.014
	p	.154	.604	**.055**	.564	.371	.143	.661	.143	.736	**.038**	.904
ApoE-ε2	AdjR2	-.135	-.085	-.012	-.098	-.045	-.143	-.143	-.003	-.116	-.083	-.142
	Beta	.084	-.225	.339	-.198	-.293	-.001	.012	-.305	.154	.228	.022
	p	.830	.560	.372	.609	.444	.997	.976	.356	.693	.555	.955
ApoE-ε3	AdjR2	.011	-.007	**.073**	-.017	-.016	**.051**	-.018	.024	-.014	**.059**	-.019
	Beta	.168	.105	**.299**	-.030	-.042	**-.260**	.024	-.204	.064	**.275**	-.002
	p	.207	.433	**.023**	.825	.757	**.049**	.860	.125	.636	**.039**	.991
ApoE-ε4	AdjR2	-.071	-.066	-.057	-.064	-.018	-.071	-.054	-.068	-.071	-.059	-.070
	Beta	-.006	.071	-.116	-.081	-.224	-.023	.126	.057	-.020	.109	.039
	p	.983	.794	.669	.765	.404	.932	.642	.835	.941	.689	.886
Adjusted analyses for covariates												
Total	AdjR2	**.035**	-.013	**.048**	.182	-.007	.365	.190	.284	.042	.191	-.013
	Beta	**.216**	.008	**.245**	.083	-.074	.035	.165	.009	-.017	.045	-.004
	p	**.052**	.945	**.028**	.425	.514	.700	.112	.927	.876	.666	.973
ApoE-ε2	AdjR2	-.094	-.136	-.044	-.167	.026	-.166	-.160	.418	-.009	-.085	-.152
	Beta	.249	-.162	.324	.009	-.407	-.031	-.077	-.231	.367	.265	.112
	p	.551	.702	.434	.983	.318	.943	.885	.460	.371	.527	.792
ApoE-ε3	AdjR2	**.033**	-.017	**.107**	.103	-.018	.383	.197	.375	-.018	.075	-.018
	Beta	**.224**	.034	**.351**	.092	.016	-.035	.182	-.008	.015	.137	-.041
	p	**.093**	.799	**.007**	.480	.910	.750	.146	.938	.915	.300	.769
ApoE-ε4	AdjR2	.161	-.066	.304	.306	.248	.669	.247	-.068	.183	-.059	-.070
	Beta	.027	.071	-.156	-.041	-.189	.202	-.186	.057	-.054	.109	.039
	p	.909	.794	.483	.851	.415	.349	.480	.835	.820	.689	.886

Note. RAVLT=Rey Auditory Verbal Learning Test; DS= Digit Span; LNS= Letter-number sequencing; TMT= Trail-Making Test.; ApoE = Apolipoprotein E.

Table 3.5.
Regression analyses with cortisol (mean or delta), ApoE, and cortisol (mean or delta) *ApoE as predictors, and cognitive outcomes (RAVLT delayed recall, Rivermead immediate recall and TMT A) as dependent variables, adjusted for covariates. . Decomposition of significant mean cortisol*ApoE interactions were performed with regression analyses with mean cortisol, ApoE-ε4 vs ε2 and ApoE-ε4 vs ε3, and mean cortisol*ApoE-ε4 vs ε2 and mean cortisol*ApoE-ε4 vs ε3 as predictors, and RAVLT delayed recall, as dependent variable, adjusted for covariates. Values in bold represent significant or marginal p values

	RAVLT delayed recall			Rivermead immediate recall			TMT A		
	Beta	p	95% CI	Beta	p	95% CI	Beta	p	95% CI
Mean cortisol	**110.126**	**.043**	3.481, 216.771	7.680	.688	-30.330, 45.691	-33.542	.421	-116.301, 49.216
ApoE	**31.608**	**.012**	7.051, 56.165	3.949	.371	-4.800, 12.699	-3.172	.740	-22.230, 15.885
Mean cortisol*ApoE	**-41.062**	**.013**	-73.459, -8.665	-4.252	.464	-15.793, 7.288	5.916	.640	-19.225, 31.057
Mean cortisol	**-49.719**	**.004**	-83.242, -16.196						
ApoE-ε4 vs ε2	-52.174	.188	-130.610, 26.262						
ApoE-ε4 vs ε3	**-30.050**	**.037**	-58.328, -1.773						
Mean cortisol* ApoE-ε4 vs ε2	69.679	.156	-27.400, 166.758						
Mean cortisol* ApoE-ε4 vs ε3	**34.897**	**.085**	-4.986, 74.781						
Delta cortisol	59.664	.183	-28.856, 148.185						
ApoE	3.425	.288	-2.953, 9.804						
Delta cortisol*ApoE	-13,667	.360	-43.248, 15.914						

Note. RAVLT=Rey Auditory Verbal Learning Test; TMT= Trail-Making Test.; ApoE = Apolipoprotein E.

CHAPTER 4

THE RELATIONSHIP BETWEEN LONELINESS AND COGNITION IN HEALTHY OLDER MEN AND WOMEN: THE ROLE OF CORTISOL

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4.1. INTRODUCTION

The world population is aging, and projections indicate that the number of people over 60 years old will reach two billion in 2050 (United Nations Population Fund [UNFPA], 2012). Although dementia it is not a normal part of healthy ageing, it affects around 50 million people worldwide, and it has been recognized as a public health priority (WHO, 2019). Therefore, it is important to identify the factors that may contribute to these health problems in older people, and loneliness has been proposed as one of them (WHO, 2013).

Loneliness is defined as a subjective feeling of dissatisfaction with social relationships (Young, 1982). It is particularly important in older adults because one-third of them will experience it to some degree (Victor et al., 2005), especially women, who are at greater risk (see review: Pinquart & Sorensen, 2001). In fact, loneliness has been considered a major risk factor for physical and mental health problems in later life (see review: Ong et al., 2015), including reduced longevity (Holt-Lunstad et al., 2015).

4.1.1. Loneliness and cognitive function

Among the health problems associated with loneliness, an increased risk of cognitive decline and dementia can be highlighted (see reviews: Cacioppo, & Hawkley, 2009; Cacioppo et al., 2014). In addition, in a review, Boss et al. (2015) concluded that in older people, greater loneliness was associated with lower cognitive functioning. However, studies included in this review reported mixed findings about the association

between loneliness and global cognition (Gow, Corley, Starr, & Deary, 2013; O'Luanaigh et al., 2012; Schnittger, Wherton, Prendergast, & Lawlor, 2012), processing speed (Gilmour, 2011; Gow et al., 2013; O'Luanaigh et al., 2012), executive function (Gilmour, 2011; Schnittger et al., 2012), and immediate and delayed memory (Gilmour, 2011; O'Luanaigh et al., 2012; Schnittger et al., 2012; Shankar, Hamer, Mcmunn, & Steptoe, 2013; Wilson et al., 2007c). Furthermore, these associations were weak, and some of them disappeared after controlling for psycho-sociodemographic variables (Boss et al., 2015).

These mixed findings may be due, at least in part, to the cognitive status of the participants. The absence of dementia was an inclusion criterion in some studies (Gilmour, 2011; Holwerda et al., 2012; O'Luanaigh et al., 2012; Schnittger et al., 2012; Wilson et al. 2007c), but it was not specified in others (Donovan et al., 2016; Gow, Pattie, Whiteman, Whalley, & Deary, 2007; Gow et al., 2013; Tilvis et al., 2004; Shankar et al., 2013). Additionally, most of the studies measured loneliness with one or two items asking participants directly if they felt lonely (Donovan et al., 2016; Gow et al., 2007; 2013; Holwerda et al., 2012; O'Luanaigh et al., 2012; Tilvis et al., 2004), which could lead to underreporting due to the social stigma associated with being identified as lonely (Pinquart & Sorensen, 2001). Gilmour (2011) measured loneliness with three items, whereas other authors used five (Wilson et al., 2007c) or six items (Schnittger et al., 2012) from the de Jong-Gierveld Loneliness Scale. Shankar et al. (2013) used the three-item short form of the R-UCLA scale, but none of the studies administered the 20-item R-UCLA scale. Moreover, most of the studies did not explore

the role of sex in the association between loneliness and cognitive function, and therefore it is an unresolved issue that should be addressed.

4.1.2. Loneliness and Hypothalamic–Pituitary–Adrenal axis

Some authors have suggested that loneliness is a psychological experience that contributes to biological stress (Steptoe et al., 2004), and several studies have explored its association with the Hypothalamic–Pituitary–Adrenal (HPA) axis. The HPA-axis is a neuroendocrine system that plays a key role in the stress response, and its end product is cortisol. In addition, cortisol follows a diurnal rhythm, reaching its highest levels early in the morning, with a decrease throughout the day, and the lowest levels in the evening. However, a dysregulation of the HPA-axis has been reported during chronic stress, showing a higher diurnal cortisol output and a flattened diurnal slope with lower morning and higher evening cortisol levels (Miller et al., 2007).

Several studies have explored the association between loneliness and both the diurnal cortisol output and the DCS, with inconsistent findings. These relationships have been studied in young (Cacioppo et al., 2000; Doane & Adam, 2010; Drake et al., 2016; Lai et al., 2018; Pressman et al., 2005), middle-aged (Ebrecht et al., 2004; Grant et al., 2009; Steptoe et al., 2004), or older participants (Adam et al., 2006; Cole et al., 2007; Schutter et al., 2017). In this latter age group, two studies did not observe an association between loneliness and awakening cortisol levels or the DCS (Adam et al., 2006; Schutter et al., 2017), whereas another study found that lonelier individuals showed a blunted cortisol rhythm (Cole et al., 2007). None of these three studies

analyzed the association between loneliness and the diurnal cortisol output or took sex differences into account. Therefore, more research is needed to clarify these issues.

4.1.3. Hypothalamic–Pituitary–Adrenal axis and cognitive function

In addition to stress and loneliness, a flatter DCS and higher diurnal cortisol levels, which reflect HPA-axis dysregulation, have been associated with physical and mental health problems, including poorer cognition (Adam et al., 2017; Lupien et al., 2005). The hippocampus and prefrontal cortex are brain areas with a high density of glucocorticoid receptors, and they play a role in memory and attention and executive function, respectively. Thus, HPA-axis dysregulation is one of the mediators proposed to explain impaired cognitive function in older people (see review: Lupien et al., 2007).

Flatter DCS has been associated with poorer cognition in older adults with memory deficits and depressive symptoms (Fiocco, Wan, Weekes, Pim, & Lupien, 2006). Previous studies analyzing the association between the DCS and cognitive function in non-demented older people reported that worse cognitive performance or cognitive decline was related to both a steeper (O'Hara et al., 2007) and flatter DCS in cross-sectional (Stawski et al., 2011; Gerritsen et al., 2011) and follow-up (Beluche et al., 2010) studies. However, the absence of associations has also been reported (Ennis et al., 2016; Hidalgo et al., 2016; Singh-Manoux et al., 2014).

Analyzing the two components (i.e. awakening vs evening) of the DCS independently, mixed findings have also been found for the association between cortisol and cognition. Higher awakening cortisol levels have been associated with better (Ennis et al., 2016; Singh-Manoux et al., 2014) and worse cognitive performance

in both cross-sectional (Beluche et al., 2010; O'Hara et al., 2007) and follow-up studies (Beluche et al., 2010). On the other hand, the relationship between higher evening cortisol levels and worse cognitive performance has been reported in cross-sectional studies (Gerritsen et al., 2011; Stawski et al., 2011), in follow-up studies (Li et al., 2006), and only in women (Singh-Manoux et al., 2014), with these results being more consistent.

Moreover, some studies in older people observed that higher diurnal cortisol levels were related to poorer cognitive performance, specifically on verbal memory and executive function (Li et al., 2006; Ouanes et al., 2017a), but other studies in middle-aged and older people did not find these results (Ennis et al., 2016; Harris et al., 2017; Singh-Manoux et al., 2014). Therefore, the association between different cortisol indexes and cognitive performance during aging is complex and poorly understood.

4.1.4. Biological pathways mediating the association between loneliness and cognitive function

HPA-axis dysregulation is one of the mechanisms proposed as biological pathways underlying the association between loneliness and cognitive function (Boss et al., 2015; Ong et al., 2015; Cacioppo, & Hawkley, 2009; Cacioppo et al., 2014). However, to our knowledge, the mediating role of the HPA-axis in the relationship between loneliness and cognitive function has not been explored.

Our main goal was to analyze the role of the HPA-axis as a mediator in the relationship between loneliness and cognitive function in non-demented healthy older people. To do so, first, we explored the association between loneliness and both (i)

HPA-axis functioning indexes and (ii) different cognitive domains, as well as (iii) the association between HPA-axis functioning and the different cognitive domains. Finally, we explored sex differences in these relationships, given that sex differences in loneliness are unclear, and that sex may play a role in both loneliness and health (see review: Brown, Gallagher, & Creaven, 2017).

Due to the inconsistent results from previous studies on the relationship between loneliness and the DCS (Adam et al., 2006; Cole et al., 2007; Schutter et al., 2017) and the lack of studies on the relationship between loneliness and the diurnal AUCg in older people, we were not able to define the direction of these relationships. We hypothesized a weak but significant negative association between loneliness and performance on different cognitive domains (see review: Boss et al., 2015). We also expected a negative association between cognitive function and awakening cortisol (Beluche et al., 2010; O'Hara et al., 2007), bedtime cortisol (Li et al., 2006; Stawski et al., 2011), the DCS (Beluche et al., 2010; Stawski et al., 2011), and the diurnal cortisol output (Li et al., 2006; Ouanes et al., 2017a). In addition, we hypothesized that this dysregulated HPA-axis pattern would mediate the relationship between loneliness and poorer cognitive performance. Finally, we hypothesized that these associations would be more pronounced in women than in men because some authors have suggested that loneliness affects women's health more than men's (Christiansen et al., 2016).

4. 2. METHODS

4.2.1. Participants

The final sample was composed of 86 participants of both sexes (41 men and 45 women), ranging from 60 to 80 years old (M= 67.44, SD= 4.37). Participants' characteristics are presented in Table 4.1. There were no significant differences between men and women in age, loneliness, or depression (all $p \geq 0.184$), but there were differences in educational level (p = .001) and marital status (p = .025). All the women were postmenopausal and had their last menstrual period more than 3 years before the testing time. None of the participants scored less than 27 on the Mini-Mental State Examination (MMSE), indicating the absence of cognitive impairment.

Participants were Caucasians, and they were recruited from classrooms in a study program at the University of Valencia (Spain) for people over 55 years of age. As compensation, they received a pen drive for participating in the study. Exclusion criteria were: age below 60 years old, smoking more than 10 cigarettes a day, alcohol or other drug abuse, non-corrected visual or hearing problems, diabetes, neurological or psychiatric disease, using any medication directly related to emotional or cognitive functioning or able to influence hormonal levels such as glucocorticoids, psychotropic substances, or sleep medications, having been under general anesthesia once or more than once in the past year, and the presence of a stressful life event during the past year, such as the death of a loved one, an accident, an important change in their habits, such as retirement, or any other event that they subjectively felt had affected them in a significant way. Of the 150 participants assessed, 42 were eliminated because they did not meet the inclusion criteria. Of the 108 participants who met the inclusion criteria, 22 participants were eliminated because they did not perform the neuropsychological evaluation (16 participants) and/or provide the saliva samples (7 participants).

Therefore, the final sample was composed of 86 participants who met the inclusion criteria and completed the whole study.

Table 4.1. Characteristics of the study population for the total sample, and for men and women.

	Total (N =86)	Men (N =41)	Women (N =45)	
Sex, (%)		47.7	52.3	
				p
Age, *M (SD)*	67.44 (4.37)	67.29 (3.97)	67.58 (4.74)	.764
Educational level, (%)				.001
Without studies	1.2	0	2.3	
Primary school	20.7	2.6	37.2	
Secondary school	18.3	15.4	20.9	
Graduate (3 years degree)	24.4	30.8	18.6	
Graduate (5 years degree)	32.9	46.2	20.9	
PhD	2.4	5.1	0	
Marital status N (%)				.025
Single	11.9	2.5	20.5	
Married	66.7	80.0	54.5	
Divorced	9.5	5.0	13.6	
Widower	11.9	12.5	11.4	
Depression, *M (SD)*	5.30 (4.22)	5.00 (3.58)	5.58 (4.75)	.529
Loneliness, *M (SD*)	34.05 (6.98)	35.10 (6.53)	33.09 (7.30)	.184

Note. M= mean; *SD*=standard deviation; %= percentages. Sex differences in age, depression, and loneliness were analyzed with Student-t tests, and in educational level and marital status with Chi-square tests.

4.2.2. Procedure

Participants who met the criteria were asked to attend one session that took place from 10:00 to 12:00 hours in the Laboratory of Social Cognitive Neuroscience at the University of Valencia. Before the session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol from the night before the first session. They were also instructed to drink only water, and not eat, smoke, take any stimulants

(such as coffee, cola, caffeine, tea or chocolate), or brush their teeth at least 1 hour prior to the session. All the participants provided written informed consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Committee of the University of Valencia. In this session, a neuropsychological battery was administered. Additionally, participants were asked to provide saliva samples at home on two consecutive weekdays in order to determine cortisol levels immediately after waking (awakening cortisol) and before going to sleep (bedtime cortisol). The participants were thoroughly instructed about how to provide the saliva samples, and they were given written instructions to drink only water and not eat or brush their teeth at least 1 h prior to each saliva sample. In addition, participants were asked to write down the time they provided the saliva samples at awakening and bedtime in a diary. A week later, participants returned to the laboratory to deliver the saliva samples and complete the questionnaires to obtain loneliness and depression scores.

4.2.3. Psychological tests

4.2.3.1. Loneliness. Loneliness was assessed with the Spanish adaptation (Vázquez & Jiménez, 1994) of the revised University of California Los Angeles Loneliness Scale (R-UCLA) (Russell, Peplau, & Cutrona, 1980). This scale contains 20 items rated on a four-point Likert scale ranging from one (never) to four (often), producing a total score ranging from 20 (low) to 80 (high). The internal consistency (Cronbach's α) of the Spanish adaptation was .94, and in this sample, it was .82.

4.2.3.2. Depression. Depression was assessed with the Spanish version (Fernández-San Martín et al., 2002) of the Geriatric Depression Scale (GDS)

(Yesavage et al., 1982) for detecting depression in older people. It consists of 30 items formulated as questions, with a dichotomous yes/no response. The lowest possible score is 0, and the highest is 30. The internal consistency (Cronbach's α) of the Spanish adaptation was .82, and in this sample, it was .81.

4.2.4. Neuropsychological tests

4.2.4.1. Global cognition. To measure global cognition, the Spanish version of the Mini-Mental State Examination (MMSE) (Lobo et al., 1999) was used. It includes eleven questions that measure cognitive functions, with higher values indicating better global cognitive function. The maximum score is 30, and a score of 23 or less is indicative of cognitive impairment.

4.2.4.2. Processing Speed, Attention, and Executive Function. These cognitive functions were measured with the Trail-Making Test (TMT). This test consists of two trials, TMT-A and TMT-B, each composed of 25 circles distributed on a white sheet of paper (Reitan, 1992). On TMT-A, the circles were numbered from 1 to 25, and the participant was asked to trace a line connecting the circles in numerical sequence as quickly as possible. The TMT-B included numbers from 1 to 13 and letters from A to L, and the participant was instructed to alternate between numbers and letters in ascending order. The score obtained was the number of seconds required to finish each trial. Errors were pointed out instantly by the examiner and contributed to the score due to the additional time needed for corrections. Two outcomes were obtained: (i) TMT-A: total number of seconds required to finish the TMT-A, which assesses attention and psychomotor processing speed, and (ii) TMT-B: total number of seconds required to

finish the TMT-B, which evaluates attention-switching and executive function performance.

4.2.4.3. Working memory (WM). To measure the participants' working memory performance, the Spanish version of the Letter-number sequencing (LNS) test from the Wechsler Memory Scale III was administrated (Wechsler, 1997). The experimenter read aloud series of mixed numbers (from 0 to 9) and letters (from A to Z) of increasing lengths (from 2 to 8 items). The participant had to repeat the series, ordering the numbers in ascending order and the letters in alphabetical order. Each set length was tested three times. The test ended when the participant failed three consecutive trials of the same length. One point was given for each correctly recalled attempt. One outcome was obtained: LNS (total number of correctly recalled attempts).

4.2.4.4. Verbal memory. To measure verbal memory performance, the story recall subtest from the Spanish version of the Rivermead Behavioral Memory Test (Wilson et al., 1985) was used. The experimenter read two short stories aloud, and participants had to recall as many memory units or ‘‘ideas’’ as possible, immediately after their oral presentation and after a 20-min delay. Participants' answers were audio recorded and corrected by an expert, and the sum of the correctly recalled ‘‘ideas’’ from the two stories was calculated. From this test, two outcomes were used for subsequent analysis: (i) Rivermead Immediate recall: total "ideas" recalled from the two stories immediately after the oral presentation and (ii) Rivermead Delayed recall: total "ideas" recalled from the two stories after 20 min, compared to the number of "ideas" recalled from the two stories immediately after the oral presentation (Delayed recall/ Immediate recall x100).

4.2.5. Salivary cortisol

Participants provided saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany). They were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, move the swab around in a circular pattern to collect saliva from all the salivary glands, and then store the saliva samples in the refrigerator until they were delivered to the laboratory. Once in the laboratory, the samples were kept in the refrigerator until they were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was stored at -80^{o}C until the analyses of the salivary cortisol levels. HPA-axis activity was measured by analyzing the salivary cortisol levels. Salivary cortisol concentrations were determined in duplicate with the salivary cortisol ELISA kit from Salimetrics (Newmarket, UK). Assay sensitivity was < .007 ug/dL. For each subject, all the samples were analyzed in the same trial. The inter- and intra- assay variation coefficients of raw optical densities were 1.365% and 1.48%, respectively. Cortisol levels were expressed in nmol/L.

4.2.6. Statistical Analysis

Participants' characteristics were described using percentages or means (standard deviation, *SD*), when appropriate, for the total sample and for men and women independently. To investigate sex differences in age, depression, and loneliness, independent sample Student-t tests were performed, whereas sex differences in educational level and marital status were analyzed with Chi-square tests.

Although the HPA- axis functioning is expected to be stable, in order to obtain better reliability, saliva was collected on two consecutive days to ensure that the cortisol data truly reflected the baseline functioning of the HPA-axis. This was verified

with the correlation analyses, which allowed us to work with the average cortisol levels for both days (for awakening cortisol: r=.517, p< .001 and for bedtime cortisol: r=.483, p< .001). Thus, we obtained four indexes: (i) awakening cortisol: mean cortisol immediately after waking on the two days (M= 6.576, SD= 3.158), (ii) bedtime cortisol: mean cortisol before going to sleep on the two days (M=1.782, SD=1.649), (iii) the DCS: bedtime cortisol minus awakening cortisol/time interval between awakening and bedtime, and (iv) the AUCg to measure the diurnal cortisol output (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). The mean awakening time was 7:15 am and the mean bedtime was 12:05 pm. If awakening or bedtime cortisol values were only obtained for one of the two days (n= 6 and n=10, respectively), data from only one day were included in the analysis, rather than the average. Bedtime cortisol levels were not obtained from three participants on either of the two days. Thus, awakening and bedtime cortisol levels were obtained from 86 and 83 participants, respectively. Missing values were treated by ignoring these data when performing the statistical analyses.

Before performing the statistical analyses, participants who scored ±3 SD from the mean were identified, and z scores were winsorized. Moreover, standardized residuals were used to detect multivariate outliers (±3 SD). Specifically, we detected, and excluded from the respective analysis, one outlier for each of the following associations: (i) loneliness and bedtime cortisol, (ii) loneliness and AUCg, (iii) loneliness and TMT-A, (iv) awakening cortisol and TMT-A, (v) awakening cortisol and TMT-B, (vi) DCS and MMSE, (vii) DCS and TMT-A, (viii) DCS and TMT-B, (ix) AUCg and MMSE, and, finally, (x) AUCg and TMT-B.

Linear regression analyses were performed to study the association between (i) loneliness and cortisol indexes, (ii) loneliness and cognitive function outcomes, and (iii) cortisol indexes and cognitive function outcomes. All these analyses were performed unadjusted and adjusted for covariates. Adjusted analyses controlling for age, sex, marital status, educational level, and depression were performed because most of the studies on loneliness and cognitive function have included these covariates (see review: Boss et al., 2015). In addition, we tested whether sex moderated these relationships.

First, to investigate whether there was an association between loneliness and the cortisol indexes, separate analyses were performed for each cortisol index (awakening cortisol, bedtime cortisol, DCS, and AUCg) as dependent variable. For unadjusted analyses, we included loneliness in step one. For adjusted analyses, we conducted hierarchical analyses, including the covariates in step one and loneliness in step two. Then, in order to analyze whether there was a moderating effect of sex on the association between loneliness and cortisol indexes, we repeated these analyses, including the covariates, loneliness, and sex in step one, and the interaction between loneliness and sex (loneliness*sex) in step two.

Second, to investigate the associations between loneliness and the different cognitive outcomes, separate linear regression analyses were performed for each cognitive domain outcome (MMSE, TMT-A, TMT-B, LNS, Rivermead Immediate recall, and Rivermead Delayed recall) as dependent variable. For the unadjusted analyses, we included loneliness in step one. For adjusted analyses, we conducted hierarchical analyses including the covariates in step one, and loneliness in step two.

Then, in order to analyze whether there was a moderating effect of sex on the association between loneliness and each cognitive domain, we repeated these analyses, including the covariates, loneliness, and sex in step one, and the interaction between loneliness and sex (loneliness*sex) in step two.

Third, to investigate the associations between cortisol indexes and the different cognitive domains, separate analyses were performed for each cognitive domain outcome as dependent variable. For unadjusted analyses, we included one cortisol index (awakening cortisol, bedtime cortisol, DCS and AUCg) in step one. For adjusted analyses, we conducted hierarchical analyses, including the covariates in step one, and one cortisol index in step 2. To analyze whether sex moderated the association between the cortisol indexes and each cognitive domain, we repeated these analyses, including the covariates, one cortisol index, and sex in step one, and in step 2, the interaction between one cortisol index and sex (i.e. awakening cortisol*sex, bedtime cortisol*sex, DCS*sex or AUCg *sex) in step two.

Finally, when we observed a significant association between loneliness and a particular cortisol index (awakening cortisol, bedtime cortisol, DCS or AUCg) in the adjusted analyses, along with a significant association between this same cortisol index and a cognitive domain (MMES, TMT-A, TMT-B, LNS, Rivermead Immediate recall, or Rivermead Delayed recall), we analyze whether this cortisol index mediated the relationship between loneliness and a specific cognitive domain. For this purpose, we conducted regression models using PROCESS (v2.13.6), with the cognitive measure as the dependent variable, loneliness as the independent variable, and the cortisol index as the mediator variable, adjusted for covariates.

To perform these statistical analyses, version 25.0 of SPSS was used. All *p* values were two-tailed, and the level of significance was taken as $p<0.05$.

4.3. RESULTS

First, Pearson correlation analyses of the cortisol indexes were performed. Thus, the DSC was negatively related to awakening cortisol ($r= -.853$, $p\leq.001$) and positively related to bedtime cortisol ($r=.366$, $p\leq.001$). The AUCg was positively related to awakening ($r= .871$, $p\leq.001$) and bedtime cortisol ($r=.572$, $p= p\leq.001$), and negatively to the DCS ($r= -.496$, $p\leq.001$). No association was observed between awakening and bedtime cortisol ($r=.118$, $p=.287$).

4.3.1. Relationship between loneliness and cortisol indexes

When analyzing the relationship between loneliness and the cortisol indexes, results showed a significant positive association between loneliness and bedtime cortisol ($B= .365$, $p= .001$ and $B= .366$, $p= .001$), for unadjusted and adjusted analyses, respectively). No significant associations were observed between loneliness and awakening cortisol, DCS, or AUCg in unadjusted or adjusted analyses (all $p\geq .215$). In addition, there were no significant interactions between loneliness and sex for any of the cortisol indexes in either unadjusted or adjusted analyses (all $p\geq .172$) (Table 4.2).

Table 4.2. Regression analyses with loneliness or loneliness*sex as predictors, and the cortisol indexes (awakening cortisol, bedtime cortisol, DCS or AUCg cortisol) as dependent variables, unadjusted and adjusted for covariates.

	Unadjusted analyses		Adjusted analyses	
	Loneliness			
	R^2 change	Beta	R^2 change	Beta
Awakening cortisol	.002	-.050	.001	-.034
Bedtime cortisol	.133	.365 **	.129	.366**
DCS	.010	.101	.009	.092
AUCg cortisol	.013	.114	.022	.149
	Loneliness*sex			
Awakening cortisol	.000	-.011	.000	.018
Bedtime cortisol	.016	-.169	.022	-.212
DCS	.002	.058	.001	.052
AUCg cortisol	.017	-.174	.014	-.177

Note. DCS: diurnal cortisol slope. AUCg: area under the curve with respect to the ground; *$p < .05$. **$p < .01$.

4.3.2. Relationship between loneliness and cognitive function

Results showed that loneliness scores were significantly and positively associated with time performing the TMT-B and, therefore, with worse performance on executive function in the unadjusted analyses (B= .223, p= .039), but this association was marginal in the adjusted analyses (B= .196, p= .064). Likewise, loneliness scores were marginally and positively associated with time performing the TMT-A and, therefore, with worse performance on processing speed and attention in unadjusted (B= .208, p= .056) and adjusted analyses (B= .179, p= .088). None of the other associations between loneliness and cognitive outcomes were statistically significant (all $p\geq$ .191). In addition, there were no significant interactions between loneliness and sex in any of the cognitive domains (all $p\geq$. 314) (Table 4.3).

Table 4.3. Regression analyses with loneliness or loneliness*sex as predictors, and cognitive outcomes (MMSE, TMT-A, TMT-B, LNS, Rivermead immediate recall or Rivermead delayed recall) as dependent variables, unadjusted and adjusted for covariates.

	Unadjusted analyses		Adjusted analyses	
	Loneliness			
	R^2 change	Beta	R^2 change	Beta
MMSE	.015	.122	.022	.150
TMT-A	.043	.208#	.029	.179#
TMT-B	.050	.223*	.035	.196#
LNS	.002	-.048	.000	-.016
Rivermead Immediate Recall	.004	.065	.001	-.025
Rivermead Delayed Recall	.019	-.139	.007	-.083
	Loneliness*sex			
MMSE	.000	-.029	.001	-.031
TMT-A	.011	-.138	.010	-.139
TMT-B	.001	-.030	.001	-.040
LNS	.000	-.029	.000	.003
Rivermead Immediate Recall	.000	-.002	.001	.053
Rivermead Delayed Recall	.000	-.028	.006	-.106

Note. MMSE: Mini-Mental State Examination; TMT: Trail-Making Test; LNS: Letter-number sequencing. #$p < .09$. *$p < .05$. **$p < .01$.

4.3.3. Relationship between cortisol indexes and cognitive function

When investigating the association between the cortisol indexes and the different cognitive domain outcomes, results showed that bedtime cortisol levels were significantly and negatively related to performance on the MMSE in unadjusted analyses (B = -.235, p= .035) and marginally in adjusted analyses (B = -.218, p= .056). Furthermore, bedtime cortisol levels were significantly and negatively related to performance on the LNS (B= -.230, p= .036 and B = -.209, p= .047, for unadjusted and adjusted analyses, respectively) and on Rivermead immediate recall (B = -.323, p= .003 and B = -.326, p= .002, for unadjusted and adjusted analyses, respectively). In addition, bedtime cortisol levels were significantly and positively associated with time

performing (i.e. worse performance) the TMT-A (*B*= .403, *p* ≤.001 and *B* = .371, *p*≤ .001, for unadjusted and adjusted analyses, respectively) and the TMT-B (*B*= .352, *p*= .001 and *B* = .303, *p*= .003, for unadjusted and adjusted analyses, respectively). Moreover, the results showed a significant, negative association between the DSC and the MMSE in unadjusted analyses (*B* = -.262, *p*= .022); that is, a smaller decrease in cortisol levels during the day was related to worse cognitive function performance, but the association was marginal in adjusted analyses (*B* = -.218, *p*= .057). In addition, although not reaching significance, in unadjusted analyses, the DCS was marginally and positively associated with time performing TMT-B (*B* = .199, *p*= .082) and negatively with Rivermead Immediate Recall performance (*B* = -.215, *p*= .059). Furthermore, the AUCg cortisol index was only significantly and positively associated with time performing the TMT-B (i.e. worse performance) (*B*= .217, *p*= .048) in adjusted analyses. The AUCg was also marginally and positively associated with time performing the TMT-A (*B* = .204, *p*= .076 and *B* = .186, *p*= .082, for unadjusted and adjusted analyses, respectively), and negatively with Rivermead immediate recall performance (*B* = -.193, *p*= .091 and *B* = -.204, *p*= .071, for unadjusted and adjusted analyses, respectively). Moreover, sex only significantly moderated the association between AUCg cortisol, and both TMT B (*B*= -.351, *p*=.011) and LNS (*B*=.310, *p*=.032) performance in unadjusted analyses. However, in adjusted analyses, the association between AUCg cortisol and TMT-B remained marginal (*B*=-.244, *p*=.075), and the association with LNS disappeared (*B*=-.142, *p*=.321). Likewise, sex marginally moderated the association between DCS and TMT-A performance in adjusted analyses (*B*=-.299, *p*=.079), and with Rivermead Delayed Recall performance (*B*= -.292, *p*=.095

and *B*=-.357, *p*=.058, in unadjusted and adjusted analysis, respectively). None of the other associations were statistically significant or marginal (all *p*≥ .104) (Table 4.4 a and b).

Table 4a. Regression analyses with cortisol indexes (awakening cortisol, bedtime cortisol, DCS or AUCg cortisol) or cortisol indexes*sex interaction terms, as predictors and cognitive outcomes (MMSE, TMT-A, TMT-B, LNS, Rivermead immediate recall and Rivermead delayed recall) as dependent variables, unadjusted and adjusted for covariates.

	Unadjusted analyses		Adjusted analyses	
		Awakening cortisol		
	R^2 change	Beta	R^2 change	Beta
MMSE	.028	.167	.029	.170
TMT-A	.000	.012	.000	.014
TMT-B	.001	-.106	.002	-.049
LNS	.010	.100	.003	.058
Rivermead Immediate Recall	.001	.032	.000	-.019
Rivermead Delayed Recall	.004	-.064	.004	-.062
		Bedtime cortisol		
	R^2 change	Beta	R^2 change	Beta
MMSE	.055	-.235*	.047	-.218#
TMT-A	.162	.403**	.134	.371**
TMT-B	.124	.352**	.087	.303**
LNS	.053	-.230*	.043	-.209*
Rivermead Immediate Recall	.104	-.323**	.106	-.326**
Rivermead Delayed Recall	.018	.133	.015	.121
		DCS		
	R^2 change	Beta	R^2 change	Beta
MMSE	.068	-.262*	.046	-.218#
TMT-A	.029	.171	.024	.157
TMT-B	.040	.199#	.022	.150
LNS	.034	-.186	.010	-.103
Rivermead Immediate Recall	.046	-.215#	.030	-.176
Rivermead Delayed Recall	.029	.172	.030	.173
		AUCg cortisol		
	R^2 change	Beta	R^2 change	Beta
MMSE	.014	-.118	.008	-.089
TMT-A	.041	.204#	.034	.186#
TMT-B	.030	.175	.046	.217*
LNS	.006	-.078	.015	-.125
Rivermead Immediate Recall	.037	-.193#	.042	-.204#
Rivermead Delayed Recall	.002	.046	.002	.047

Note. DCS: diurnal cortisol slope; AUCg: area under the curve with respect to the ground; MMSE: Mini-Mental State Examination; TMT: Trail-Making Test; LNS: Letter-number sequencing. #*p* <.09. **p* < .05. ***p* < .01.

Table 4b. Regression analyses with cortisol indexes (awakening cortisol, bedtime cortisol, DCS or AUCg cortisol) or cortisol indexes*sex interaction terms, as predictors and cognitive outcomes (MMSE, TMT-A, TMT-B, LNS, Rivermead immediate recall and Rivermead delayed recall) as dependent variables, unadjusted and adjusted for covariates.

	Unadjusted analyses		Adjusted analyses	
	Awakening cortisol*sex			
	R^2 change	Beta	R^2 change	Beta
MMSE	.000	-.005	.001	-.036
TMT-A	.000	-.006	.004	.096
TMT-B	.015	-.185	.002	-.076
LNS	.006	.121	.002	-.067
Rivermead Immediate Recall	.003	.083	.000	.002
Rivermead Delayed Recall	.001	.056	.003	.086
	Bedtime cortisol*sex			
	R^2 change	Beta	R^2 change	Beta
MMSE	.000	-.007	.001	-.044
TMT-A	.000	.008	.000	-.005
TMT-B	.002	.049	.003	.060
LNS	.001	.052	.002	-.051
Rivermead Immediate Recall	.000	.024	.000	.004
Rivermead Delayed Recall	.005	-.086	.005	-.087
	DCS*sex			
	R^2 change	Beta	R^2 change	Beta
MMSE	.020	.219	.033	.287
TMT-A	.019	-.211	.036	-.299$^{\#}$
TMT-B	.020	.216	.005	.108
LNS	.010	-.152	.000	.008
Rivermead Immediate Recall	.002	.065	.009	.147
Rivermead Delayed Recall	.036	-.292$^{\#}$	.052	-.357$^{\#}$
	AUCg cortisol*sex			
	R^2 change	Beta	R^2 change	Beta
MMSE	.021	.188	.006	.103
TMT-A	.008	-.116	.002	-.051
TMT-B	.074	-.351*	.034	-.244$^{\#}$
LNS	.057	.310*	.012	.142
Rivermead Immediate Recall	.021	.187	.007	.110
Rivermead Delayed Recall	.000	.006	.001	.049

Note. DCS: diurnal cortisol slope; AUCg: area under the curve with respect to the ground; MMSE: Mini-Mental State Examination; TMT: Trail-Making Test; LNS: Letter-number sequencing. $^{\#}p$ <.09. *p < .05. **p < .01.

4.3.4. Mediation models

Because we observed significant associations between loneliness and bedtime cortisol, and between bedtime cortisol and several cognitive domains (assessed by TMT-A, TMT-B, LNS, and Rivermead immediate recall), separate mediation analyses adjusted for covariates were performed to examine the indirect effect of loneliness on these cognitive domains via bedtime cortisol. First, the model assessing the mediation effect of bedtime cortisol in the association between loneliness and TMT-A performance revealed significant effects of loneliness on bedtime cortisol (path *a:* B = .217, SE= .081, t= 2.654, p= .009) and of bedtime cortisol on TMT-A (path *b*: B= .458, SE= .160, t= 2.864, p= .005), but not a significant total effect (path *c*: B = .211, SE= .113, t= 1.860, p= .067) or a direct effect of loneliness on TMT-A (path *c'*: B = .111, SE= .113, t= .984, p= .328). However, there was a significant indirect effect of loneliness on TMT-A via bedtime cortisol levels (path *ab*: B = .099, *SE*= .055, 95% CI: .000, .219) (Figure 4.1).

Likewise, the model assessing the mediation effect of bedtime cortisol in the association between loneliness and TMT-B performance revealed significant effects of loneliness on bedtime cortisol (path *a:* B = .213, SE= .081, t= 2.611, p= .011) and of bedtime cortisol on TMT-B (path *b*: B= .384, SE= .161, t= 2.384, p= .019), but neither the total effect (path *c*: B = .114, SE= .112, t= 1.017, p= .312) nor the direct effect of loneliness on TMT-B was significant (path *c'*: B = .033, SE= .114, t= .288, p= .774). Finally, a significant indirect effect of loneliness on TMT-B via bedtime cortisol levels was found (path *ab*: B = .081, SE= .055, 95% CI: .000, .211) (Figure 4.2).

Similarly, the model assessing the mediation effect of bedtime cortisol in the association between loneliness and Rivermead immediate recall performance revealed significant direct effects of loneliness on bedtime cortisol (path *a:* B = .213, SE= .081, t= 2.747, p= .011) and of bedtime cortisol on Rivermead immediate recall (path *b*: B= -.508, SE= .184, t=- 2.691, p= .007), but not a total effect (path *c*: B = -.019, SE= .131, t= -.150, p= .880) or a direct effect of loneliness on Rivermead immediate recall (path *c'*: B = .088, SE= .131, t= .673, p= .502). However, a significant indirect effect of loneliness on Rivermead immediate recall via bedtime cortisol levels (path *ab*: B = -.108, *SE*= .071, 95% CI: -.273, -.004) was found (Figure 4.3). By contrast, a significant indirect effect of loneliness on LNS via bedtime cortisol levels was not observed (path ab: B = -.055, SE= .057, 95% CI: -.199, .021) (Table 4.5).

4.4. DISCUSSION

Our results showed that loneliness was significantly associated with higher bedtime cortisol levels, but not with the other cortisol indexes analyzed. Moreover, higher bedtime cortisol levels were related to worse cognitive performance on processing speed and attention, executive function, working memory, and verbal memory immediate recall. Furthermore, although we did not find a direct association between loneliness and cognitive function, we observed that bedtime cortisol levels mediated the association between loneliness and worse performance on attention and processing speed, executive function, and verbal memory immediate recall. No sex differences were observed in these associations.

Table 4.5. Mediation models to test the indirect effect of loneliness on cognitive performance (TMT-A, TMT-B, LNS and Rivermead immediate recall) via bedtime cortisol.

	B	SE	*t*	*p*
Effect of loneliness on bedtime cortisol (a)	**.217**	**.081**	**2.654**	**.009**
Effect of bedtime cortisol on TMT-A (b)	**.458**	**.160**	**2.864**	**.005**
Total effect of loneliness on TMT-A (c)	.211	.113	1.860	.067
Direct effect of loneliness on TMT-A (c')	.111	.113	.984	.328
	B	*SE*	*CI*	*95%*
Indirect effect of loneliness on TMT-A via bedtime cortisol *(ab)*	**.099**	**.055**	**.004**	**.219**
	B	SE	*t*	*p*
Effect of loneliness on bedtime cortisol *(a)*	**.213**	**.081**	**2.611**	**.011**
Effect of bedtime cortisol on TMT-B *(b)*	**.384**	**.161**	**2.384**	**.019**
Total effect of loneliness on TMT-B *(c)*	.114	.112	1.017	.312
Direct effect of loneliness on TMT-B *(c')*	.033	.114	.288	.774
	B	*SE*	*CI*	*95%*
Indirect effect of loneliness on TMT-B via bedtime cortisol *(ab)*	**.081**	**.055**	**.000**	**.211**
	B	SE	*t*	*p*
Effect of loneliness on bedtime cortisol *(a)*	**.213**	**.081**	**2.611**	**.011**
Effect of bedtime cortisol on LNS *(b)*	-.261	.159	-1.634	.106
Total effect of loneliness on LNS *(c)*	.122	.109	1.116	.268
Direct effect of loneliness on LNS *(c')*	.178	.113	1.567	.121
	B	*SE*	*CI*	*95%*
Indirect effect of loneliness on LNS via bedtime cortisol *(ab)*	-.055	.057	-.199	.021
	B	SE	*t*	*p*
Effect of loneliness on bedtime cortisol *(a)*	**.213**	**.081**	**2.611**	**.011**
Effect of bedtime cortisol on Rivermead immediate recall *(b)*	**-.508**	**.184**	**-2.747**	**.007**
Total effect of loneliness on Rivermead immediate recall *(c)*	-.019	.131	-.150	.880
Direct effect of loneliness on Rivermead immediate recall *(c')*	.088	.131	.673	.502
	B	SE	*CI*	*95%*
Indirect effect of loneliness on Rivermead immediate recall via bedtime cortisol *(ab)*	**-.108**	**.071**	**-.273**	**-.004**

Note. TMT: Trail-Making Test; LNS: Letter-number sequencing. Values in bold represent significant *p* values.

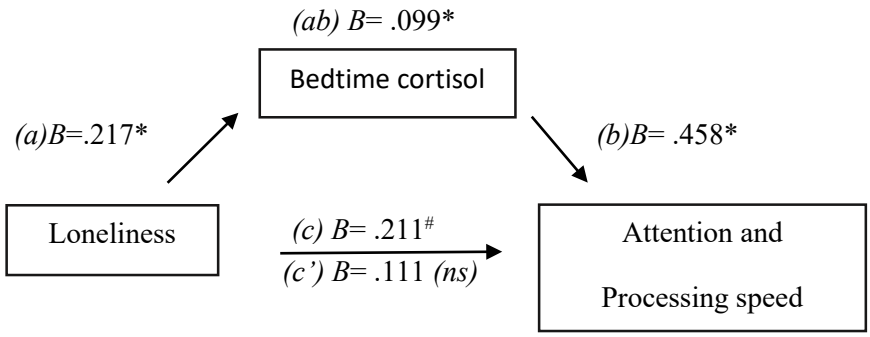

Figure 4.1. Mediation model to test the indirect effect of loneliness on attention and processing speed (Trail-Making Test A), via bedtime cortisol.

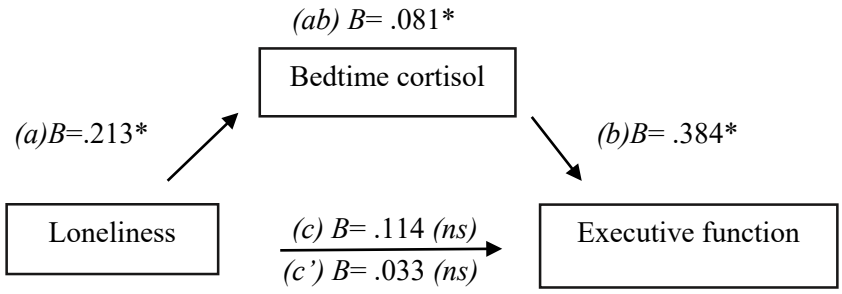

Figure 4.2. Mediation model to test the indirect effect of loneliness on executive function (Trail-Making Test B), via bedtime cortisol.

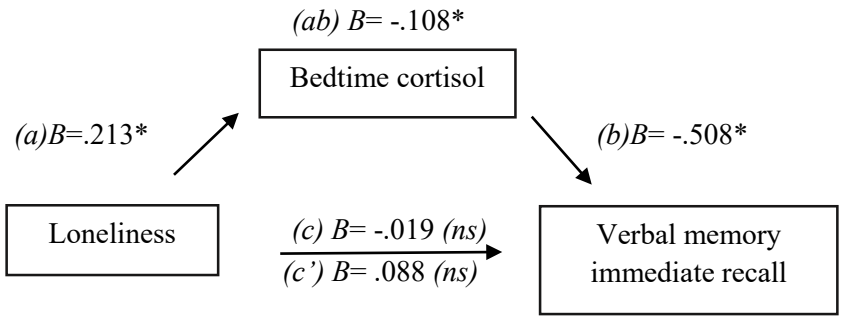

Figure 4.3. Mediation model to test the indirect effect of loneliness on verbal memory immediate recall (Rivermead immediate recall), via bedtime cortisol.

Our results showed that loneliness was related to higher bedtime cortisol levels, but not to awakening cortisol, the DCS or the AUCg. These results are consistent with previous studies in older people that did not find an association between loneliness and awakening cortisol levels (Schutter et al., 2017) or the DCS (Adam et al., 2006; Schutter et al., 2017). However, Cole et al. (2007), in a smaller and selected sample of individuals with extreme high and low loneliness scores that were stable over 3 years, observed that highly lonely individuals showed a blunted DCS compared to less lonely participants. To our knowledge, this is the first study to analyze the relationships between loneliness and both bedtime cortisol and the AUCg in older people.

We did not observe significant associations between loneliness and cognitive function. We only found that higher loneliness was marginally associated with worse performance on processing speed and attention (TMT-A), and significantly with executive function (TMT-B), but after controlling for sociodemographic factors, these associations disappeared. Schnittger et al. (2012) observed that emotional loneliness was related to worse performance on TMT-A, but not on TMT-B and verbal fluency, whereas social loneliness was related to worse performance on verbal fluency, but not on TMT-A and B. Other studies also observed that loneliness was related to worse performance on processing speed or executive function in cross-sectional studies (Gow et al., 2013; Gilmour, 2011; O'Luanaigh et al., 2012; Shankar el al., 2013) and in a follow-up study (Wilson et al. 2007c, except Shankar et al., 2013). However, as in our study, other studies found that the association between loneliness and processing speed or executive function did not persist after controlling for sociodemographic factors and depression (Gilmour, 2011; Gow et al., 2013).

In line with this study, Schnittger et al. (2012) failed to find an association between loneliness and global cognition. By contrast, other studies did find this association (Gow et al., 2013; O'Luanaigh et al., 2012), but it disappeared after controlling for depression (Gow et al., 2013). In addition, no association between loneliness and working memory has been reported (O'Luanaigh et al., 2012; Schnittger et al., 2012; Wilson et al. 2007c), consistent with our results. We did not find an association between loneliness and immediate or delayed verbal memory recall. Previous studies reported an association between loneliness and verbal memory at baseline (Gow et al., 2013; Shankar et al., 2013; Wilson et al., 2007c) and in the decline over time (Shankar et al., 2013), but others did not (Gow et al., 2013; Wilson et al., 2007c). In addition, an association between loneliness and verbal memory immediate recall, but not delayed recall, has been reported (Gilmour, 2011; O'Luanaigh et al., 2012). However, this association disappeared after controlling for psycho-sociodemographic variables (O'Luanaigh et al., 2012). Therefore, it is important to clarify which factors play a role in the relationship between loneliness and these cognitive domains.

Interestingly, we did not find significant associations between loneliness and any of the cognitive domains, but we observed an indirect effect of loneliness on attention and processing speed, executive function, and verbal memory immediate recall via bedtime cortisol levels. When analyzing mediation models, the indirect effect of loneliness on both TMT-B and Rivermead immediate recall was greater in magnitude than the direct effect. However, the indirect effect of loneliness on TMT-A was smaller than the direct effect. As previously noted, the power to detect an indirect

effect may be higher than the power to detect a total and direct effect (e.g., Loeys, Moerkerke, & Vansteelandt, 2014). Therefore, the results for the total and direct effects should be interpreted with caution because we cannot rule out the possibility of a direct effect of loneliness on these cognitive domains that could be observed with greater statistical power. Several studies have proposed that HPA-axis functioning is one of the biological mechanisms that could mediate between loneliness and cognition (reviews: Boss et al., 2015; Cacioppo, & Hawkley, 2009; Cacioppo et al., 2014; Ong et al., 2015). To our knowledge, this is the first study to explore this relationship, verifying a mediating effect of bedtime cortisol on the associations between loneliness and some cognitive functions. With all this in mind, the mediating effects may explain mixed findings regarding the relationship between loneliness and cognitive function.

Although it has been reported that healthier daily cortisol profiles, including a steeper DCS, higher morning cortisol levels, and lower afternoon and evening cortisol levels, are related to higher cognitive function (Stawski et al., 2011), this relationship has not always been observed. Some studies reported that a flatter DCS was related to poorer executive function (Stawski et al., 2011) and memory performance (Gerritsen et al., 2009), and a decline in visuospatial and visual memory performance in men and verbal fluency in women (Beluche et al., 2010). However, other studies reported no association between DCS and cognitive performance (Ennis et al., 2016; Hidalgo et al., 2016; Singh-Manoux et al., 2014), or even an association between a steeper DCS and worse performance on verbal memory delayed recall (O'Hara el al., 2007). In our study, we only observed that a higher DCS was marginally related to poorer

performance on global cognition, but not on the rest of the cognitive domains measured.

No association between awakening cortisol levels and cognitive function was observed; however, higher bedtime cortisol levels were related to worse cognitive performance on processing speed and attention, executive functioning, working memory, and verbal memory immediate recall. Although previous literature on the relationship between awakening cortisol levels and cognition shows heterogeneous results, the relationship between evening cortisol levels and cognitive function seems more consistent. Stawski et al. (2011) reported that lower cognitive function (i.e. episodic memory and executive function) was associated with lower awakening and higher bedtime cortisol levels. However, after performing these analyses independently for these two components, worse executive function performance was only related to higher bedtime cortisol levels, but not to awakening cortisol levels. In addition, other studies reported that higher bedtime cortisol levels were associated with poorer performance on memory, processing speed, and executive functioning (Geerlings et al., 2015; Li et al.,2006; Tene et al., 2018), but awakening levels were not (Geerlings et al., 2015; Tene et al., 2018). Furthermore, Geerlings et al. (2015) also observed that higher bedtime cortisol levels were associated with smaller total brain volume, whereas higher morning cortisol levels were associated with slightly greater than normal white matter volume and better cognitive performance. Moreover, similarly to Li et al.'s study (2006), we observed that higher diurnal cortisol output (AUCg) was only related to worse performance on executive function measured with the TMT-B test, but not to verbal recall, working memory, or global cognition. However, a recent study (Ouanes

et al., 2017a) observed that higher diurnal cortisol levels were related to poorer global cognitive performance, specifically on episodic memory. Nevertheless, no association has been found between the diurnal cortisol output and cognitive performance in middle-aged and older people (Ennis et al., 2016; Harris et al., 2017; Singh-Manoux et al., 2014).

Finally, it is worth noting that there were no sex differences in the relationship between loneliness and HPA-axis functioning or cognitive function, or in the association between HPA-axis functioning and cognitive function. Our study is the first to explore sex differences in the association between loneliness and awakening cortisol, bedtime cortisol, and the DCS in older adults, showing no significant differences. Steptoe et al. (2004) did not observe a sex interaction in the association between loneliness and HPA-axis function in middle-aged adults either. Sex differences in the loneliness-poorer cognitive function link have been explored very little. Although Gow et al. (2007) found this association only in women, but not in men, conclusions about sex differences in this relationship cannot be drawn because they did not perform interaction tests. As in Holwerda et al. (2012), we did not observe a moderating effect of sex in the relationship between loneliness and cognitive function, either directly or indirectly, via cortisol levels. We expected these associations to be more pronounced in women than in men because loneliness has been associated with greater health problems in women than in men (Christiansen et al., 2016). One possible explanation for this lack of sex differences in the association between loneliness and HPA-axis functioning and cognitive function is that we did not find differences in loneliness

between men and women, even though being a woman has been associated with greater loneliness (see review: Pinquart & Sorensen, 2001).

One limitation of our study is that, due to the correlational nature of the results, we cannot claim causal relationships. In addition, it is possible that, given the sample size, there was not enough statistical power to detect sex differences in the associations between loneliness and cortisol levels and cognition. Thus, a larger sample size would be necessary to explore sex differences in these relationships. Moreover, the fact that we ran multiple regression analyses to assess the association between the cortisol indexes and the different cognitive domains could lead to an inflation of Type 1 error. Furthermore, we did not include some neuropsychological functions that have shown an association with loneliness in previous studies, such as verbal fluency (Schnittger et al., 2012; Shankar et al., 2013), visual memory (O'Luanaigh et al., 2012; Schnittger et al., 2012), and visuospatial ability (Wilson et al., 2007c). Moreover, in our study, we only included two cortisol data points (awakening and bedtime cortisol); future studies should include more cortisol data points during the day to calculate the DCS and the CAR, which have also shown significant associations with loneliness (Adam et al., 2006; Shutter et al., 2017) and cognitive function (Evans, Hucklebridge, Loveday, & Clow, 2012; Hidalgo et al., 2016) in older adults. Despite these limitations, this study is the first to examine the mediating effect of HPA-axis functioning in the relationship between loneliness and poorer cognitive function in older people, providing interesting results to continue advancing in this area.

CHAPTER 5

THE ROLE OF PERSONALITY IN HPA-AXIS FUNCTIONING IN OLDER MEN AND WOMEN

5.1. INTRODUCTION

The pace of the population's aging around the world is increasing dramatically. Although as people age they are more likely to experience several health conditions, there is great heterogeneity in this process. Whereas some older adults maintain good physical and mental capacities, others experience significant decline (WHO, 2018). Therefore, it is important to identify vulnerability and protective factors in order to understand ways to improve health and reduce mortality risk.

Personality traits are among the factors that are known to influence health, subjective well-being, and mortality risk during aging (Friedman et al.,2010; Jerram & Coleman, 1999; Weston et al., 2015). Specifically, in a longitudinal study carried out in a large sample of older adults, Weston et al. (2015) reported that greater neuroticism was a risk factor for disease onset, whereas conscientiousness and openness, and to a lesser degree extraversion and agreeableness, were protective factors. Personality traits have been associated with aging-related diseases such as depression (Koorevaar et al., 2017), metabolic syndrome, heart disease, and diabetes (Mommersteeg & Pouwer, 2012), obesity (Terracciano et al., 2014), and cognitive impairment and dementia (Jaroudi et al., 2017; Luchetti, Terracciano, Stephan, & Sutin, 2016; Terracciano et al., 2014; Wilson et al., 2007b), among others.

This evidence has aroused interest in studying the biological pathways that could explain the relationship between personality and health. Because personality plays a role in ways of coping with psychological stress (Kaur et al., 2013; Soliemanifar et al.,

2018), several studies have analyzed how personality influences the biological reactivity to stressful events, which involves the autonomic nervous system, the immune system, sex hormones, the sympathetic-adrenal-medulla system, and the hypothalamic–pituitary–adrenal axis (HPA-axis) (see review: Soliemanifar et al., 2018). The HPA-axis is a neuroendocrine system that plays a key role in the stress response. The prefrontal cortex, hippocampus, and amygdala, with a high density of glucocorticoid receptors, are crucial in the regulation of this system (Fries et al., 2009). It has been suggested that personality moderates an age-related decline in prefrontal and medial temporal regions (Jackson et al., 2011). In healthy humans, cortisol, the end product of the HPA-axis, follows a diurnal rhythm, with a rapid increase upon awakening, peaking between 30 and 45 min post-awakening (conceptualized as the cortisol awakening response; CAR) (Fries et al., 2009), and followed by a steady decrease throughout the day, reaching the lowest levels in the evening (the difference between awakening and evening cortisol is conceptualized as the diurnal cortisol slope; DCS) (Adam et al., 2017). A dysregulation of the diurnal cortisol pattern has been associated with poorer mental and physical health (see review and meta-analysis: Adam et al., 2017; Clow et al., 2004; Fries et al., 2009).

Although a dysregulation of HPA-axis functioning has also been reported in aging (Heaney et al., 2010), to our knowledge, only three studies have analyzed the association between the diurnal cortisol pattern and personality in older people (Gerritsen et al., 2009; Ouanes et al., 2017b; Puig-Perez et al., 2016a). Each of these studies assessed personality traits with different questionnaires, and they considered different cortisol indexes to measure HPA-axis functioning. Gerritsen et al. (2009)

assessed neuroticism with the abbreviated subscale of the Dutch Personality Questionnaire, and they observed that higher neuroticism was related to higher evening cortisol levels, but not to post-awakening cortisol or diurnal cortisol variability. By contrast, in the Puig-Perez et al. (2016a) study, neuroticism and extraversion were measured with the Eysenck Personality Questionnaire-Revised short form (EPQ-RS). The authors reported that higher neuroticism was related to lower overall morning cortisol concentrations (AUCg) (i.e., area under the curve with respect to the ground), but not to the CAR; however, extraversion was not associated with the AUCg or with the CAR. Only Ouanes et al. (2017b) administered the NEO Five-Factor Inventory to measure the big five personality traits (i.e. neuroticism, extraversion, conscientiousness, openness, and agreeableness), and they reported that higher extraversion and lower openness were associated with increased diurnal cortisol (AUCg).

Although it has been reported that sex is an important moderator in the relationship between personality and HPA-axis functioning (DeSoto & Salinas, 2015), only Puig-Perez et al. (2016a) analyzed sex differences in the association between neuroticism and extraversion and morning cortisol levels, and they reported that higher neuroticism was related to a greater CAR only in women. Therefore, more research is needed to clarify the association between the big five personality traits and HPA-axis functioning in older people, as well as sex differences in these relationships.

The aim of this study was to analyze the relationships between the big five personality traits and the diurnal cortisol pattern in older people, as well as the moderating effect of sex in these relationships. We hypothesized that dysregulation of

the HPA-axis functioning (i.e., lower awakening cortisol, greater CAR, flatter DCS, and higher bedtime cortisol) would be associated with higher scores on neuroticism, a personality trait considered to be a risk factor, and lower scores on personality traits considered protective factors (i.e. conscientiousness, extraversion, openness, and agreeableness) for health (Weston et al., 2015). In addition, we expected these associations to be more pronounced in older women than in older men, at least in the case of neuroticism (Puig-Perez et al., 2016a).

5.2. METHODS

5.2.1. Participants

Participants belonged to a larger research study designed to explore relationships between cognitive performance and the HPA-axis in older people (MNEME Project). In 2011, 128 participants were recruited from a study program at the University of Valencia (Spain) for people over 55 years of age. Exclusion criteria have been described elsewhere (Montoliu et al., 2018). Four years later, participants were contacted by telephone and invited to take part in a follow-up study, and 87 individuals agreed to participate.

Therefore, the sample was composed of 87 participants (44 men and 43 women), ranging in age from 59 to 81 years (M= 69.20, SD= 4.50). Participants' characteristics are presented in Table 5.1. There were no significant differences between men and women in age and BMI (all $p \geq .466$), but men showed a significantly higher socio-economic status (SES) than women ($p = .006$).

Table 5.1.
Characteristics of the study population for the total sample and for men and women separately.

	Total	Men	Women	*p*
N	87	44	43	
Gender, (%)		50.6	49.4	
Age, *M (SD)*	69.20 (4.50)	69.55 (4.72)	68.84 (4.28)	.466
SES, *M (SD)*	5.75 (1.29)	6.13 (1.26)	5.35 (1.22)	.006
BMI, *M (SD*)	27.29 (3.60)	27.41 (2.63)	27.17 (4.41)	.762

Note. BMI= body mass index; *M*= mean; SES= socio-economic status*; SD*=standard deviation; %= percentages. Gender differences in age, SES and BMI were analyzed with Student-*t* tests.

5.2.2. Procedure

Participants who agreed to participate were asked to attend one session that took place in the Laboratory of Social Cognitive Neuroscience at the University of Valencia. They were asked to fill out the Spanish version (Costa & McCrae, 1999) of the NEO-FFI (Costa & McCrae, 1992b) and provide a total of 15 saliva samples at home on three consecutive days, one on the weekend (Sunday) and two weekdays (Monday and Tuesday). The saliva samples were collected immediately after awakening, 15, 30, and 45 min post-awakening, and immediately before bedtime. The participants were thoroughly instructed about how to provide the saliva samples, and they were given written instructions to drink only water and not eat or brush their teeth at least 1 h prior to each saliva sample. In addition, participants were asked to write down the time they provided the saliva samples in a diary.

All the participants provided written informed consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki. The

protocol was approved by the Research Ethics Committee of the University of Valencia.

5.2.2.1.*NEO-Five Factor Inventory***.** The Spanish version (Costa & McCrae, 1999) of the NEO-FFI (Costa & McCrae, 1992b) was used to measure the Big Five personality traits. The NEO-FFI consists of 60 items measuring neuroticism, conscientiousness, extraversion, openness, and agreeableness, with 12 items each. The items are answered on 5-point scales, and higher scores indicate a higher degree of the trait. The internal reliabilities for the subscales in the present study were good; Cronbach's alphas: .840 (neuroticism), .793 (conscientiousness), .824 (extraversion), .703 (openness), and .786 (agreeableness).

5.2.2.2.*Salivary cortisol*. Participants provided saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany). They were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all the salivary glands, and then store the saliva samples in the refrigerator until they were delivered to the laboratory. Once in the laboratory, the samples were kept in the refrigerator until they were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was stored at -80^{o}C until the analyses of the salivary cortisol levels. HPA-axis activity was measured by analyzing the salivary cortisol levels. Salivary cortisol concentrations were determined in duplicate with the salivary cortisol enzymeimmunoassay kit from Salimetrics (Newmarket, UK). Assay sensitivity was < .007 ug/dL. For each subject, all the samples were analyzed in the same trial. The inter- and intra- assay variation coefficients were all below 10%. Cortisol levels were expressed in nmol/L.

5.2.3. Statistical Analysis

Participants' characteristics were described using percentages or means (standard deviation, *SD*), when appropriate, for the total sample and for men and women independently. To investigate sex differences in age, SES, and BMI, independent sample Student-*t* tests were performed.

Before the statistical analyses were performed, cortisol data were checked for normal distribution and homogeneity of variance using Shapiro-Wilks and Levene's test. These analyses revealed significant deviations in cortisol values; therefore, cortisol data were logarithm 10 (Log10) transformed. For each of the three days (Sunday, Monday and Tuesday), we obtained four cortisol indexes: (i) awakening cortisol (cortisol immediately after waking), (ii) the area under the curve with respect to the increase (AUCi) to measure the CAR (Pruessner et al., 2003), (iii) the DCS: bedtime cortisol minus awakening cortisol/time interval between awakening and bedtime, and (iv) bedtime cortisol (cortisol immediately before going to sleep). The mean awakening time was 7:22 am, and the mean bedtime was 12:15 am.

Although it has been reported that cortisol concentrations are relatively stable when assessed at the same time on subsequent days, some studies have reported weekend versus weekday differences in the CAR (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). However, because our participants were retired, we did not expect to find differences between weekend and weekday cortisol indexes. To check this, we first calculated cortisol indexes for the weekdays (Monday and Tuesday), and then we compared them to the weekend indexes (Sunday). We averaged the two weekdays' cortisol indexes, and all of them showed a good correlation (awakening

cortisol, $p < .001$; CAR, $p = .001$; DCS, $p = .002$; bedtime cortisol, $p < .001$). After that, repeated-measures ANOVAs were performed to detect differences between the weekend and weekday cortisol indexes. Because the results showed no differences between the weekend and the weekday on any of the cortisol indexes (all $p \geq .228$), we averaged values from all three days for each cortisol index. All the cortisol indexes showed good correlations between the weekend and the weekday (awakening cortisol, $p < .001$; CAR, $p < .001$; DCS, $p = .002$; bedtime cortisol, $p < .001$). Three women were excluded from the analyses because they scored ± 3 *SD* from the mean on some of the cortisol indexes (two for awakening cortisol, and one for the CAR).

To investigate whether there was an association between personality traits and the cortisol indexes, separate linear regression analyses were performed for each cortisol index (awakening cortisol, CAR, DCS, or bedtime cortisol) as dependent variable. For unadjusted analyses, we included one personality trait (neuroticism, conscientiousness, extraversion, openness, or agreeableness) in step one. For adjusted analyses, we conducted hierarchical analyses, including the covariates (age, sex, SES, BMI, and awakening time) in step one and one personality trait in step two. Then, to analyze whether there was a moderating effect of sex on the association between personality traits and cortisol indexes, we repeated these analyses, including the covariates, one personality trait and sex in step one, and the interaction between one personality trait and sex in step two.

To perform these statistical analyses, version 25.0 of SPSS was used. All *p* values were two-tailed, and the level of significance was taken as $p<0.05$.

5.3. RESULTS

Results showed that neuroticism was negatively related to awakening cortisol levels (B= -.257, p= .024 and B = -.289, p= .013, for unadjusted and adjusted analyses, respectively) and positively related to the CAR (B= .290, p= .011 and B = .321, p= .006, for unadjusted and adjusted analyses, respectively) and the DCS (B= .336, p= .004 and B =.294, p= .012, for unadjusted and adjusted analyses, respectively). Moreover, conscientiousness was positively related to awakening cortisol levels (B= .240, p= .041, for adjusted analyses) and negatively related to the CAR (B = -.272, p= .020, for adjusted analyses) and the DCS (B= -.320, p= .007 and B= -.306, p= .008, for unadjusted and adjusted analyses, respectively). However, no significant associations were found between neuroticism and conscientiousness and bedtime cortisol levels, or between extraversion, openness, and agreeableness and any of the cortisol indexes (all $p \geq .104$) (Table 5.2).

Furthermore, sex only moderated the association between extraversion and bedtime cortisol levels. Specifically, higher scores on extraversion were related to higher bedtime cortisol in men (B =1.853, p= .030) and lower bedtime cortisol in women (B =-1.844, p= .030). Sex did not moderate any of the other associations between personality traits and cortisol indexes (all $p \geq .063$)**.**

Table 5.2.
Regression analyses with personality traits (neuroticism, conscientiousness, extraversion, openness, and agreeableness) as predictors, and the cortisol indexes (awakening cortisol, CAR, DCS, and bedtime cortisol) as dependent variables, unadjusted and adjusted for covariates.

	Unadjusted analyses		Adjusted analyses	
	R^2 change	Beta	R^2 change	Beta
		Neuroticism		
Awakening cortisol	.066	-.257*	.084	-.289*
CAR	.084	.290*	.103	.321**
DCS	.113	.336**	.083	.294*
Bedtime cortisol	.026	.161	.016	.127
		Conscientiousness		
Awakening cortisol	.030	.173	.058	.240*
CAR	.043	-.207$^{\#}$	.074	-.272*
DCS	.102	-.320**	.093	-.306**
Bedtime cortisol	.036	-.189	.020	-.140
		Extraversion		
Awakening cortisol	.005	-.068	.011	.105
CAR	.011	-.105	.020	-.140
DCS	.000	-.003	.000	-.005
Bedtime cortisol	.001	.038	.007	.082
		Openness		
Awakening cortisol	.002	-.049	.006	-.077
CAR	.003	-.052	.001	-.034
DCS	.001	-.032	.008	-.093
Bedtime cortisol	.005	-.068	.010	-.099
		Agreeableness		
Awakening cortisol	.012	-.110	.006	-.075
CAR	.001	.031	.000	-.007
DCS	.006	-.078	.021	-.150
Bedtime cortisol	.031	-.177	.018	-.134

Note. CAR: cortisol awakening response; DCS: diurnal cortisol slope. *$p < .05$. **$p < .01$.

5.4. DISCUSSION

Our results showed that in the whole sample, higher neuroticism was related to lower awakening cortisol levels and greater CAR and DCS. By contrast, greater conscientiousness was related to higher awakening cortisol levels and lower CAR and

DCS. Furthermore, sex only moderated the association between extraversion and bedtime cortisol. No significant associations were found between openness and agreeableness and any of the cortisol indexes we measured.

Individuals who score higher on neuroticism are more likely to experience stressful events (Lahey, 2009), and chronic stress has been related to a dysregulation of the HPA-axis pattern (lower awakening cortisol, greater CAR, flatter DCS, and greater evening/bedtime cortisol) (Miller et al., 2007). Supporting this evidence, we observed that higher neuroticism was related to lower awakening cortisol levels and greater CAR and DCS (i.e. a smaller decrease in cortisol levels throughout the day), indicating a less healthy diurnal cortisol pattern. Three previous studies analyzed the association between neuroticism and the diurnal cortisol pattern in older adults (Gerritsen et al., 2009; Puig-Perez, 2016a; Ouanes et al., 2017b). Similar to our findings, Puig-Perez et al. (2016a) found that higher neuroticism was marginally related to lower awakening cortisol levels and a greater CAR only in women, indicating less healthy HPA-axis functioning. Moreover, contrary to our results, Gerritsen et al. (2009) reported that greater neuroticism was related to higher bedtime cortisol levels in participants who aged less than 75, but not to the DCS. Although the results of Puig-Perez et al. (2016a) and Gerritsen et al. (2009) do not exactly match ours, they also suggest a relationship between greater neuroticism and less healthy HPA-axis functioning. The discrepancies between these two studies and ours could be due to methodological differences, such as the use of different questionnaires to assess neuroticism (Eysenck Personality Questionnaire-Revised in Puig-Perez et al.'s (2016a), and the abbreviated subscale of the Dutch Personality Questionnaire in

Gerritsen et al.'s (2009) study). In a meta-analysis, Munafò, Clark, & Flint (2005) reported that the questionnaire used to assess the relationship between the serotonin transporter gene and anxiety-related personality traits may moderate this association, suggesting that psychometric equivalence does not necessarily guarantee that different instruments index the same construct (Mufanò et al., 2006). In addition, unlike in our study, in Gerritsen et al.'s (2009) study the DCS was measured as the difference between the 30 min peak and bedtime cortisol, and they did not divide this difference by the total time between the two samples, as we have done (see review and meta-analysis: Adam et al., 2017). Only one of these three studies assessed neuroticism with the NEO-FFI, as in our case, but they failed to observe an association with the diurnal cortisol pattern (Ouanes et al., 2017b). However, in Ouanes et al.'s (2017b) study, they considered a different cortisol index (AUCg), and they measured cortisol levels on a single day.

Other studies also analyzed the association between neuroticism and the diurnal cortisol pattern, but in samples with a wide age range, including both young and older people, and they failed to find an association between neuroticism and awakening cortisol, the CAR, or the DCS (Bogg & Slatcher, 2015; Hill, Billington, & Krägeloh, 2013; Van Santen et al., 2011). However, it has been suggested that the influence of personality traits on health may accumulate with time, and, consequently, personality may have a greater influence on disease in old age (Weston et al., 2015). This could explain why the association between neuroticism and a less healthy diurnal cortisol pattern is observed in older adults, but not in samples that also include younger participants.

We did not observe a moderating sex effect on the association between neuroticism and the cortisol indexes, unlike Puig-Perez et al.'s study (2016a), which observed that the CAR was only related to neuroticism in older women. In line with our results, Portella, Harmer, Flint, Cowen, & Goodwin (2005), in a sample of adults, observed that higher neuroticism was related to greater morning cortisol levels, but they did not find a sex effect. However, other studies also in younger samples reported that the relationship between neuroticism and HPA-axis dysregulation is more pronounced in men than in women (DeSoto & Salinas, 2015; Hauner et al., 2008; Zobel et al., 2004). It has been suggested that the heritability of neuroticism decreases across the life span more in men than in women (see review: Lahey, 2009), possibly as a result of cultural differences where men are socialized to suppress neurotic tendencies, unlike women (DeSoto & Salinas, 2015). Thus, it is possible that sex differences in the relationship between neuroticism and HPA-axis functioning in older people tend to disappear, supporting our results, or are even more pronounced in women (Puig-Perez et al., 2016a).

Second, regarding the association between conscientiousness and the diurnal cortisol pattern, one previous study in older adults did not observe an association between this personality trait and the AUCg cortisol index (Ouanes et al., 2017b). Conversely, we observed that higher conscientiousness was related to higher awakening levels, a lower DCS (a greater decrease in cortisol levels throughout the day), and a lower CAR, indicating a healthier diurnal cortisol rhythm. Supporting our results, in a recent study in a large sample of older adults, Steptoe, Easterlin, & Kirschbaum (2017) found that greater conscientiousness was related to low hair

cortisol concentrations, which is an indicator of tonic cortisol output over several weeks, indicating healthier HPA-axis functioning. Studies in samples that included both young and older participants found that greater conscientiousness was related to a steeper DCS (Bogg & Slatcher, 2015), as in our study, but not to awakening cortisol levels or CAR (Bogg & Slatcher, 2015; Hill et el., 2013; Van Santen et al., 2011). In addition, we did not observe a moderating sex effect on the association between conscientiousness and the cortisol indexes. To our knowledge, no previous study has explored sex interactions in the association between conscientiousness and the diurnal cortisol pattern, and our results suggest that sex does not play a role in these relationships.

Thus, we observed that higher neuroticism and lower conscientiousness were associated with a less healthy HPA-axis pattern. Supporting our results, in a study carried out in middle-aged and older healthy adults, greater neuroticism and lower conscientiousness were related to a greater decline in prefrontal and medial temporal regions (Jackson et al., 2011), which are brain areas that contribute to HPA-axis regulation functioning (Fries et al., 2009). Furthermore, of the big five personality traits, neuroticism and conscientiousness have shown the strongest links with health. Neuroticism has been highly correlated with negative feelings and chronic stress because individuals tend to perceive more stressors and respond with intense emotional reactions. In addition, neuroticism is considered a robust predictor of many mental and physical health problems, such as depression and anxiety disorders, cardiovascular disease, and greater mortality. In contrast, conscientiousness has been related to better coping and emotion regulation ability and positive healthier behaviors (less smoking

and alcohol consumption, healthier food, and physical exercise), and it plays a very significant role in health and longevity (see reviews: Friedman & Kern, 2014; Lahey, 2009).

In the whole sample, we did not find any relationship between agreeableness, openness, or extraversion and the cortisol indexes measured. In line with our results, studies in older people (Ouanes et al., 2107b), or in samples that that included both young and older participants (Hill et el., 2013; Van Santen et al., 2011), failed to observe an association between agreeableness and the cortisol indexes, suggesting that this personality trait has no influence on HPA-axis functioning. Ouanes et al. (2017b) reported that higher openness was related to lower AUCg in older adults. However, we did not observe a significant association between openness and cortisol indexes, coinciding with studies that included both young and older participants (Hill et el., 2013; Van Santen et al., 2011). Although we failed to find a relationship between extraversion and cortisol indexes, coinciding with Puig-Perez et al. (2016a), Ouanes et al. (2017b) observed that higher extraversion was related to higher AUCg, suggesting an association between extraversion and the diurnal cortisol pattern in older adults. Moreover, results of studies carried out in samples that included young and older people reported mixed findings about the association between extraversion and morning cortisol levels (Hill et al., 2013; Mufanò et al., 2006; Van Santen et al., 2011).

Although we did not find a significant relationship between extraversion and cortisol indexes in the entire sample, we did observe a moderating sex effect in the relationship between extraversion and bedtime cortisol. Specifically, greater extraversion was related to higher bedtime cortisol in men, indicating HPA-axis

dysregulation, and to lower bedtime cortisol in women, indicating a healthier HPA-axis profile. Friedman et al. (2010) observed that extraversion was related to physical health in woman, but not in men, when performing correlations. Additionally, although extraversion is the tendency to be outgoing and sociable and experience positive emotions, it has been linked to both positive (diet, exercise) and negative health behaviors (alcohol, smoking) (Booth-Kewley & Vickers, 1994). Thus, it is possible that in this age cohort (59 to 81 years old), men have performed less healthy behaviors than women. In addition, in a recent study (Montoliu, Hidalgo, & Salvador, 2019), we observed that in older adults, greater loneliness, which has been associated with lower extraversion (Cacioppo et al., 2006), was associated with higher bedtime cortisol levels. Therefore, only in men who feel lonely, low extraversion may be related to higher bedtime cortisol levels and, hence, to a less healthy HPA-axis profile. Future research should explore whether the association between loneliness and bedtime cortisol is mediated by extraversion, and whether there is a moderating sex effect in this association.

In conclusion, our results suggest that, in older adults, neuroticism, and extraversion only in men, are vulnerability factors in HPA-axis dysregulation, with possible adverse effects on health. By contrast, conscientiousness, and extraversion only in women, are protective factors of HPA-axis functioning, with potential beneficial effects on health. Finally, agreeableness and openness are not related to HPA-axis functioning, at least based on the cortisol indexes we considered.

CHAPTER 6

PERSONALITY AND COGNITIVE CHANGE IN OLDER PEOPLE: THE ROLE OF THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS AND COGNITIVE RESERVE

6.1 INTRODUCTION

The pace of the population's aging around the world is increasing dramatically, and cognitive decline and dementia have been recognized as a public health priority (WHO, 2019). However, there is great heterogeneity in the way people age. Some older adults maintain good cognitive abilities until advanced ages ("healthy/normal aging"), whereas others experience a significant decline ("pathological aging"). Therefore, it is important to understand which factors could account for individual differences in cognitive decline, and personality has been proposed as one of these factors.

The big five personality traits (neuroticism, extraversion, openness, agreeableness, and conscientiousness) measure individual differences in relatively enduring patterns of thoughts, feelings, and behavior, and increased age is associated with changes in personality traits (Roberts et al., 2006). Personality traits are known to influence health during aging (Friedman et al., 2010; Weston et al., 2015), including the risk of AD (Terracciano et al., 2014). In their review, these authors reported evidence suggesting that higher neuroticism and lower conscientiousness, and to a lesser degree, less openness and agreeableness, are associated with higher risk of AD (Terracciano et al., 2014).

Although several studies have examined the role of personality as a predictor of cognitive decline in older non-demented adults, this question remains unclear (Curtis et al., 2015). In a review, Curtis et al. (2015) reported that openness and conscientiousness were the personality traits most consistently related to cognitive

ability. Openness has been related to several cognitive abilities (Aiken-Morgan et al., 2012; Baker & Bichsel, 2006; Booth et al., 2006; Chapman et al., 2012; Sharp et al., 2010), but not to cognitive decline (Chapman et al., 2012; Sharp et al., 2010). In contrast, conscientiousness has not generally been related to any cognitive ability (Chapman et al., 2012; Ouanes et al., 2017b), but higher conscientiousness has been associated with reduced rates of cognitive decline (Chapman et al., 2012; Wilson et al., 2007b). In addition, extraversion seems to be related to better long-term memory in cross-sectional studies (Allen et al., 2011; Baker & Bichsel, 2006). Moreover, agreeableness seems to be unrelated to cognitive function and/or cognitive decline (Booth et al., 2006; Chapman et al., 2012), but some studies have observed an association between agreeableness and better verbal and working memory (Aiken-Morgan et al., 2012), but also worse cognitive abilities (Baker & Bichsel, 2006). In addition, the association between neuroticism and cognitive abilities has also shown mixed results, with some studies reporting no association (Baker & Bichsel, 2006; Ouanes et al., 2017b), but other studies finding that higher neuroticism was related to worse verbal memory, executive function, and/or cognitive decline (Aiken-Morgan et al., 2012; Booth et al., 2006; Caselli et al., 2016; Chapman et al., 2012; Klaming et al., 2017; Meier et al., 2002). Therefore, these inconsistent results show that the association between the big five personality traits and cognitive function/decline remains unclear.

These heterogeneous results could be explained by different mechanisms that might influence the associations between personality traits and cognitive decline, such as the stress response, health behaviors, and cognitively stimulating activity. For

example, highly conscientious individuals tend to engage in healthier behaviors (such as a healthy diet, physical exercise, or non-smoking), which would provide protection from age-related brain changes. Moreover, openness has been linked to better cognitive ability because individuals who are high in openness have a greater predisposition to cognitively stimulating activities, which would contribute to greater cognitive reserve. Similarly, highly extroverted individuals also tend to engage in social and intellectually stimulating abilities. In addition, extraversion has been associated with lower arousal, which could have beneficial effects on cognitive performance, but highly extroverted individuals could also be more easily distracted and, therefore, show impaired performance. Moreover, extraversion could be indirectly linked to better cognitive ability because highly extraverted individuals may compensate for the loss of social interactions and the decline in sensory functions related to aging, which would protect them from cognitive decline. Moreover, highly neurotic individuals tend to perceive and experience greater stress in daily life. Consequently, these individuals are prone to chronic stress (see review: Curtis et al., 2015). However, neuroticism and the other personality traits have also been associated with stress reactivity (Soliemanifar et al., 2018).

Chronic stress has been related to a dysregulation of the HPA-axis, leading to increased diurnal cortisol levels and a flattened DCS (Miller et al., 2007). Because the hippocampus and prefrontal cortex are brain structures with a high density of glucocorticoid receptors and play a role in memory and executive functions, increased cortisol levels would lead to neurotoxic effects on these brain structures, leading to cognitive impairment (Lupien et al., 2007). Moreover, cortisol follows a diurnal rhythm

with high levels upon awakening and a steady decrease throughout the day, reaching the lowest levels in the evening. The DCS is conceptualized as the difference between awakening and evening cortisol levels. A flattened DCS has been associated with poorer mental and physical health (Adam et al., 2017) and poorer cognition in older adults with memory deficits and depressive symptoms (Fiocco et al., 2006) and in non-demented adults (Beluche et al., 2010; Gerritsen et al., 2011; Stawski et al., 2011), although not in all of them (Ennis et al., 2016; Hidalgo et al., 2016; O'Hara et al., 2007; Singh-Manoux et al., 2014).

These findings suggest that HPA-axis functioning could be one of the biological mechanisms underlying the association between personality and cognitive function. Only one recent study has explored this association in older adults, without observing a mediating effect of personality in the association between the HPA-axis and cognitive functioning (Ouanes et al., 2017b). However, this study measured the HPA-axis functioning using the total diurnal cortisol output and the CAR, and it is possible that other cortisol indexes, such as the DCS, could mediate the association between personality and cognitive functioning. Additionally, Ouanes et al. (2017b) did not take sex differences into account, which could be an important moderator of these associations. In addition to the HPA-axis, other factors related to life experience could also play a role. For example, it has been suggested that greater cognitive reserve could contribute to explaining the positive association between greater openness and better cognitive ability (Chapman et al., 2012; Sharp et al., 2010).

Thus, the main goal of this study was to analyze the mediating role of the DCS and cognitive reserve in the relationship between the big five personality traits and the change in cognitive functioning in non-demented older people, as well as the moderating role of sex in these associations. To do so, first, we explored (i) the association between personality traits and change in different cognitive domains, and sex differences in these associations. We also explored the association between (ii) personality traits and the DCS and cognitive reserve, as well as (iii) the associations between the DCS and cognitive reserve and cognitive change.

On the one hand, we hypothesized that (i) higher neuroticism and lower conscientiousness, and to a lesser degree, less openness and agreeableness, would be associated with greater cognitive decline (Terracciano et al., 2014). On the other hand, we hypothesized that (ii) higher neuroticism would be related to a flattened DCS, whereas higher openness would be related to greater cognitive reserve. Moreover, we expected (iii) an association between a flattened DCS and lower cognitive reserve and greater cognitive decline. Finally, (iv) we hypothesized a mediating effect of the DCS in the association between neuroticism and cognitive change, as well as a mediating effect of cognitive reserve in the association between openness and cognitive change.

6.2. METHODS

6.2.1. Participants

Participants belonged to a larger research study designed to explore relationships between cognitive performance and the HPA-axis in older people (MNEME Project). At baseline, 128 participants were recruited from a study program

at the University of Valencia (Spain) for people over 55 years of age. For more information about the sample, see Montoliu et al. (2018). Four years later, participants were contacted by telephone and invited to take part in a follow-up study, and 87 individuals agreed to participate (44 men and 43 women), ranging in age from 59 to 81 years (*M*= 69.20, *SD*= 4.50) at follow-up. None of the participants scored less than 27 on the MEC (Spanish version of the Mini-Mental State Examination [MMSE]; Lobo et al., 1999), indicating the absence of cognitive impairment.

There were no significant differences between men and women in age (t (85) = -.732, p=.466), but there were marginal differences in educational level (X^2=8.283, p = .082). Moreover, there were no differences in conscientiousness, extraversion, or openness between men and women (all $p \geq .222$), but women showed significantly higher scores on neuroticism (t (84) =- 2.715, p=.008) and, marginally, on agreeableness (t (84) = 1.882, p=.063) than men (Table 6.1).

Table 6.1.
Characteristics of the study population for the total sample. and for men and women.

	Total	Men	Women	*p*
Sex *N (%)*	87(100%)	43 (50.6%)	44 (49.4%)	
Age *M (SD)*	69.20 (4.50)	69.55 (4.72)	68.84 (4.28)	.466
Educational level (%)				.082
Primary school	19.8%	9.1%	31%	
Secondary school	19.8%	20.5%	19%	
Graduate (3 years degree)	29.1%	29.5%	28.6%	
Graduate (5 years degree)	30.2%	38.6%	21.4%	
PhD	1.2%	2.3%	0%	
Neuroticism *M (SD)*	28.27 (6.88)	26.33 (6.49)	30.21 (6.77)	.008
Conscientiousness *M (SD)*	45.60 (5.63)	46.35 (5.71)	44.86 (5.50)	.222
Extraversion *M (SD)*	40.84 (6.59)	40.40 (7.18)	41.28 (5.99)	.537
Openness *M (SD)*	41.73 (5.55)	41.47 (6.02)	42.00 (5.09)	.658
Agreeableness *M (SD)*	43.97 (5.59)	42.85 (5.56)	45.09 (5.46)	.063

Note. M= mean; *SD*=standard deviation; %= percentages.

6.2.2. Procedure

At baseline and at the four-year follow-up, participants were asked to attend a neuropsychological session that took place at 10:00 and at 12:00 hours in the Laboratory of Social Cognitive Neuroscience at the University of Valencia. Before the session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol from the night before the first session. They were also instructed to drink only water, and not eat, smoke, take any stimulants (such as coffee, cola, caffeine, tea or chocolate), or brush their teeth at least 1 hour prior to the session. In addition, at follow-up, participants were also asked to fill out the Spanish version (Costa & McCrae, 1999) of the NEO-FFI (Costa & McCrae, 1992b) and the Cognitive Reserve Questionnaire (CRQ) (Ramí et al., 2011). They were also asked to provide a total of 6 saliva samples at home on three consecutive days, one on the weekend (Sunday) and on two weekdays (Monday and Tuesday), immediately after awakening and before bedtime. The participants received thorough instructions about how to provide the saliva samples, and they were given written instructions to drink only water and not eat or brush their teeth at least 1 h prior to each saliva sample. In addition, participants were asked to write down the time they provided the saliva samples in a diary.

All the participants provided written informed consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Committee of the University of Valencia.

6.*2.3. Neuropsychological assessment.*

To measure *declarative memory*, the Spanish version of the Rey Auditory Verbal Learning Test (RAVLT) (Miranda & Valencia, 1997) and Story recall subtest from the Spanish version of the Rivermead Behavioral Memory Test (Wilson et al., 1985) were administered. The RAVLT test consists of a target list (List A) of 15 neutral words repeated five times by the experimenter (trials I–V: Total Learning) that participants had to learn. Then, an interference list (List B) was presented only once, and participants had to repeat it. Participants were asked to recall the target list again immediately after the interference list (trial VI), and again after a delay of 20 min (trial VII). Three outcomes were used in subsequent analyses: (i) RAVLT Total Learning: total number of words recalled on the first five trials (trial I to V); (ii) RAVLT Immediate Recall: percentage of total number of words recalled after the interference trial compared to the number of words recalled on trial V (trial VI/trial V x 100); and (iii) RAVLT Delayed Recall: percentage of total number of words recalled after the 20-min delay compared to the number of words recalled on the immediate recall trial (trial VII/trial V x 100). On the Rivermead Stories Subtest, the experimenter read two short stories aloud, and participants had to recall as many memory units or ‘‘ideas’’ as possible immediately after their oral presentation and after a 20-min delay. Participants’ answers were audio recorded and corrected by an expert, and the sum of the correctly recalled ‘‘ideas’’ from the two stories was calculated. From this test, two outcomes were used for the subsequent analysis: (i) Rivermead Immediate recall: total “ideas” recalled from the two stories immediately after the oral presentation; and (ii) Rivermead Delayed recall: total “ideas” recalled from the two stories after 20 min,

compared to the number of "ideas" recalled from the two stories immediately after the oral presentation (Delayed recall/ Immediate recall x100).

To measure *working memory*, the Digit Span (DS) test and the Letter-number sequencing (LNS) test from the Spanish version of the Wechsler Memory Scale III (Wechsler, 1997) were administered. On the DS test, the experimenter read aloud a series of numbers (from 0 to 9) of increasing length (from 2 to 9 digits) at a rate of one digit per second. The participant had to repeat the numbers, first in the same order (DS-Forward), and then in reverse order (DS-Backward). Each set length was tested twice. The test ended when the participant failed two consecutive trials of the same length. For each correctly repeated digit set, one point was given. Two outcomes were obtained: (i) DS-Forward: total number of correctly recalled attempts in the same order; and (ii) DS-Backward: total number of correctly recalled attempts in the reverse order. DS-Forward was used as a measure of the attention and memory span component of WM, whereas DS-Backward was used as a measure of the executive component of WM (Conklin et al., 2000). In the LNS test, the experimenter read aloud a series of mixed numbers (from 0 to 9) and letters (from A to Z) of increasing length (from 2 to 8 items). The participant had to repeat the series, ordering the numbers in ascending order and the letters in alphabetical order. Each set length was tested three times. The test ended when the participant failed three consecutive trials of the same length. One point was given for each correctly recalled attempt. One outcome was obtained: LNS (total number of correctly recalled attempts).

To measure *attention and executive function*, the Trail-Making Test (TMT) (Reitan, 1992) and the Stroop Color-Word Interference test (Golden, 1978) were

administered. The TMT consists of two trials, TMT-A and TMT-B, each composed of 25 circles distributed on a white sheet of paper. In TMT-A, the circles were numbered from 1 to 25, and the participant was asked to trace a line connecting the circles in numerical sequence as quickly as possible. TMT-B included numbers from 1 to 13 and letters from A to L, and the participant was instructed to alternate between numbers and letters in ascending sequence. The score obtained was the number of seconds required to finish each trial. Errors were pointed out instantly by the examiner and contributed to the score due to the additional time needed for corrections. Two outcomes were obtained: (i) TMT-A: total number of seconds required to finish the TMT-A; and (ii) TMT-B: total number of seconds required to finish the TMT-B. The TMT-A was used to assess attention and general psychomotor speed, whereas the TMT-B was used to evaluate attention-switching performance. The Stroop Color-Word Interference test is composed of three trials. In each trial, participants had to name as many words as possible in 45 seconds. In the first trial, participants had to read the written word (W), which was red, blue, or green. In the second trial, participants had to name the printed color (C), red, blue, or green, of the XXX letters. In the third trial, participants had to name the color of the printed word (red, blue, or green), which was different from the written word (red, blue, or green) (WC), for example, the word green printed in red color. Afterwards, the WC' was calculated (WC' = (W x C) / (W + C)). Finally, the Stroop Interference outcome was obtained (Stroop Interference = WC – WC'), which is a measure of the ability to inhibit automatic responses.

6.2.4. Personality traits.

The Spanish version (Costa & McCrae, 1999) of the NEO-FFI (Costa & McCrae, 1992b) was used to measure the Big Five personality traits. The NEO-FFI consists of 60 items measuring neuroticism, conscientiousness, extraversion, openness, and agreeableness, with 12 items each. The items are answered on 5-point scales, and higher scores indicate a higher degree of the trait. The internal reliabilities for the subscales in the present study were good; Cronbach's alphas: .840 (neuroticism), .793 (conscientiousness), .824 (extraversion), .703 (openness), and .786 (agreeableness).

6.2.5. Cognitive reserve.

To measure cognitive reserve, the Cognitive reserve questionnaire (CRQ) (Ramí et al., 2011) was administered. It includes 8 items that measure intellectual activity. The maximum score is 25, and the higher the score, the higher the cognitive reserve. A score of 6 or less is considered the lowest cognitive reserve. Cronbach's alpha in this sample was .754.

6.2.6. Salivary cortisol.

Participants provided saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany). They were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, move the swab around in a circular pattern to collect saliva from all the salivary glands, and then store the saliva samples in the refrigerator until they were delivered to the laboratory. Once in the laboratory, the samples were kept in the refrigerator until they were centrifuged at 3000 rpm for 5 min, resulting in a clear

supernatant of low viscosity that was stored at -80ºC until the analyses of the salivary cortisol levels. HPA-axis activity was measured by analyzing the salivary cortisol levels. Salivary cortisol concentrations were determined in duplicate with the salivary cortisol enzymeimmunoassay kit from Salimetrics (Newmarket, UK). Assay sensitivity was < .007 ug/dL. For each subject, all the samples were analyzed in the same trial. The inter- and intra- assay variation coefficients were all below 10%. Cortisol levels were expressed in nmol/L.

6.2.7. Statistical Analysis

Participants' characteristics were described using percentages or means (standard deviation, *SD*), when appropriate, for the total sample and for men and women independently. To investigate sex differences in age and personality traits, independent sample Student-*t* tests were performed, whereas differences in educational level were analyzed with Chi-square tests. In addition, dependent sample Student-t tests were performed to assess differences in cognitive domain scores at baseline and at follow-up.

Before the statistical analyses were performed, cortisol data were checked for normal distribution and homogeneity of variance using Shapiro-Wilks and Levene's test. These analyses revealed significant deviations in cortisol values; therefore, cortisol data were logarithm 10 (Log10) transformed. For each of the three days (Sunday, Monday, and Tuesday), we obtained the DCS index by subtracting bedtime cortisol minus the awakening cortisol/time interval between awakening and bedtime. The mean awakening time and bedtime for the three days were 7:22 am and 12:15 am,

respectively. Although cortisol concentrations are relatively stable when assessed at the same time on subsequent days, some studies have reported weekend versus weekday differences (Kunz-Ebrecht et al., 2004). However, because our participants were retired, we did not expect to find differences between weekend and weekday cortisol levels. To check this, we calculated a weekday DCS index (averaging Monday and Tuesday DCS) and compared it to the weekend DCS (Sunday). Because the weekend and weekday DCS showed a good correlation (r=.365, p= .002), and repeated-measures ANOVAs showed no differences between the two indexes (p=.983), we averaged the DCS from all three days. If DCS values were only obtained for one or two of the three days (n= 3 and n=7, respectively), data from the available days were averaged and included in the analyses. Of the 87 participants, DCS data were obtained from 73 participants. There was one missing value for the NEO-FFI (N=86), and there were five missing values for the Stroop Color-Word Interference test (N=82). Before performing the statistical analyses, participants who scored ±3 SD from the mean were identified, and z scores were winsorized.

Linear regression analyses were performed to study the association between (i) personality traits and cognitive change (ii) personality traits, and the DCS or cognitive reserve, and (iii) DCS or cognitive reserve, and cognitive change. All these analyses were performed adjusted for the covariates age, sex, and educational level.

First, we conducted hierarchical analyses to investigate the associations between a personality trait (neuroticism, conscientiousness, extraversion, openness, or agreeableness) and change in a cognitive domain (RAVLT Total Learning, RAVLT Immediate recall, RAVLT Delayed recall, Rivermead Immediate recall, Rivermead

Delayed recall, DS-Forward, DS-Backward, LNS, TMT-A, TMT-B, and Stroop Interference). We conducted separate analyses, including one cognitive outcome at follow-up as dependent variable, the covariates in step 1, the baseline cognitive score in step two, and one personality trait in step three. Then, we tested whether there was a moderating effect of sex on the associations between personality and cognitive change using PROCESS (v2.13.6). For this purpose, we included a cognitive measure as the dependent variable, a personality trait as the independent variable, sex as the moderator variable, and the age, educational level, and baseline cognitive score as covariates. Second, we investigated whether there was an association between personality traits, and the DCS or cognitive reserve. Separate analyses were performed, including the DCS or cognitive reserve as dependent variable, the covariates in step one, and one personality trait in step two. Third, to investigate the associations between the DCS or cognitive reserve and cognitive change in different domains, separate analyses were performed for each cognitive domain outcome at follow-up as dependent variable, including the covariates in step one, the baseline cognitive score in step two, and the DCS in step three.

Finally, when we observed a significant association between a personality trait and the DCS or cognitive reserve, along with a significant association between the DCS or cognitive reserve and a cognitive change in a specific domain, we analyze whether DCS or cognitive reserve mediated the relationship between that personality trait and cognitive change in a specific domain using PROCESS (v2.13.6). To do so, we included one cognitive outcome at the follow-up measure as the dependent variable, a personality trait as the independent variable, DCS or cognitive reserve as the mediator

variable, and age, educational level, and baseline cognitive score as covariates. Then, we explored whether there was a moderating effect of sex in these mediation analyses. To perform these statistical analyses, version 25.0 of SPSS was used. All *p* values were two-tailed, and the level of significance was taken as $p<0.05$.

6.3. RESULTS

6.3.1. Cognitive change/decline in four-years follow-up

Results showed differences between baseline and follow-up on the RAVLT and Rivermead Immediate recall, and on TMT-B performance. Specifically, a decline was observed in verbal memory immediate recall when assessed with the RAVLT test (t (85) = 2.787, p= .007), whereas an improvement was found when assessed with the Rivermead test (t (85) = -3.893, $p \leq .001$). Similarly, results showed an improvement in executive function assessed with the TMT-B test (t (85) = 2.193, p= .031). The rest of the cognitive domains showed no changes between baseline and follow-up (all $p\geq$ .110) (Table 6.2). In this study, we have used the concepts of cognitive change/decline as synonyms, even though the concept of change is broader and covers both improvement and worsening of cognitive function. However, participants in this study only showed a decline in one cognitive domain.

Table 6.2.
Mean scores on different cognitive domains at baseline and follow-up.

	Baseline *M (SD)*	Follow-up *M (SD)*	*p*
RAVLT Total Learning	51.15 (8.04)	50.05 (9.09)	.202
RAVLT Immediate recall	87.90 (16.35)	81.85 (18.57)	.007
RAVLT Delayed recall	86.35 (18.31)	82.93 (17.34)	.110
Rivermead Immediate recall	17.30 (5.73)	19.29 (5.28)	≤ .001
Rivermead Delayed recall	103.15 (20.24)	106.70 (15.88)	.123
DS-Forward	8.91 (2.27)	8.62 (1.97)	.159
DS-Backward	6.02 (1.97)	6.19 (2.00)	.436
LNS	9.97 (2.27)	10.15 (2.34)	.442
TMT-A	39.23 (12.45)	40.27 (15.13)	.454
TMT-B	98.78 (43.33)	91.81 (37.52)	.031
Stroop Interference	-1.88 (7.33)	-2.02 (7.41)	.874

Note. M= mean; *SD*=standard deviation; RAVLT=Rey Auditory Verbal Learning Test; DS= Digit Span; LNS= Letter-number sequencing; TMT= Trail-Making Test.

6.3.2. Personality traits as predictors of cognitive change

Results showed that higher extraversion was related to less cognitive decline in verbal memory delayed recall, measured with the Rivermead test (*B*= -232, *p*= .025). Similarly, higher agreeableness was related to less cognitive decline in verbal memory delayed recall, measured with both the RAVLT (*B*= .265, *p*= .009) and the Rivermead test (*B*= .242, *p*= .021). Neuroticism, conscientiousness, and openness were not significantly related to cognitive change in any cognitive domain, although higher neuroticism was marginally related to higher cognitive decline in verbal memory delayed recall, measured with the RAVLT test (*B*= -.197, *p*= .053). None of the other associations between personality traits and change in the rest of the cognitive domains were statistically significant (all *p*≥ .82) (Table 6.3) (see page 172), and sex did not moderate these associations.

6.3.3. Mediating effect of the DCS on the association between personality traits and cognitive change

Higher DCS, indicating a flattened DCS, was related to higher neuroticism and lower conscientiousness (*B*= .324, *p*= .005 and *B*= -.275, *p*= .018, respectively), but not to the other personality traits (all *p*≥ .566). Moreover, higher DCS was only significantly associated with greater cognitive decline in working memory, measured with the LNS test (*B*= -.179, *p*= .044), and marginally associated with longer times performing the TMT-A and, therefore, greater cognitive decline in attention (*B*= .168, *p*= .077). None of the other associations between the DCS and cognitive change in the rest of the cognitive domains were statistically significant or marginal (all *p*≥ .130) (Table 6.4). Based on these findings, we explored the mediating effect of the DCS on the association between neuroticism and conscientiousness and cognitive change in LNS and TMT-A performance.

Results did not show a significant indirect effect of neuroticism on change in LNS via the DCS (path ab: *B*= -.055, SE= .037, 95% CI: -.143, .000), but sex significantly moderated the indirect effect of neuroticism on change in LNS via the DCS (*B* = -.163, SE= .095, 95% CI: -.379, -.003). Specifically, the indirect effect of neuroticism on change in LNS via the DCS was observed for men (*B*= -.162, SE= .090, 95% CI: -.361, -.013), but not for women (*B* = .000, SE= .029, 95% CI: -.056, .072). By contrast, a significant indirect effect of neuroticism on change on the TMT-A via the DCS was observed (path ab: *B*= .055, SE= .032, 95% CI: .002, .125), but not a moderating sex effect.

Regarding conscientiousness, the DCS did not mediate the association with change in LNS (path ab: *B*= .044, SE= .030, 95% CI: -.002, .112) or with change in TMT-A performance (path ab: *B*= -.047, SE= .025, 95% CI: -.099, .001) (Table 6.5), and sex did not moderate these associations.

6.3.4.Mediating effect of cognitive reserve on the association between personality traits and cognitive change

Higher cognitive reserve was related to higher extraversion and openness (*B*= .221, *p*= .021 and *B*= .315, *p*= .001, respectively), but not to the other personality traits (all *p*≥ .111). Moreover, higher cognitive reserve was related to lower cognitive decline on the RAVLT Total Learning index (*B*= .181, *p*= .050) and on Rivermead Immediate recall performance (*B*= .194, *p*= .025), and to shorter times performing the TMT-B and, therefore, to less cognitive decline in executive function (*B*= -.191, *p*= .025) (Table 6.3). Therefore, we explored the mediating effect of cognitive reserve on the association between extraversion and openness and the change on RAVLT Total Learning, Rivermead Immediate recall, and the TMT-B (Table 6.4).

Results reveled a significant indirect effect of extraversion on change on the TMT-B via cognitive reserve (path ab: *B* = -.057 SE= .032, 95% CI: -.129, -.002), but not an indirect effect of extraversion on change on RAVLT Total Learning (path ab: *B*= .035 SE= .029, 95% CI: -.011, .101), or on Rivermead Immediate recall (path ab: *B*= .064 SE= .037, 95% CI: -.001, .143), via cognitive reserve. Interestingly, when controlling for cognitive reserve, a negative direct effect of extraversion on change on Rivermead Immediate recall was observed (path c': *B*=-.210, SE= .083, *t*=-2.531, *p*=.013). Finally, a significant indirect effect of openness on change in Rivermead

Immediate recall performance via cognitive reserve was observed (path ab: *B*= .088 SE= .040, 95% CI: .018, .177), but not an indirect effect of openness on change on RAVLT Total Learning (path ab: *B*= .035 SE= .038, 95% CI: -.032, .121), or TMT-B (path ab: *B*= -.060 SE= .035, 95% CI: -.132, .003) (Table 6.6). Moreover, sex did not significantly moderate any of these relationships.

Table 6.4.
Regression analyses with the DCS and cognitive reserve as predictors and cognitive function at follow-up as dependent variable, adjusted for sex, age, educational level, and baseline cognitive function.

		DCS			Cognitive reserve	
	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*
RAVLT Total Learning	.001	-.036	.706	.031	.181	.050
RAVLT Immediate recall	.774	-.102	.382	.007	.082	.434
RAVLT Delayed recall	.000	.011	.919	.008	.089	.380
Rivermead Immediate recall	.020	-.146	.130	.033	.194	.029
Rivermead Delayed recall	.001	.030	.794	.000	.022	.836
DS-Forward	.009	-.097	.274	.013	.119	.162
DS-Backward	.018	-.135	.158	.007	.097	.362
LNS	.032	-.179	.044	.009	.102	.238
TMT-A	.026	.168	.077	.009	-.098	.335
TMT-B	.009	.101	.209	.025	-.191	.025
Stroop Interference	.001	.034	.750	.009	-.094	.312

Note. DCS= Diurnal cortisol slope; RAVLT=Rey Auditory Verbal Learning Test; DS= Digit Span; LNS= Letter-number sequencing; TMT= Trail-Making Test**.**

Table 6.5.
Mediation models to test the indirect effect of neuroticism and conscientiousness on cognitive change (LNS and TMT-A) via the DCS.

	B	*SE*	*t*	*p*
Effect of neuroticism on the DCS (a)	.319	.124	2.563	.012
Effect of the DCS on LNS (b)	-.174	.087	-1.989	.050
Total effect of neuroticism on LNS (c)	.035	.091	-.392	.696
Direct effect of neuroticism on LNS (c')	.019	.093	.212	.832
	B	*SE*	*CI*	*95%*
Indirect effect of neuroticism on LNS via the DCS *(ab)*	-.055	.037	-.143	.000
	B	*SE*	*t*	*p*
Effect of neuroticism on the DCS (a)	.308	.121	2.535	.013
Effect of the DCS on TMT-A (b)	.180	.092	1.952	.055
Total effect of neuroticism on TMT-A (c)	-.012	.093	-.129	.897
Direct effect of neuroticism on TMT-A (c')	-.067	.095	-.707	.481
	B	*SE*	*CI*	*95%*
Indirect effect of neuroticism on TMT-A via the DCS *(ab)*	.055	.032	.002	.125
	B	*SE*	*t*	*p*
Effect of conscientiousness on the DCS (a)	-.276	.119	-2.312	.023
Effect of the DCS on LNS (b)	-.161	.087	-1.853	.068
Total effect of conscientiousness on LNS (c)	.073	.086	.851	.397
Direct effect of conscientiousness on LNS (c')	.029	.088	.330	.742
	B	*SE*	*CI*	*95%*
Indirect effect of conscientiousness on LNS via the DCS *(ab)*	.044	.030	-.002	.112
	B	*SE*	*t*	*p*
Effect of conscientiousness on the DCS (a)	-.268	.115	-2.313	.023
Effect of the DCS on TMT-A (b)	.117	.091	1.936	.057
Total effect of conscientiousness on TMT-A (c)	.012	.088	.140	.888
Direct effect of conscientiousness on TMT-A (c')	.060	.090	.668	.506
	B	*SE*	*CI*	*95%*
Indirect effect of conscientiousness on TMT-A via the DCS *(ab)*	-.047	.025	-.099	.001

Note. DCS= Diurnal cortisol slope; LNS= Letter-number sequencing; TMT= Trail-Making Test.

Table 6.6.
Mediation models to test the indirect effect of extraversion and openness on cognitive change (RAVLT Total Learning, Rivermead Immediate recall, and TMT-B) via the cognitive reserve.

	B	*SE*	*t*	*p*
Effect of extraversion on cognitive reserve (a)	.218	.091	2.395	.018
Effect of the cognitive reserve on RAVLT Total Learning (b)	.160	.108	1.474	.144
Total effect of extraversion on RAVLT Total Learning (c)	-.001	.089	-.019	.984
Direct effect of extraversion on RAVLT Total Learning (c')	-.036	.091	-.402	.688
	B	*SE*	*CI*	*95%*
Indirect effect of extraversion on RAVLT Total Learning via the cognitive reserve *(ab)*	.035	.029	-.011	.101
	B	*SE*	*t*	*p*
Effect of extraversion on the cognitive reserve (a)	.205	.093	2.194	.031
Effect of the cognitive reserve on Rivermead Immediate recall (b)	.311	.097	3.197	.002
Total effect of extraversion on Rivermead Immediate recall (c)	-.146	.085	-1.715	.090
Direct effect of extraversion on Rivermead Immediate recall (c')	-.210	.083	-2.531	.013
	B	*SE*	*CI*	*95%*
Indirect effect of extraversion on Rivermead Immediate recall via the cognitive reserve *(ab)*	.064	.037	-.001	.143
	B	*SE*	*t*	*p*
Effect of extraversion on the cognitive reserve (a)	.247	.090	2.728	.007
Effect of the cognitive reserve on TMT-B (b)	-.233	.088	-2.631	.010
Total effect of extraversion on TMT-B (c)	.017	.073	.239	.811
Direct effect of extraversion on TMT-B (c')	.075	.074	1.014	.313
	B	*SE*	*CI*	*95%*
Indirect effect of extraversion on TMT-B via the cognitive reserve *(ab)*	-.057	.032	-.129	-.002
	B	*SE*	*t*	*p*
Effect of openness on the cognitive reserve (a)	.303	.087	3.464	≤ .001
Effect of the cognitive reserve on RAVLT Total Learning (b)	.115	.112	1.025	.308
Total effect of openness on RAVLT Total Learning (c)	.112	.087	1.287	.201
Direct effect of openness on RAVLT Total Learning (c')	.077	.094	.827	.410
	B	*SE*	*CI*	*95%*
Indirect effect of openness on RAVLT Total Learning via the cognitive reserve *(ab)*	.035	.038	-.032	.121
	B	*SE*	*t*	*p*
Effect of openness on the cognitive reserve (a)	.315	.090	3.482	≤ .001
Effect of the cognitive reserve on Rivermead Immediate recall (b)	.281	.105	2.671	.009
Total effect of openness on Rivermead Immediate recall (c)	.019	.087	.223	.823
Direct effect of openness on Rivermead Immediate recall (c')	-.069	.090	-.763	.447
	B	*SE*	*CI*	*95%*
Indirect effect of openness on Rivermead Immediate recall via the cognitive reserve *(ab)*	.088	.040	.018	.177
	B	*SE*	*t*	*p*
Effect of openness on the cognitive reserve (a)	.340	.084	4.027	≤ .001
Effect of the cognitive reserve on TMT-B (b)	-.179	.093	-1.914	.059
Total effect of openness on TMT-B (c)	-.116	.071	-1.638	.105
Direct effect of openness on TMT-B (c')	-.055	.076	-.721	.472
	B	*SE*	*CI*	*95%*
Indirect effect of openness on TMT-B via the cognitive reserve *(ab)*	-.060	.035	-.132	.003

Note. DCS= Diurnal cortisol slope; RAVLT=Rey Auditory Verbal Learning Test; LNS= Letter-number sequencing; TMT= Trail-Making Test.

6.4. DISCUSSION

Our results showed that higher agreeableness was related to less decline in verbal memory delayed recall, whereas higher extraversion was related to both less decline in verbal memory delayed recall and greater decline in verbal memory immediate recall. In contrast, neuroticism, conscientiousness, and openness were not directly related to cognitive change. Moreover, higher neuroticism was indirectly related to greater decline in attention via DCS, whereas higher extraversion and openness were indirectly related to less decline in executive function and immediate verbal recall, respectively, via cognitive reserve. Sex only moderated the indirect effect of neuroticism on working memory decline.

Regarding neuroticism, we observed that higher neuroticism was only marginally related to greater decline in verbal memory immediate recall. Some cross-sectional studies observed an association between higher scores on this personality trait and lower verbal memory performance (Aiken-Morgan et al., 2012; Klaming et al., 2017; Meier et al., 2002), but others did not (Baker & Bichsel, 2006; Ouanes et al., 2017b). In addition, two longitudinal studies observed that higher neuroticism was related to cognitive decline only on episodic memory (Wilson et al., 2003; 2007a). Although our results showed that higher neuroticism was only marginally related to greater decline in verbal memory immediate recall, it is possible that with a larger sample size and higher statistical power, we would observe a significant association between higher neuroticism and a decline in verbal memory, supporting previous studies.

Moreover, because neuroticism is a personality trait characterized by proneness to psychological stress, it has been hypothesized that it could affect memory and executive function through its effect on HPA-axis dysregulation, which would exert neurotoxic effects on the hippocampus and prefrontal cortex (Lupien et al., 2007). Interestingly, we observed that neuroticism was related to a flattened DCS, indicating a dysregulation of the HPA-axis. Some studies in older people also observed an association between neuroticism and less healthy HPA-axis functioning (Gerritsen et al., 2009; Puig-Perez et al., 2016a), except Ouanes et al. (2017b). Although we did not observe a significant direct effect on the association between neuroticism and change in any cognitive domain, we found an indirect effect of neuroticism on processing speed and attention via DCS. Furthermore, we observed a significant indirect effect of neuroticism on working memory via the DCS only in men, but not in women. Interestingly, Wilson et al. (2007a) observed that the association between neuroticism and risk of Mild Cognitive Impairment (MCI) was stronger in men than in women.

In cross-sectional studies, higher neuroticism has also been related to worse performance on cognitive functions that depend on the prefrontal cortex, such as executive function (Booth et al., 2006) and working memory (Wilson et al., 2007a). Moreover, our results show that neuroticism is associated with a decline in cognitive abilities related to the prefrontal cortex via a dysregulation of the HPA-axis.

Because two longitudinal studies observed that higher conscientiousness was related to a reduced rate of decline in several cognitive domains, such as general cognitive ability, verbal ability, working memory, episodic memory, and processing speed (Chapman et

al., 2012; Wilson et al., 2007b), we expected to find an association between higher conscientiousness and reduced cognitive decline. However, contrary to our hypothesis, in our study, conscientiousness was not related to change in any cognitive ability. This could be explained by the fact that these two longitudinal studies were performed over seven and eight years. Therefore, it is possible that in a four-year follow-up study of healthy older people, cognitive decline was not severe enough to observe an effect of conscientiousness on this association. On the other hand, we observed that conscientiousness was related to a steeper DCS, indicating healthier HPA-axis functioning, as some studies observed in samples that also included older people (Bogg & Slatcher, 2015; Steptoe et al., 2017), but not all of them (Ouanes et al., 2017b). Conscientiousness has been related to better coping and emotion regulation ability (see reviews: Friedman & Kern, 2014), which would explain a healthier diurnal cortisol profile. However, we did not observe an indirect effect of conscientiousness on cognitive change. Because individuals high in conscientiousness tend to engage in healthier behaviors, leading to better health and longevity (Friedman & Kern, 2014), it is possible that the association between conscientiousness and cognitive change may be mediated by specific health behaviors (less smoking, moderate alcohol consumption, physical exercise, and eating healthier food) that would have protective effects on vascular health and age-related brain changes.

We observed that higher extraversion was related to better performance on verbal memory delayed recall, as in other studies (Allen et al., 2011; Baker & Bichsel, 2006), but also to a greater decline in verbal memory immediate recall. It has been

hypothesized that because highly extraverted individuals tend to experience high positive affect, this would enhance memory encoding and retrieval (Curtis et al., 2015). However, other studies also observed that extraversion was negatively related to episodic memory, as we did (Luchetti et al., 2016; Meier et al., 2002). Thus, the fact that extraversion seems to both improve and impair some cognitive domains may explain the lack of association between this personality trait and risk of dementia (Low, Harrison, & Lackersteen, 2013; Terracciano et al., 2014). In addition, it has been suggested that highly extraverted individuals could engage greater social and intellectually stimulating activities (Curtis et al.,2015). Interestingly, we observed that higher extraversion was related to higher cognitive reserve. Moreover, our results showed that cognitive reserve mediated the association between extraversion and executive function, supporting this hypothesis.

Openness has been consistently related to several cognitive abilities (Aiken-Morgan et al., 2012; Baker & Bichsel, 2006; Booth et al., 2006; Chapman et al., 2012; Sharp et al., 2010), but not to cognitive decline (Chapman et al., 2012; Sharp et al., 2010). In line with these results, we did not observe a significant association between openness and cognitive change. In addition, it has been suggested that openness is associated with better cognitive ability because individuals who score high on this personality trait have a greater predisposition toward cognitively stimulating activities, which would contribute to greater cognitive reserve and, therefore, to coping better with age-related changes and pathology in the brain. One previous study analyzed the role of activity engagement in the association between openness and cognition in a

sample that included young and older adults (Soubelet, & Salthouse, 2010). Although Soubelet, & Salthouse (2010) observed that more open individuals tended to have higher cognitive functioning, and that higher openness was related to engagement in activities such as writing and reading, they did not observe that greater engagement in cognitively stimulating activities mediated the association between openness and cognitive functioning. However, we observed that openness was positively related to cognitive reserve, and that cognitive reserve was related to less cognitive decline on verbal memory immediate recall and learning and executive function. Furthermore, our results showed that cognitive reserve mediated the association between greater openness and less decline in verbal memory immediate recall.

Finally, although we did not expect to find a significant association between agreeableness and cognitive function change, as Chapman et al. (2012) did, we observed that higher agreeableness was related to less cognitive decline on verbal memory delayed recall. Similar to our results, in a cross-sectional study, Aiken-Morgan et al. (2012) observed that higher agreeableness was related to better verbal and working memory, but other studies reported an association between agreeableness and poorer cognitive performance (Ouanes et al., 2017b; Baker & Bichsel, 2006). However, in their meta-analysis, Terracciano et al. (2014) reported that agreeableness was related to a reduced risk of AD. This could be explained by the fact that higher agreeableness facilitates the formation of interpersonal connections and the stability of social networks (Bennett, Schneider, Tang, Arnold, & Wilson, 2006), or that individuals with less agreeableness tend to be aggressive, competitive, and antagonistic, which would

increase the risk of cardiovascular disease (Dembroski, MacDougall, Costa, & Grandits,1989; Sutin et al. 2010b), and contribute to the risk of AD (Terracciano et al., 2014). Therefore, our results support a protective effect of agreeableness on verbal memory decline.

In conclusion, in older people, neuroticism was related to a greater decline in attention and working memory, whereas openness and agreeableness were related to less cognitive decline in verbal memory immediate and delayed recall, respectively. Extraversion was associated with less decline in executive function and verbal memory delayed recall, but greater decline in verbal memory immediate recall. In addition, HPA-axis functioning, cognitive reserve, and possibly sex explain some of these associations.

Table 6.3.
Regression analyses with personality traits as predictors, and cognitive function at follow-up, the DCS or cognitive reserve as dependent variables, adjusted sex, age, educational level, and cognitive function at baseline.

	Neuroticism			Conscientiousness			Extraversion			Openness			Agreeableness		
	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*
RAVLT Total Learning	.011	-.106	.247	.000	.006	.951	.000	.000	.996	.014	.119	.190	.004	-.061	.502
RAVLT Immediate recall	.015	-.123	.240	.001	.031	.781	.020	-.142	.180	.003	.054	.610	.019	.140	.182
RAVLT Delayed recall	.038	-.197	.053	.001	.029	.784	.002	-.004	.670	.001	.034	.740	.070	.265	.009
Rivermead Immediate recall	.000	-.014	.876	.003	-.059	.498	.020	-.144	.093	.000	.018	.830	.016	-.132	.134
Rivermead Delayed recall	.010	-.102	.330	.017	.129	.211	.053	.232	.025	.014	.120	.251	.056	.242	.021
DS-Forward	.001	-.026	.766	.002	.048	.569	.021	-.144	.082	.011	-.103	.215	.008	-.094	.268
DS-Backward	.000	.018	.850	.000	.016	.868	.008	-.091	.337	.001	.036	.705	.000	-.011	.908
LNS	.005	.070	.433	.001	.024	.794	.001	-.037	.681	.000	-.021	.818	.004	.069	.458
TMT-A	.000	-.006	.943	.001	-.033	.706	.001	-.034	.702	.005	-.072	.410	.018	-.140	.123
TMT-B	.002	-.043	.573	.001	-.039	.597	.000	.018	.812	.014	-.117	.105	.002	-.041	.589
Stroop Interference	.004	.062	.517	.001	-.032	.737	.000	-.005	.959	.000	-.001	.995	.001	.033	.724
DCS	.105	.324	.005	.076	-.275	.018	.003	-.055	.644	.005	-.068	.566	.003	-.052	.661
Cognitive reserve	.023	-.153	.111	.005	.070	.470	.048	.221	.021	.099	.315	.001	.004	-.065	.505

Note. DCS= Diurnal cortisol slope; RAVLT=Rey Auditory Verbal Learning Test; DS= Digit Span; LNS= Letter-number sequencing; TMT= Trail-Making Test .

CHAPTER 7

SEX DIFFERENCES IN THE RELATIONSHIPS BETWEEN PERSONALITY TRAITS AND OBJECTIVE AND SUBJECTIVE HEALTH MEASURES IN OLDER ADULTS

7.1. INTRODUCTION

The world population is getting older, and this older age group is more likely to experience several health conditions (WHO, 2018). However, there is great heterogeneity in the way people age, and, therefore, it is important to identify factors that may contribute to health problems during aging, in order to improve health and reduce mortality risk. Among these health problems, central obesity, dysglycemia, dyslipidemia, and arterial hypertension stand out as a cluster of risk factors for metabolic syndrome whose prevalence increases with age (Hildrum et al., 2007). These health outcomes are related to HPA-axis dysregulation (Björntorp, & Rosmond, 2000), and they are risk factors for CVDs, the number one cause of death globally (WHO, 2017), and for cognitive impairment and dementia (Frisardi et al., 2010). Therefore, increasing the knowledge about factors associated with obesity, dysglycemia, dyslipidemia, and arterial hypertension would help to prevent these adverse health outcomes.

Among the factors proposed are personality traits, which make it possible to measure individual differences in relatively enduring patterns of thoughts, feelings, and behaviors, although increased age is associated with changes in these traits (Roberts et al., 2006). Personality traits are known to influence health, subjective well-being, and mortality risk (Friedman et al., 2010; Friedman & Kern, 2014; Lahey, 2009). However, previous studies analyzing the relationship between personality traits and health outcomes show inconsistent findings, possibly due to sex and age differences (Sutin &

Terracciano, 2016b). In this line, Weston et al. (2015) suggested that the link between personality and health may be accumulative over time, and so personality could have a greater influence on health in old age. Therefore, the study of health indexes (obesity, dysglycemia, dyslipidemia, and arterial hypertension), taking into account the moderating effect of sex and using samples that include only older people, could help to clarify the relationship between personality and health in this age group.

Some studies have analyzed the associations between the big five personality traits and the risk of obesity in older adults (Mõttus et al. 2013; Otonari et al., 2012) or in samples with a wide age range that considered the group of older people independently (Sutin & Terracciano, 2016b; Terracciano et al., 2014) (18-91, and 14-94, respectively), with inconsistent findings. Neuroticism or impulsiveness (a facet of neuroticism) was associated with higher BMI (Otonari et al., 2012; Sutin & Terracciano, 2016B; Terracciano et al., 2014). However, Terracciano et al. (2014) also observed an association between neuroticism and being underweight, whereas Mõttus et al. (2013) failed to observe an association. However, all these studies observed that conscientiousness or order (a facet of conscientiousness) was related to lower BMI/risk of obesity. Mixed findings were observed in the association between extraversion and BMI/risk of obesity; some studies observed a positive association (Otonari et al., 2012), a negative association (Sutin & Terracciano, 2016B), or no association (Mõttus et al., 2013; Terracciano et al., 2014). In addition, Sutin & Terracciano (2016B) also observed a negative association between openness and agreeableness and BMI, but the other studies did not (Mõttus et al., 2013; Terracciano et al., 2014). Furthermore, Terracciano

et al. (2014) also included an indicator of abdominal adiposity distribution, the waist-hip ratio (WHR), and they observed that higher impulsiveness (a facet of neuroticism) and lower order (a facet of conscientiousness) were related to higher WHR. Moreover, Sutin & Terracciano (2016B) also reported important sex differences in the associations between personality traits and BMI when considering the entire sample (age range: 18-91 years old). Specifically, they observed that the association between BMI and neuroticism and conscientiousness was stronger in women, whereas the association between extraversion and BMI was only observed in women.

Regarding the relationship between cholesterol and psychological traits, Sutin et al. (2010a), in a sample with a broad age range (14-102 years old), observed that high conscientiousness and low openness were related to a healthier cholesterol profile (higher HDL-cholesterol and lower triglycerides), but not to LDL-cholesterol. Moreover, they observed that the association between conscientiousness and higher HDL-cholesterol was stronger in women than in men. In addition, when analyzing the moderator effect of age, they reported that the association between conscientiousness and triglycerides was supported in older participants, but not in young participants. Despite this, to our knowledge, no previous study has analyzed the relationship between the big five personality traits and cholesterol levels, and the moderating effect of sex, in older people. Nonetheless, in older adults, Chapman et al. (2013) observed that personality traits, specifically higher neuroticism and lower conscientiousness and agreeableness, were associated with illness burden morbidity, and they reported that cholesterol explained part of these effects.

Regarding the association between the two other components of metabolic syndrome (high blood pressure and high glucose), Weston et al. (2015) reported that, in older adults, higher neuroticism and lower conscientiousness, extraversion, and openness were associated with a higher risk of developing high blood pressure, whereas lower conscientiousness was related to a higher risk of developing diabetes. However, these measures were collected by telephone, and no biomarkers were analyzed. In addition, Ohseto et al. (2018) administered the Eysenck personality questionnaire-revised, short form (EPQ-RS), to a sample with a broad age range (30->70 years old), and they observed that higher extraversion, but not neuroticism, was related to higher blood pressure and glucose.

A good proxy for hypertension is the pulse pressure index, which reflects arterial stiffness and is measured as systolic minus diastolic blood pressure. It is potentially a better measure of chronic effects of hypertension than blood pressure itself (Nation et al., 2012; Power et al., 2013). Pulse pressure increases with age and has been associated with cardiovascular risk factors (Franklin et al.,1999) and cognitive decline (McDade et al., 2016). Moreover, HbA1c assesses average glucose control over the previous 2-3 months. Compared to fasting glucose, HbA1c has the advantage that it can be measured at any time of day, regardless of the duration of the fast. In addition, HbA1c has been similarly associated with a risk of diabetes and more strongly associated with risk of cardiovascular disease and death from any cause, compared to fasting glucose (Selvin et al., 2010). To our knowledge, no previous study has analyzed

the relationship between pulse pressure and HbA1c and the five major personality traits in older people.

Subjective health status has also been associated with health outcomes and mortality (Idler & Benyamini, 1997). The Short Form Health Survey (SF-36) questionnaire has been widely administered to assess subjective physical and mental health (Ware, & Sherbourne, 1992). Several studies have analyzed the relationship between personality and subjective health. Duberstein et al. (2003) reported that this association appeared to be more pronounced with age. All the studies that analyzed the relationship between subjective health and personality in older people observed a relationship between higher neuroticism and worse subjective physical and/or mental health (Chapman et al., 2006; 2007; Duberstein et al., 2003; Jaconelli et al., 2012; Jerram & Coleman, 1999; Löckenhoff et al., 2008). Moreover, most studies reported an association between higher conscientiousness and better subjective physical and/or mental health (Chapman et al., 2006; 2007; Jaconelli et al., 2012; Löckenhoff et al., 2008), but others did not (Jerram & Coleman, 1999; Duberstein et al., 2003). Regarding extraversion, most studies observed an association between this personality trait and better subjective physical and/or mental health (Chapman et al., 2006; Duberstein et al., 2003; Jaconelli et al., 2012; Jerram & Coleman, 1999), but not all of them (Chapman et al., 2007; Löckenhoff et al., 2008). Openness has been associated with better subjective physical health (Duberstein et al., 2003; Jerram & Coleman, 1999) and worse mental health (Chapman et al. 2007), but not in all studies (Chapman et al., 2006; Jaconelli et al., 2012; Löckenhoff et al., 2008). In addition, most studies failed

to observe this association (Chapman et al., 2006; 2007; Duberstein et al., 2003; Jaconelli et al., 2012; Löckenhoff et al., 2008), although Jerram & Coleman (1999) observed a positive association between agreeableness and subjective health. These discrepancies could be explained by the fact that these studies assessed subjective health considering different subscales of the SF-36 questionnaire. Three studies considered one (Chapman et al., 2006; Jaconelli et al., 2012) or two (Duberstein et al., 2003) physical subscales, whereas two studies considered four (Chapman et al., 2007) or eight (Jerram & Coleman, 1999) physical and mental subscales. Moreover, only one study considered its two main components, physical and mental health (Löckenhoff et al., 2008). In addition, these mixed findings may also be explained by sex differences in these associations. Only Jerram & Coleman (1999) analyzed these associations separately for men and women, and they suggested that the results for the whole sample were a poor reflection of the results for men and women separately. However, Jerram & Coleman (1999) did not analyze the moderating effect of sex on the association between the big five personality traits and subjective health, and so the study of sex differences in these associations in older people is a pending issue.

The aim of this study was to analyze, in older people, the relationships between the big five personality traits and objective and subjective health indexes, as well as the moderating effect of sex in these relationships. We hypothesized that worse objective and subjective health would be associated mainly with higher neuroticism and lower conscientiousness, and to a lesser degree, with lower extraversion, openness, and agreeableness. In addition, we expected the associations between the personality traits

and health to be more pronounced in older women than in older men, at least for neuroticism and conscientiousness, as found in previous studies in other age ranges (Armon et al., 2013; Brumett et al., 2006; Faith et al., 2001; Sutin, Ferrucci, Zonderman, & Terracciano, 2011; Sutin & Terracciano, 2016a;2016b).

7.2. METHODS

7.2.1. Participants

Participants belonged to a larger research study designed to explore relationships between cognitive performance and the HPA-axis in older people (MNEME Project). At baseline, 128 participants were recruited from a study program at the University of Valencia (Spain) for people over 55 years of age. For more details, see (Montoliu et al., 2018). Four years later, participants were contacted by telephone and invited to take part in a follow-up study, and 87 individuals agreed to participate.

Therefore, the sample was composed of 87 participants of both sexes (43 women and 44 men), ranging in age from 59 to 81 years (M= 69.20, SD= 4.50; M= 68.84, SD= 4.28 for women and M= 69.55, SD= 4.72 for men). Eight participants (9.2%) were diabetic, 33 (37.9%) took medication to control dyslipidemia, and 38 (43.7%) took medication for hypertension. There were no significant differences between men and women in age, conscientiousness, extraversion, or openness (all $p \geq .222$). However, women showed significantly higher scores on neuroticism (t (84) = -2.715, p=.008) and marginally higher scores on agreeableness (t (84) =1.882, p=.063) than men. Moreover, there were no significant sex differences in BMI, HbA1c, or LDL-

cholesterol (all $p \geq .376$). However, men showed higher WHR (t (84) = -6.152, $p \leq .001$) and pulse pressure (t (85) = -2.907, p=.005) than women, whereas women showed higher HDL-cholesterol (t (73) = 4.055, $p \leq .001$). Regarding subjective health, women showed worse physical (t (85) = 5.420, $p \leq .001$) and mental (t (85) = 13.234, p=.002) health than men (Table 7.1).

Table 7.1.
Characteristics of the study population for the total sample, and for men and women.

	Total	Men	Women	*p*
Sex *N (%)*	87(100%)	43 (50.6%)	44 (49.4%)	
Age *M (SD)*	69.20 (4.50)	69.55 (4.72)	68.84 (4.28)	.466
Neuroticism *M (SD)*	28.27 (6.88)	26.33 (6.49)	30.21 (6.77)	.008
Conscientiousness *M (SD)*	45.60 (5.63)	46.35 (5.71)	44.86 (5.50)	.222
Extraversion *M (SD)*	40.84 (6.59)	40.40 (7.18)	41.28 (5.99)	.537
Openness *M (SD)*	41.73 (5.55)	41.47 (6.02)	42.00 (5.09)	.658
Agreeableness *M (SD)*	43.97 (5.59)	42.85 (5.56)	45.09 (5.46)	.063
BMI *M (SD)*	27.29 (3.57)	27.41 (2.63)	27.16 (4.36)	.741
WHR *M (SD)*	0.91 (.08)	0.96 (0.06)	0.86 (0.08)	≤.001
HbA1c *M (SD)*	5.64 (0.41)	5.63 (0.43)	5.64 (0.40)	.992
LDL-cholesterol *M (SD)*	96.21 (31.05)	93.23 (29.53)	99.84 (32.90)	.376
HDL-cholesterol *M (SD)*	53.69 (14.99)	47.88 (11.66)	60.71 (15.70)	≤.001
Pulse pressure *M (SD)*	59.66 (11.83)	12.24 (1.84)	10.36 (1.58)	.005
SF-36 Physical health *M (SD)*	386.93 (70.99)	414.77 (51.30)	358.44 (77.34)	≤.001
SF-36 Mental health *M (SD)*	409.82 (56.48)	428.60 (33.27)	390.60 (68.19)	.002

Note. M= mean; *SD*=standard deviation; %= percentages. BMI= Body mass index; WHR= Waist-Hip Ratio; HbA1c= Glycated hemoglobin; LDL = Low-density lipoprotein; HDL=High-density lipoprotein. SF= Short Form Health Survey.

7.2.2. Procedure

Participants who agreed to participate were asked to attend one session that took place at 10:00 and at 12:00 hours in the Laboratory of Social Cognitive Neuroscience

at the University of Valencia in order to obtain objective health variables such as BMI, WHR, HbA1c, LDL and HDL cholesterol, and pulse pressure. In addition, participants were asked to fill out the Spanish version (Alonso, Prieto, & Antó, 1995) of the Short Form Health Survey (SF-36) (Ware, & Sherbourne, 1992) to measure subjective health status, and the Spanish version (Costa & McCrae, 1999) of the NEO-FFI (Costa & McCrae, 1992b) to measure personality traits. Data on BMI, pulse pressure, and the SF-36 were obtained for the entire sample (N=87), but there was one missing value for the NEO-FFI and WHR (N=86), two for HbA1c (N=85), and sixteen for LDL and HDL-cholesterol (N=71).

All the participants provided written informed consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Committee of the University of Valencia.

7.2.3 Personality traits

The Spanish version (Costa & McCrae, 1999) of the NEO-Five Factor Inventory (NEO-FFI) (Costa & McCrae, 1992b) was used to measure the Big Five personality traits. The NEO-FFI consists of 60 items that measure neuroticism, conscientiousness, extraversion, openness, and agreeableness, 12 items for each. The items are answered on 5-point scales, and higher scores indicate higher degree of the trait. The internal reliabilities for the subscales in the present study were good, with Cronbach's alphas of .84 (neuroticism), .79 (conscientiousness), .82 (extraversion), .70 (openness), and .79 (agreeableness).

7.2.4 Objective health indexes

Body weight measures. *Body Mass Index (BMI*) was calculated as weight (kg)/height (m)2. *Waist-Hip Ratio (WHR)* was obtained by dividing the waist circumference by the hip-circumference.

Blood biomarkers. *Glycated Hemoglobin (HbA1c)* and *Low-density lipoprotein (LDL)* ("bad cholesterol") and *High-density lipoprotein (HDL) cholesterol* ("good cholesterol" were determined from blood samples with the Cobas b 101 system (Roche Diagnostics, Spain).

Blood pressure. Blood pressure was measured approximately 20 minutes after the participant's arrival to the laboratory, using the ORMON M6W automatic blood pressure monitor (ORMON Healthcare, Japan). The participant was in a seated position, and three measures of systolic blood pressure (SBP) and diastolic blood pressure (DBP) (mm Hg) were taken at 30-second intervals. Average levels for the three measures of SBP and DBP were obtained, and the *pulse pressure* index was calculated as the difference between the SBP and DBP average levels.

7.2.5 Subjective health indexes

The Spanish version (Alonso et al., 1995) of the Short Form Health Survey (SF-36) (Ware, & Sherbourne, 1992) was administered to measure subjective health. It consists of 36 items distributed in eight subscales: physical functioning (PF), role-physical (RF), bodily pain (BP), general health (GH), vitality (V), social functioning (SF), role-emotional (RE), and mental health (MH). The 8 subscales were grouped into

two summary measures: physical health (PF, RF, BP, GH, and V) and mental health (GH, V, SF, RE, and MH). The internal consistency (Cronbach's α) in this sample was .78 for the physical health scale and .76 for the mental health scale.

7.2.6. Statistical Analysis

Participants' characteristics were described using percentages or means (standard deviation, *SD*), when appropriate, for the total sample and for men and women independently. To investigate sex differences in age, personality traits, and objective and subjective health measures, independent sample Student-t tests were performed.

To investigate whether there were associations between personality traits (neuroticism, conscientiousness, extraversion, openness, or agreeableness) and objective (BMI, WHR, HbA1c, LDL and HDL cholesterol, and pulse pressure) and subjective (physical and mental health) health indexes, separate linear regression analyses were performed for each health index as dependent variable. We conducted hierarchical analyses, including the covariates (age and sex) in step one and one personality trait in step two. In addition, as covariates, we included the diabetic condition or the use of medication for dyslipidemia or hypertension when the dependent variable analyzed was HbA1c, LDL and HDL-cholesterol, or pulse pressure, respectively.

Then, in order to analyze whether there was a moderating effect of sex on the association between personality traits and objective and subjective health indexes, moderated regression analyses and bootstrapped bias-corrected 95% confidence

intervals of the interaction effect were computed using the PROCESS macro in SPSS (Model 1) with 5000 bootstrapped samples. We included one personality trait as the independent variable, one health index as the dependent variable, sex as moderator, and age and the diabetic condition or the use of medication for dyslipidemia or hypertension as covariates, only when the dependent variable analyzed was HbA1c, LDL and HDL-cholesterol, or pulse pressure, respectively.

Outliers were defined as values ±3 SD and winsorized by replacing their values with values equal to the mean ±3 SD. One outlier was detected for agreeableness, one for BMI, one for HbA1c, one for pulse pressure, two for physical health, and four for mental health.

To perform these statistical analyses, version 25.0 of SPSS was used. All *p* values were two-tailed, and the level of significance was taken as $p<0.05$.

7.3. RESULTS

7.3.1. Relationships between personality traits and objective and subjective health indexes

Results showed that neuroticism was significantly and positively related to WHR ($B= .198$, $p= .036$) and HbA1c ($B= .309$, $p= .001$), and marginally to BMI ($B= .184$, $p= .091$) and LDL-cholesterol ($B= .195$, $p= .090$), indicating a worse objective health status. Similarly, neuroticism was significantly and negatively related to physical ($B= -.323$, $p= .002$) and mental ($B = -.381$, $p\leq.001$) health, indicating a worse

subjective health status. No association was observed between neuroticism and HLD-Cholesterol or pulse pressure (all $p \geq .364$).

Regarding conscientiousness, a significant negative association with BMI ($B = -.230$, $p = .033$), WHR ($B = -.201$, $p = .027$), HbA1c ($B = -.269$, $p = .004$), and LDL-cholesterol ($B = -.234$, $p = .040$) was observed, indicating a better objective health status. No associations were observed between conscientiousness and HLD-Cholesterol or pulse pressure (all $p \geq .291$). Moreover, conscientiousness was significantly and positively related to physical health ($B = .230$, $p = .022$), indicating a better subjective health status, but it was not related to mental health ($B = .132$, $p = .196$).

In addition, extraversion was significantly and negatively related to LDL-cholesterol ($B = -.228$, $p = .048$), indicating a better objective health status. No associations were observed between extraversion and the rest of the objective health indexes (all $p \geq .140$). Similarly, extraversion was significantly and positively related to subjective physical ($B = .313$, $p = .002$) and mental ($B = .236$, $p = .019$) health, indicating a better subjective health status.

Openness was not significantly associated with any objective or subjective health index (all $p \geq .287$), whereas agreeableness was only significantly and positively related to HDL-cholesterol ($B = .228$, $p = .035$), indicating a better objective health status, but it was not related to the rest of the objective and subjective health indexes (all $p \geq .261$) (Table 7.2, see page 189).

7.3.2. Moderation effect of sex on the relationships between personality traits and objective and subjective health indexes.

When performing moderation analyses, results showed a significant interaction effect of sex and neuroticism on BMI (ΔR^2=.065, p=.016, CI 95% [-.964, -.097]), HbA1c (ΔR^2=.043, p=.048, CI 95% [-.847, -.003]), and physical (ΔR^2=.051, p=.016, CI 95% [.085, .818]) and mental (ΔR^2=.100, $p\leq$.000, CI 95% [.285, .976]) health. Specifically, results showed a significant positive relationship between neuroticism and both BMI (Est.=.469, SE= .150, p=.002, CI 95% [.169, .768]) and HbA1c (Est.=.438, SE= .145, p=.003, CI 95% [.149, .728]) in women, but not in men (Est.=-.062, SE= .157, p=.694, CI 95% [-.375, .251] and Est.=.013, SE= .153, p=.931, CI 95% [-.293, .319], respectively). Similarly, a significant negative relationship was observed between neuroticism and physical (Est.= -.519, SE= .127, $p\leq$.001, CI 95% [-.772, -.266]) and mental (Est.=-.664, SE= .120, $p\leq$.001, CI 95% [-.903, -.426]) health in women, but not in men (Est.= -.067, SE= .133, p=.611, CI 95% [-.332, .196] and Est.=-.034, SE= .125, p=.786, CI 95% [-.283, .215], respectively).

Moreover, sex only moderated the associations between conscientiousness and pulse pressure (ΔR^2=.060, p=.012, CI 95% [.112, .888]). Results showed a significant positive relationship between conscientiousness and pulse pressure in men (Est.=.335, SE= .135, p=.015, CI 95% [.066, .605]), but not in women (Est.=-.165, SE= .138, p=.227, CI 95% [-.440, .111]).

Additionally, sex moderated the association between extraversion and BMI (ΔR2=.049, p=.042, CI 95% [.016, .892]), HbA1c (ΔR^2=.038, p=.040, CI 95% [.002,

.114]) and physical (ΔR^2=.041, *p*=.030, CI 95% [-.754, -.037]) and mental (ΔR^2=.071, *p*=.005, CI 95% [-.888, -.155]) health. However, results showed no significant association between extraversion and BMI for men (Est.=.202, SE= .140, *p*=.152, CI 95% [-.076, .482]) or for women (Est.=-.251, SE= .169, *p*=.142, CI 95% [-.589, .086]). Similarly, no significant association was observed between extraversion and HbA1c for women (Est. =-.032, SE= .021, *p*=.141, CI 95% [-.074, .010]) or for men (Est.=.026, SE= .0181, *p*=.144, CI 95% [-.009, .0629]). Furthermore, a significant positive relationship between extraversion and physical (Est.=.545, SE= .139, *p*≤.000, CI 95% [.268, .821]) and mental (Est.=.535, SE= .142, *p*≤.000, CI 95% [.253, .818]) health was observed in women, but not in men (Est.=.149, SE= .114, *p*=.197, CI 95% [-.079, .377], and Est.=.013, SE= .117, *p*=.908, CI 95% [-.220, .247], respectively).

Furthermore, sex did not moderate any association between openness and the health indexes. Finally, sex only moderated the association between agreeableness and LDL-cholesterol (ΔR^2=.060, *p*=.032, CI 95% [.044, .962]). Results showed a significant positive association between agreeableness and LDL-cholesterol for men (Est. =.356, SE= .155, *p*=.025, CI 95% [.045, .667]), but not for women (Est.=-.147, SE= .167, *p*=.383, CI 95% [-.481, .187]).

7.4. DISCUSSION

Our results showed that higher neuroticism and lower conscientiousness were related to a worse objective health status (higher BMI, WHR, HbA1c, and LDL-cholesterol). In addition, higher extraversion and agreeableness were related to a better

objective health status (lower LDL-cholesterol and higher HDL- cholesterol, respectively). Regarding the subjective health status, higher neuroticism and lower extraversion were related to worse subjective physical and mental health, whereas higher conscientiousness was associated with better subjective physical health. Sex moderated some of these associations. Only in women, higher neuroticism was related to worse objective health (BMI, HbA1c), whereas higher neuroticism and lower extraversion were related to worse subjective physical and mental health. By contrast, only in men, higher conscientiousness and agreeableness were related to worse objective health (higher pulse pressure and LDL-cholesterol, respectively).

Neuroticism was related to worse objective health (higher WHR and HbA1c, and marginally to BMI and LDL-cholesterol). Findings on the association between neuroticism and BMI/risk of obesity in older adults were mixed. Two studies observed that higher neuroticism was related to higher BMI (Otonari et al., 2012; Sutin & Terracciano, 2016b), but also to being underweight (Terracciano et al., 2014), or that there was no association (Mõttus et al., 2013). We observed that neuroticism was marginally related to higher BMI, but sex moderated this association. Interestingly, we observed a positive association between neuroticism and BMI only in older women, as reported in samples including young, middle-aged, and older adults (Armon et al., 2013; Brumett et al., 2006; Faith et al., 2001; Sutin & Terracciano, 2016b). Although we observed a marginal positive association between neuroticism and BMI, we found a significant positive association between neuroticism and WHR, as in other studies with samples including young and older people (Armon et al., 2013; Terracciano et al.,

2009). Although we failed to find sex differences in the association between neuroticism and WHR, Armon et al. (2013) observed that the association between neuroticism and both BMI and WHR was stronger in women than in men. We also observed a positive association between neuroticism and higher HbA1c, indicating worse glycemic control. Again, sex moderation analyses indicated that this association was observed only in women, suggesting that women are more vulnerable to the adverse effects of neuroticism on these health outcomes. Although, to the best of our knowledge, no previous studies have analyzed this relationship in healthy older people, Weston et al. (2015) failed to find an association between neuroticism and greater diabetes disease onset in old age. However, a recent study in young adulthood observed that higher neuroticism was related to higher levels of fasting blood sugar (Sutin, Stephan, & Terracciano, 2019), in line with our results. No significant associations were observed between neuroticism and cholesterol levels, but higher neuroticism was marginally related to higher LDL-cholesterol, indicating worse health. Similar to our results, Sutin et al. (2010a) failed to find this association in a sample that included participants ranging from young to older ages. However, in older adults, Chapman et al. (2013) reported that total cholesterol explained the association between neuroticism and morbidity. Nor did we observe a relationship between neuroticism and pulse pressure. However, Weston et al. (2015) observed that higher neuroticism was related to a higher risk of developing hypertension in older adults.

Regarding conscientiousness, this trait was related to worse objective health (higher BMI, WHR, HbA1c, and LDL-cholesterol). Conscientiousness was

significantly and negatively related to both BMI and WHR, which is consistent with other studies with older adults (Mõttus et al., 2013; Terracciano et al., 2009; Sutin & Terracciano, 2016b). Furthermore, Sutin & Terracciano (2016b) observed that this association was stronger in older people than in younger participants. Although we expected this association to be stronger in women than in men, as reported in samples that included younger participants (Brummett et al., 2006; Jokela et al., 2013; Sutin & Terracciano, 2016a; 2016b), we failed to find a moderator effect of sex in older adults. Higher conscientiousness was also related to lower HbA1c, indicating better glycemic control; again, no sex differences were observed in this association. Supporting our findings, lower conscientiousness was a risk factor for developing diabetes in older adults (Weston et al., 2015), and it was related to higher levels of fasting blood sugar in both men and women in young adulthood (Sutin et al., 2019). Higher conscientiousness was also associated with lower LDL-cholesterol, indicating a healthier lipidic profile, but not with HDL-cholesterol, in both men and women. Similarly, in a sample from adolescence to late adulthood, Sutin el at. (2010a) observed that high conscientiousness was related to a healthier cholesterol profile. Weston et al. (2015) also reported that lower conscientiousness was related to a higher risk of developing hypertension in older adults. Although we did not observe a significant association between conscientiousness and pulse pressure for the total sample, sex moderated this association. Contrary to our hypothesis, we observed that higher conscientiousness was associated with higher pulse pressure, but only in men. This may be due to the fact that blood pressure was measured before performing a neuropsychological assessment, which may be considered a stressful situation for older

people (Lupien et al., 2007). Therefore, this could explain why, in our study, the most conscientiousness men obtained higher pulse pressure scores. However, this result should be interpreted with caution because in our study conscientiousness was consistently related to other better health outcomes, and no sex differences were observed. Therefore, this outcome could be due to an inflation of Type 1 error.

We failed to observe a significant association between extraversion and both BMI and WHR, as in other studies (Mõttus et al., 2013; Terracciano et al., 2009; Sutin & Terracciano, 2016b), and with Hb1Ac, in line with Weston et al. (2015), who did not observe an association between extraversion and risk of diabetes in older adults. Several studies in samples that included younger participants have reported sex differences in the associations; extraversion has been associated with higher BMI in men (Brummett et al., 2006; Faith et al., 2001; Jokela et al., 2013; Shim et al., 2014; Sutin & Terracciano, 2016a) and lower BMI in women (Faith et al., 2001; Sutin & Terracciano, 2016b). Supporting these results, we observed a significant moderation effect of sex in the association between extraversion and both BMI and Hb1Ac. Although these associations were not significant for men and women separately, we observed that the direction of this association was positive for men, but negative for women. We also failed to observe an association between extraversion and pulse pressure, although Weston et al. (2015) reported that extraversion was related to a lower risk of hypertension in older adults. However, we observed that higher extraversion was related to lower LDL-cholesterol, indicating a healthier lipidic

profile, contrary to what was observed in a sample that also included younger participants (Sutin et al., 2010a).

In our study, the personality traits of openness and agreeableness showed little or no association with health. Similar to our findings, Sutin et al. (2019) failed to observe an association between these two personality traits and the four components of metabolic syndrome (glucose, blood pressure, cholesterol, and waist circumference) in young adults. Likewise, in older adults, these two personality traits were not associated with BMI and/or WHC (Mõttus et al., 2013; Terracciano et al., 2014), except Sutin & Terracciano (2016b), who reported a positive association. In our study, openness was not related to any objective health index, whereas higher agreeableness was only significantly associated with cholesterol levels. Specifically, higher agreeableness was related to higher HDL-cholesterol, indicating a healthier lipidic profile. Furthermore, only in men, higher agreeableness was related to higher LDL-cholesterol, indicating a less healthy lipidic profile, contrary to our hypothesis. However, these results should be interpreted with caution because, in our study, agreeableness was not consistently related to other health outcomes, and these results could stem from an inflation of Type 1 error.

Regarding subjective health, we observed that higher neuroticism and lower extraversion were related to worse physical and mental health, whereas lower conscientiousness was only associated with worse physical health. By contrast, we did not observe any significant associations between agreeableness and openness and subjective physical and mental health. Only one study considered, as in our study, the

two main scales of the SF-36 questionnaire, physical and mental health, and similar to our results, the authors observed that higher neuroticism was related to worse physical and mental health, but agreeableness and openness were not significantly associated with either of the two subscales in older people (Löckenhoff et al., 2008). In addition, as in our case, in this study they observed that lower conscientiousness was associated with worse physical health, but also with worse subjective mental health. However, other studies, although considering a few subscales of the SF-36 questionnaire, also failed to observe an association between conscientiousness and subjective mental health (Chapman et al., 2007; Jerram & Coleman, 1999), in line with our results. Regarding extraversion, contrary to our results, Löckenhoff et al. (2008) did not observe an association between extraversion and either of the two subjective physical and mental scales in a sample of healthy older people (39.9% women). However, other studies that considered only a few subscales of the SF-36 questionnaire observed that higher extraversion was related to better physical/mental health (Chapman et al., 2006; Duberstein et al., 2003; Jaconelli et al., 2012; Jerram & Coleman, 1999) but not all studies (Chapman et al., 2007).

These discrepancies could be explained by sex differences in the association between personality traits and subjective health. We explored the moderating effect of sex in the associations between personality and subjective physical and mental health, and we observed that the association between higher extraversion and better subjective physical and mental health was only observed for women. This could explain why Löckenhoff et al. (2008) failed to observe an association between extraversion and the

two subjective physical and mental scales in a sample of healthy older people composed mainly of men (60.1 % men), but they observed that extraversion was related to better subjective mental health in a sample of unhealthy older people composed mainly of women (75.5%). We also observed that the association between higher neuroticism and worse subjective physical and mental health was only observed for women. To our knowledge, no previous study has analyzed the moderating effect of sex in the relationship between personality and subjective health in older adults. Therefore, this study highlights the importance of taking sex differences into account in this association, mainly in the personality traits of neuroticism and extraversion.

In conclusion, in line with previous research, our study shows that conscientiousness and neuroticism are the personality traits more consistently and strongly related to health (Friedman & Kern, 2014; Lahey, 2009), using both objective and subjective indexes. There is evidence that conscientious individuals tend to engage in healthier behaviors, such as smoking less, moderate alcohol consumption, physical exercise, and eating healthier food, and they are better educated and have higher incomes and more successful careers (Friedman & Kern, 2014), which would explain the link between conscientiousness and better objective and subjective physical health. In addition, individuals with high neuroticism tend to engage less in healthy behaviors, have a less healthy diet (Mõttus et al., 2013), do less exercise (Sutin & Terracciano, 2016b), and smoke and abuse alcohol (Lahey, 2009). Moreover, individuals higher in neuroticism appear to be more likely to experience stressful events and show more pronounced and less well-regulated emotional responses, which would lead to HPA-

axis dysregulation (Lahey, 2009), also involved in metabolic syndrome (Björntorp, & Rosmond, 2000). Therefore, this would explain the association between higher neuroticism and worse objective and subjective physical and mental health. Interestingly, our results showed that this association was observed only in women, as in other studies (Armon et al., 2013; Brumett et al., 2006; Faith et al., 2001; Sutin & Terracciano, 2016a; 2016b), which could explain the inconsistent findings in previous literature on the association between neuroticism and health. By contrast, in our study, no sex differences were observed in the association between conscientiousness and health in older adults, even though other studies with samples that included younger participants suggested that this association was stronger in women (Brummett et al., 2006; Jokela et al., 2013; Sutin & Terracciano, 2016a; 2016b).

We also observed that extraversion was related to better objective (lower LDL-cholesterol) and subjective physical and mental health. Interestingly, sex moderated these associations, and extraversion was related to better subjective health only in women. Although the associations between neuroticism and conscientiousness and health are more consistent, the relationship between extraversion and health is less clear. This could be explained by the fact that extraversion could be associated with negative health outcomes in men (Brummett et al., 2006; Faith et al., 2001; Jokela et al., 2013; Shim et al., 2014; Sutin & Terracciano, 2016a), but have a protective health effect in women, as our results and other studies (Faith et al., 2001; Sutin & Terracciano, 2016b) suggest. In addition, extraversion has been linked to more positive affect and well-being and better perceived health (Costa & McCrae, 1984; McCrae &

Costa, 1991). This personality trait has been related to better health behaviors (diet and exercise), but also to negative (alcohol and smoking) health behaviors (Booth-Kewley & Vickers, 1994). Therefore, it is possible that women may benefit from the positive effects of extraversion on health, whereas the opposite occurs in men. Finally, our study suggests that agreeableness and openness have little or no effect on objective and subjective health in older adults, at least on the health indexes we considered.

In summary, our results suggest that mainly lower neuroticism and higher conscientiousness, and to a lesser extent, higher extraversion, are associated with better objective and subjective health. In addition, sex is an important moderator of these relationships. Specifically, lower neuroticism and higher extraversion are protective factors for health only in women.

Our study has some limitations. First, due to the correlational nature of the results, we cannot claim causal relationships. In addition, cholesterol levels were not obtained in fasting conditions, and blood pressure assessment was taken before a neuropsychological assessment that could be considered an acute stressor. Moreover, the fact that we ran multiple regression analyses could lead to an inflation of Type 1 error.

Table 7.2.
Regression analyses with personality traits as predictors and objective and subjective health indexes as dependent variables, adjusted for covariates.

	Neuroticism			Conscientiousness			Extraversion			Openness			Agreeableness		
	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*	ΔR^2	Beta	*p*
Objective health indexes															
BMI	.034	.184#	.091	.053	-.230*	.033	.000	.010	.928	.000	-.011	.920	.003	-.054	.662
WHR	.036	.198*	.036	.040	-.201*	.027	.005	-.069	.457	.004	-.066	.476	.007	-.086	.357
HbA1c	.093	.309**	.001	.072	-.269**	.004	.000	.007	.940	.006	.080	.409	.006	-.081	.404
LDL-Cholesterol	.038	.195#	.090	.055	-.234*	.040	.051	-.228*	.048	.002	.040	.732	.017	.129	.261
HDL-Cholesterol	.010	-.102	.364	.001	-.034	.755	.025	.159	.140	.012	-.110	.307	.050	.228*	.035
Pulse pressure	.008	.095	.368	.011	.108	.291	.005	.072	.481	.004	-.064	.528	.003	-.056	.589
Subjective health indexes															
Physical Health	.096	-.323**	.002	.052	.230*	.022	.097	.313**	.002	.001	.029	.772	.001	-.030	.775
Mental Health	.133	-.381**	.000	.017	.132	.196	.055	.236*	.019	.012	-.108	.287	.009	.097	.350

Note. BMI= Body mass index; WHR= Waist-Hip Ratio; HbA1c= Glycated hemoglobin; LDL = Low-density lipoprotein; HDL=High-density lipoprotein. # *p < .10* *p < .05.* **p < .01.*

CHAPTER 8
GENERAL DISCUSSION

The previous chapters have described the main results related to the role that genetic, situational, and individual factors play in the cognitive function of older men and women, taking in account the HPA-axis functioning. Overall, the ApoE genotype, loneliness, and personality traits explain part of the association between the HPA-axis functioning and cognitive functioning/ decline in older men and women. This final chapter presents a summary of the main findings of these studies.

8.1. MAIN FINDINGS

8.1.1. The role of the ApoE genotype in the association between HPA-axis and cognitive functioning in older adults

The evidence that the ApoE-ε4 allele is a major risk factor for developing late-onset AD, whereas the ApoE-ε2 allele has been associated with a reduced risk (Verghese et al., 2011), has aroused interest in the role that the ApoE genotype plays in the cognitive function of healthy individuals, which remains unclear (Lancaster et al., 2016). Moreover, because increased cortisol levels are observed in AD (Ouanes & Popp, 2019), the ApoE genotype may affect the association between the HPA-axis and cognitive functioning. Therefore, the first aim of this thesis, addressed in chapter 3, was to determine the impact of the three allelic variations of the ApoE polymorphism (ApoE-ε2, ApoE-ε3, and ApoE-ε4) on cognitive performance and the HPA-axis functioning. To do so, a neuropsychological battery was administered to assess attention, executive function, and working memory, and declarative verbal memory. Cortisol levels were measured before and after the neuropsychological session. Results

showed an association between the ApoE genotype and declarative verbal memory, specifically learning ability. Specifically, the ApoE-ε2 group performed better than the ApoE-ε4 and ApoE-ε3 groups, as reported previously (Helkala et al., 1995; 1996; Wisdom et al., 2011). Contrary to what was hypothesized, there were no differences in declarative verbal memory between the ApoE-ε4 and ApoE-ε3 groups. However, in line with our results, in a meta-analytic review, Lancaster et al. (2016) did not report differences in cognitive function between ApoE-ε4 carriers and non-carriers in mid-adulthood (35- 60 years). In addition, no differences in cortisol levels were obtained considering the ApoE genotype, as other studies reported (Fiocco et al., 2008; Lara et al., 2013; Li et al., 2006). Moreover, higher mean cortisol levels were related to worse performance on declarative verbal memory for the whole sample and, when considering the three allelic variations, for the ApoE-ε4 group. Therefore, these results indicate that the ApoE-ε4 allele may be a vulnerability factor in the adverse effects of HPA-axis dysregulation on cognition during aging, as reported in other studies (Gerritsen et al., 2011; Lee et al., 2008; Singh- Manoux et al., 2014). By contrast, an increase in cortisol levels during the neuropsychological session was associated with better performance on declarative verbal memory for the whole sample and for the ApoE-ε3. Moreover, the ApoE-ε3 group also showed an association between higher mean cortisol levels and better attention performance. Thus, our results suggest that the ApoE-ε3 allele could be associated with a more adaptive HPA-axis response.

8.1.2. The role of the HPA-axis in the association between loneliness and cognitive functioning in older men and women

Loneliness has been associated with an increased risk of cognitive decline and dementia (Boss et al., 2015; Cacioppo, & Hawkley, 2009; Lara et al., 2019), and with biological stress processes (Steptoe et al., 2004). The second aim of this thesis, addressed in chapter 4**,** was to analyze the mediating effect of HPA-axis functioning on the relationship between loneliness and cognitive function, as proposed in some studies (see reviews: Boss et al., 2015; Cacioppo, & Hawkley, 2009; Cacioppo et al., 2014; Ong et al., 2015). To do so, a neuropsychological battery was administered to assess global cognition, processing speed and attention, executive function, working memory, and declarative verbal memory. Participants also completed the R-UCLA loneliness scale. The HPA-axis functioning was assessed with several indexes of the diurnal cortisol pattern: awakening cortisol levels, DCS, AUCg, and bedtime cortisol levels. Results showed that loneliness was associated with higher bedtime cortisol levels (HPA-axis dysregulation), as also observed in older people (Cole et al., 2007). In turn, higher bedtime cortisol levels were associated with worse performance on attention, executive function, and working and declarative verbal memory (immediate recall), as other studies reported (Geerlings et al., 2015; Li et al., 2006; Tene et al., 2018). Furthermore, although loneliness was not directly related to any cognitive domain, it was associated indirectly with worse performance on attention, executive function, and declarative verbal memory (immediate recall), via bedtime cortisol levels. Therefore, our results confirm that HPA-axis functioning is one of the biological mechanisms that mediates the relationship between loneliness and poorer cognitive

function. Moreover, no sex differences were observed in the associations between loneliness and both HPA-axis and cognitive functioning, although it was expected these associations would be more pronounced in women than in men (Christiansen et al., 2016).

8.1.3. The role of personality in HPA-axis functioning, cognitive change, and other objective and subjective health measures

Personality traits measure individual differences in relatively enduring patterns of thoughts, feelings and behavior, and they are known to be associated with health and well-being during aging (Friedman et al., 2010; Jerram & Coleman, 1999; Weston et al., 2015), including dementia (Terracciano et al., 2014). The third aim of this thesis was to analyze the association between the big five personality traits and the HPA-axis functioning, addressed in chapter 5, as well as the mediating role of HPA-axis functioning and cognitive reserve in the association between personality traits and cognitive change, addressed in chapter 6. In addition, this thesis also aimed to explore the associations between personality traits and other objective and subjective health indexes in older adults, addressed in chapter 7. Finally, the moderating role of sex in these associations has also been investigated. To do so, at baseline and at a four-year follow-up, participants performed a neuropsychological battery to assess attention, executive function, working memory, and declarative verbal memory. At follow-up, participants also completed the NEO- FFI and CRQ, and they provided saliva samples to assess the diurnal cortisol pattern: awakening cortisol, CAR, DCS, and bedtime

cortisol. Objective (BMI, WHR, HbA1c, LDL and HDL-cholesterol, and pulse pressure) and subjective health indexes were also assessed at follow-up.

Results presented in chapter 5 showed that higher neuroticism and lower conscientiousness were related to lower awakening cortisol and greater CAR and DCS, indicating a less healthy diurnal cortisol profile. Previous studies in older people also observed an association between these two personality traits and an HPA-axis dysregulation, measured with different indexes (Gerritsen et al., 2009; Puig-Perez et al., 2016a; Steptoe et al., 2017). In addition, although extraversion was not related to the HPA-axis when considering the entire sample, sex moderated this association. Specifically, greater extraversion was related to higher bedtime cortisol in men, indicating HPA-axis dysregulation, and to lower bedtime cortisol in women, indicating a healthier HPA-axis profile. This evidence could explain the lack of association between extraversion and HPA-axis reported in previous findings (Hill et el., 2013; Ouanes et al., 2017b; Van Santen et al., 2011).

Regarding the association between personality traits and cognitive change, results presented in the chapter 6 revealed that higher agreeableness was related to less cognitive decline on declarative verbal memory (delayed recall), in line with Aiken-Morgan et al.'s (2012) study. However, higher extraversion was related to both less cognitive decline on delayed memory recall, but greater decline on immediate memory recall. Other studies also observed that extraversion was related both positively and negatively related to episodic memory (Allen et al., 2011; Luchetti et al., 2016; Meier et al., 2002). Because the association between personality and cognitive abilities in older people

remains elusive, it is important to increase the knowledge about the mechanisms underlying this association. Among these mechanisms the HPA-axis functioning and cognitive reserve have been proposed to explain the relationship between neuroticism and openness and cognitive functioning, respectively (Curtis et al., 2015). Results showed that higher neuroticism was not directly related to cognitive change, but it was indirectly related to a decline in attention via DCS. In addition, only in men an indirect effect of neuroticism on working memory change, via DCS, was observed. Supporting this finding, Wilson et al. (2007a) suggested that the association between neuroticism and risk of MCI was stronger for men. Moreover, higher extraversion and openness were related to less decline in executive function and immediate declarative verbal recall, respectively, via cognitive reserve.

Finally, results shown in chapter 7 indicated that higher neuroticism and lower conscientiousness were related to worse objective health (higher BMI, WHR, HbA1c and LDL-cholesterol), whereas higher extraversion and agreeableness were related to better objective health (lower LDL-cholesterol and higher HDL-cholesterol, respectively). In addition, higher neuroticism and lower conscientiousness and extraversion were related to worse subjective health. Regarding sex differences, only in women, higher neuroticism was related to worse objective (higher BMI and HbA1c) and subjective health, whereas higher extraversion was related to better subjective health. By contrast, only in men, higher conscientiousness and agreeableness were related to worse objective health (higher pulse pressure and LDL-cholesterol, respectively).

In summary, results addressing the third aim of this thesis showed that conscientiousness and neuroticism were the personality traits that were more consistently related to health outcomes, confirming previous results. It has been reported that highly conscientious individuals tend to engage in healthier behaviors (e.g. less smoking, moderate alcohol consumption, physical exercise, and eating healthier food), are better educated and have higher incomes, and have better emotion regulation abilities (Friedman & Kern, 2014). However, individuals with high scores on neuroticism tend to engage less in healthy behaviors and tend to experience more stressful events and less well-regulated emotional responses (Friedman & Kern, 2014; Lahey, 2009). In line with this evidence, results of this thesis showed that higher conscientiousness was related to healthier HPA-axis functioning and better objective and subjective health, with no sex differences emerging in these associations. Therefore, conscientiousness is considered a robust protective factor for health in older men and women. However, conscientiousness was not related to less cognitive decline, as expected (Chapman et al., 2012; Wilson et al., 2007b), possibly due to the fact that the sample was composed of healthy elderly adults and no pronounced cognitive decline was observed at the four-year follow-up. By contrast, neuroticism was associated with a dysregulation of the HPA-axis. In addition, a dysregulation of the HPA-axis mediated the relationship between higher neuroticism and greater decline in cognitive functions that depend on the prefrontal cortex, such as attention, and working memory but only in men. Moreover, neuroticism was associated with worse objective and subjective health, mainly in women. Therefore, neuroticism is an important risk factor for health outcomes in older people, and sex should be an important moderator

to consider when analyzing the associations of this personality trait with both general health and cognitive decline.

The association between extraversion and health in the previous literature is less consistent, which could be explained by important sex differences in these relationships. When considering the entire sample, results showed that higher extraversion was related to better objective and subjective health, suggesting beneficial effects on health, as we expected, but also to both a greater and lower cognitive decline. However, when considering sex differences, results showed that higher extraversion was related to a dysregulation of the HPA-axis in men, but to a healthier HPA-axis functioning and better subjective physical and mental health only in women. Although extraversion has been associated with better positive affect and well-being and better perceived health (Costa & McCrae, 1984; McCrae & Costa, 1991), this personality trait has been related to both positive (diet and exercise) and negative (alcohol and smoking) health behaviors (Booth-Kewley & Vickers, 1994). Thus, it is possible that women may benefit from the positive effects of extraversion on health, whereas the opposite occurs in men. Therefore, our results suggest that extraversion could be considered a protective health factor in women, but a risk factor in men.

Although openness does not seem to be related to health in older people, individuals with higher scores on openness would tend to engage in more cognitively stimulating activities, which would contribute to greater cognitive reserve and, therefore, to coping better with age-related cognitive changes. Thus, openness was not associated with health outcomes in older people, but it is suggested to be protective

factor against cognitive decline via cognitive reserve. Finally, we found that higher agreeableness is a protective factor against cognitive decline, supporting the fact that agreeableness has been associated with a lower risk of developing AD (Terracciano et al., 2014). Moreover, agreeableness showed little association with health, compared to the other personality traits assessed with the NEO-FFI, suggesting that this trait is less important than others for health in healthy older adults.

In sum, this thesis shows the importance of approaching the study of age-related cognitive changes and, specifically, the relationship between the HPA-axis and cognitive functioning, in older people, considering different moderating/mediating factors. We propose genetic (ApoE genotype), environmental (loneliness), and individual (personality) factors as important elements to consider when explaining individual differences in cognitive change/decline and health during aging. Along general lines, this thesis shows that the ApoE-ε4 allele and the loneliness and neuroticism traits are risk factors for health and cognitive function in older people. By contrast, the ApoE-ε2 and ApoE-ε3 alleles and the conscientiousness, agreeableness, and openness traits can be considered protective factors for health and cognitive function. Finally, extraversion seems to have both beneficial and harmful effects on cognition depending on sex. Thus, extraversion may be considered a risk factor for health in older men, but a protective factor in older women.

8.2. LIMITATIONS, STRENGTHS, AND FUTURE RESEARCH

In each chapter, specific limitations have been discussed. Here, some general limitations of the thesis are presented. First, although chapter 6 refers to a follow-up methodological approach, the rest of the chapters (3, 4, 5 and 7) are cross-sectional, and so no causal relationships can be claimed. In addition, a larger sample size would be necessary to increase the statistical power. This is especially important in chapter 3 because the unequal distribution of the three ApoE alleles and the low frequency of the ApoE-ε2 and ApoE-ε4 alleles in the population led to a small sample size for the ApoE-ε2 and ApoE-ε4 groups. In addition, larger samples would also be convenient to increase the statistical power when assessing sex moderator effects (chapters from 4 to 7) because it is possible that some of the hypothesized results were not observed. Nevertheless, it is important to highlight the effort made to analyze the association between the HPA-axis functioning and cognitive performance in a wide range of cognitive domains, considering the three allelic variations of the ApoE genotype, as well as sex moderating effects. However, the fact that we ran multiple regression analyses could have also led to an inflation of Type 1 error. Furthermore, in chapter 6, personality traits were assessed at follow-up, but not at baseline. Although personality traits are relatively stable across adulthood, some studies have suggested changes in these dimensions in aging (Roberts et al., 2006). Finally, the sample assessed in this thesis is characterized by having a generally good psychological and physical health condition, which cannot be generalized to all older people. However, this has the

advantage of reducing the possible effects of confounder variables when analyzing the association between HPA-axis functioning and cognition.

Therefore, future studies should replicate these findings in follow-up studies and with larger samples, in order to claim causality and increase statistical power. In addition, future research should also replicate these findings, assessing the NEO-FFI at baseline and at follow-up, to explore whether changes in personality traits are related to health outcomes and cognitive change in non-demented older adults. Finally, future studies should also assess the mediating role of some of the health outcomes related to personality traits in chapter 7 in the association between personality traits and cognitive change/decline. All of this will help to increase the knowledge about individual differences in age-related cognitive changes.

CHAPTER 9
MAIN CONCLUSIONS

In this section, the most important conclusions stemming from the objectives of this thesis are described below:

1. The ApoE-ε2 allele has been shown to be a protective factor for cognitive function, specifically for learning ability.

2. The ApoE-ε3 allele is related to a more adaptive HPA-axis response, with beneficial effects on cognitive performance (i.e. declarative verbal memory and attention). By contrast, the ApoE-ε4 allele could be a vulnerability factor in the adverse effects of HPA-axis dysregulation on cognition (i.e. declarative verbal memory).

3. Loneliness is associated with a dysregulation of the HPA-axis (i.e. higher bedtime cortisol levels), which, in turn, is associated with worse cognitive function.

4. Specifically, loneliness is not associated directly with cognitive function in healthy older adults, but it is indirectly related to worse cognitive performance via higher bedtime cortisol levels. Therefore, the dysregulation of the HPA-axis appears to be one of the mechanisms underlying the relationship between loneliness and cognitive impairment.

5. Neuroticism is an important health risk factor in aging because it is associated with a dysregulation of the HPA-axis, decline in attention via a dysregulation of the HPA-axis, and worse objective and subjective health.

6. Conscientiousness is a robust protective factor for health in older people because it is associated with healthier HPA-axis functioning and better objective and subjective health. However, it is not a predictor of cognitive change in healthy older adults.

7. Extraversion could protect against decline in executive function and delayed memory recall, but favor decline in immediate memory recall, which could be explained by cognitive reserve.

8. Openness and agreeableness are not important predictors of health outcomes in older people, but they are protective factors against cognitive decline in immediate recall via cognitive reserve, and delayed recall, respectively.

9. Sex does not play a role in the associations between loneliness and HPA-axis dysregulation and cognitive function.

10. Sex seems to be an important moderator in the relationship between neuroticism and extraversion and health and cognitive decline. Specifically,

neuroticism is a greater risk factor for cognitive decline in working memory in men, and for health in women, whereas extraversion is a protective factor for health in women, but a risk factor in men.

CHAPTER 10

GENERAL SUMMARY IN SPANISH

El porcentaje de personas mayores de 60 años está aumentando a un ritmo exponencial. Este grupo de edad se enfrenta a importantes retos para la salud, entre los que destacan los cambios cognitivos relacionados con la edad. A pesar de ello, cabe mencionar que el envejecimiento es un proceso muy heterogéneo (WHO, 2018). De hecho, algunas personas mantienen una buena salud hasta edades avanzadas, mientras que otras manifiestan un declive físico y cognitivo pronunciado, pudiendo llegar, en algunos casos al desarrollo de demencia. Por ello, es importante identificar los factores protectores y de vulnerabilidad del declive cognitivo en este grupo de edad con el objetivo de prevenirlo.

Entre estos factores destaca el funcionamiento del eje Hipotalámico-Hipofisario-Adrenal (HHA), que interviene en la respuesta de estrés. En situaciones de estrés crónico se ha descrito una desregulación del eje HHA (Miller et al., 2007), que podría contribuir al daño neurológico y al deterioro cognitivo durante el envejecimiento (McEwen, 2008). Sin embargo, hay muchas cuestiones que aún no están resultas, por lo que es necesario seguir avanzando en el conocimiento de los factores que puedan explicar las diferencias individuales en los cambios cognitivos asociados a la edad.

Esta tesis pretende abordar el estudio de las relaciones entre el funcionamiento del eje HHA y la función cognitiva, teniendo en cuenta factores genéticos (el polimorfismo genético ApoE), situacionales (la soledad percibida), e individuales (rasgos de personalidad).

El alelo ApoE-ε4 del gen de la ApoE ha sido descrito como el factor de riesgo más importante para el desarrollo de la Enfermedad de Alzheimer (EA) de inicio tardío, mientras que el alelo ApoE-ε2 ha sido descrito como un factor protector (Verghese et al., 2011). A pesar de que varios estudios han explorado el papel que juegan estos alelos en la función cognitiva en personas mayores sanas, la cuestión sigue sin estar resuelta (Lancaster et al., 2016). El genotipo ApoE también podría estar relacionado con el funcionamiento del eje HHA, ya que en la EA se han observado niveles elevados de cortisol. Además, se ha sugerido que un aumento en los niveles de cortisol podría inducir o exacerbar la patología cerebral de la EA al aumentar la carga cerebral de amiloide-β, la patología tau y el estrés oxidativo, conduciendo a la neurodegeneración. Esta evidencia sugiere que el gen de la ApoE podría intervenir en la relación entre el eje HHA y la función cognitiva (Ouanes y Popp, 2019).

La soledad percibida también ha sido descrita como un factor de riesgo para el desarrollo de demencia (ver revisiones: Cacioppo y Hawkley, 2009; Lara et al., 2019), aunque los resultados de los estudios previos en personas mayores no dementes son inconsistentes (Boss et al., 2015). Además, se ha sugerido que la soledad percibida es una experiencia psicológica que contribuye al estrés biológico (Steptoe et al., 2004), por lo que varios estudios han propuesto el funcionamiento del eje HHA como uno de los mecanismos biológicos subyacentes a la asociación entre la soledad y la cognición (Boss et al., 2015; Ong et al., 2015; Cacioppo y Hawkley, 2009; Cacioppo et al., 2014). También cabe señalar que, a pesar de que las mujeres refieren mayor soledad percibida que los hombres, y que ésta parece afectar más la salud de las mujeres que de los

hombres (Christiansen et al., 2016), las diferencias de sexo en estas relaciones han sido poco estudiadas. Por lo tanto, analizar el papel mediador del funcionamiento del eje HHA en la asociación entre la soledad y la cognición, teniendo en cuenta las posibles diferencias sexuales en personas mayores sanas, podría ayudar a aclarar los hallazgos inconsistentes reportados en la literatura.

Es sabido que la personalidad influye en la salud (Friedman et al., 2010; Jerram y Coleman, 1999; Weston et al., 2015), incluida la demencia (Terracciano et al., 2014). Sin embargo, la asociación entre los rasgos de personalidad y la función cognitiva en poblaciones no clínicas de adultos mayores no está clara (ver revisión: Curtis et al., 2015). Por lo tanto, es importante estudiar los mecanismos que subyacen a la relación entre la personalidad y la función cognitiva. Entre estos mecanismos se han propuesto el funcionamiento del eje HHA y la reserva cognitiva. Se sabe que los rasgos personalidad, principalmente el neuroticismo, están asociados con la reactividad biológica al estrés (Soliemanifar et al., 2018) y, por lo tanto, con el funcionamiento del eje HHA. Además, la personalidad también puede estar relacionada con la reserva cognitiva, ya que las personas que puntúan alto en apertura a la experiencia podrían tender a realizar más actividades intelectuales (Soubelet y Salthouse, 2010). Además, la literatura previa muestra resultados inconsistentes en la relación entre la personalidad, y otros índices objetivos y subjetivos de salud, que podrían explicarse debido a que la edad y el sexo son moderadores importantes de estas asociaciones (Jerram y Coleman, 1999; Sutin y Terracciano, 2016b).

En resumen, el objetivo general de esta tesis es avanzar en el conocimiento de los factores que pueden intervenir en la relación entre el eje HHA y la función cognitiva durante el envejecimiento, con el fin de explicar las diferencias individuales en el deterioro cognitivo. Entre estos factores, hemos propuesto variables genéticas (ApoE), situacionales (soledad) e individuales (personalidad).

10.1. OBJETIVOS E HIPÓTESIS

Debido a los resultados inconsistentes y las cuestiones pendientes expuestas en el capítulo 1 (Introducción), la presente tesis pretende abordar los objetivos generales y específicos presentados a continuación:

Objetivo general 1. Determinar el impacto de las tres variaciones alélicas del polimorfismo ApoE (ApoE-ε2, ApoE-ε3 y ApoE-ε4) en diferentes dominios cognitivos y el funcionamiento del eje HHA, evaluado a través de los niveles de cortisol durante la evaluación neuropsicológica, en adultos mayores sanos.

Objetivo específico 1.1: Estudiar las diferencias en el rendimiento cognitivo (atención y función ejecutiva, memoria de trabajo y memoria declarativa verbal), en función del grupo ApoE.

Objetivo específico 1.2: Estudiar las diferencias en los niveles de cortisol (niveles de cortisol medios y delta), en función del grupo ApoE.

Objetivo específico 1.3: Investigar la relación entre los niveles de cortisol y el rendimiento cognitivo de forma separada para los grupos ApoE, así como el papel moderador de la ApoE en estas asociaciones.

Basándonos en la literatura previa, planteamos la hipótesis de un peor rendimiento cognitivo en el grupo ApoE-ε4 y un mejor rendimiento cognitivo en el grupo ApoE-ε2 (Wisdom et al., 2011). También esperamos observar niveles más altos de cortisol en el grupo ApoE-ε4, en comparación con los grupos ApoE-ε2 y ApoE-ε3 (Peskind et al., 2001). Por último, planteamos la hipótesis de que, tanto niveles más altos de cortisol medios, como un aumento en los niveles de cortisol durante la evaluación neuropsicológica, estarían relacionados con un peor rendimiento cognitivo, principalmente en memoria declarativa verbal, y en el grupo ApoE-ε4 (Gerritsen et al., 2011; Lee et al., 2008).

Estos objetivos, así como los principales resultados obtenidos han sido planteados en el capítulo 3.

Objetivo general 2. Analizar el efecto mediador del funcionamiento del eje HHA, evaluado con diferentes índices del patrón diurno de cortisol, en la relación entre la soledad percibida y la función cognitiva, en personas mayores sanas.

Objetivo específico 2.1: Estudiar la relación entre la soledad percibida y el rendimiento cognitivo (cognición global, atención y función ejecutiva, memoria de trabajo y memoria declarativa verbal).

Objetivo específico 2.2: Estudiar la relación entre la soledad percibida y el patrón diurno de cortisol (cortisol al despertar, pendiente diurna de cortisol (DCS, por sus siglas en inglés), área bajo la curva de cortisol (AUCg, por sus siglas en inglés) y cortisol antes de dormir).

Objetivo específico 2.3: Analizar la asociación entre el patrón diurno de cortisol y el rendimiento cognitivo.

Objetivo específico 2.4: Analizar el efecto mediador del patrón diurno de cortisol en la relación entre la soledad percibida y la función cognitiva.

Objetivo específico 2.5: Investigar el papel moderador del sexo en los objetivos específicos previamente mencionados.

Debido a que los estudios previos han mostrado resultados mixtos en la relación entre la soledad percibida y la DCS (Adam et al., 2006; Cole et al., 2007; Schutter et al., 2017), y a la falta de estudios sobre la relación entre la soledad percibida y el AUCg, no se propuso una hipótesis específica con la dirección de estas relaciones. Por otra parte, hipotetizamos una asociación negativa entre la soledad percibida y el rendimiento en diferentes dominios cognitivos (Boss et al., 2015). Además, esperamos observar una asociación negativa entre la función cognitiva y los niveles de cortisol al despertar (Beluche et al., 2010; O'Hara et al., 2007), antes de dormir (Li et al., 2006; Stawski et al., 2011), la DCS (Beluche et al., 2010; Stawski et al., 2011), y los niveles de cortisol a lo largo del día (Li et al., 2006; Ouanes et al., 2017a). Asimismo, nos planteamos la hipótesis de que este patrón desregulado del eje HHA, mediaría la relación entre la soledad percibida y un rendimiento cognitivo más deficiente. Finalmente, esperamos que estas asociaciones fueran más pronunciadas en mujeres que en hombres (Christiansen et al., 2016).

Estos objetivos se han abordado en el capítulo 4 de esta tesis.

Objetivo general 3. Determinar el impacto de los cinco grandes rasgos de personalidad (neuroticismo, responsabilidad, extraversión, apertura a la experiencia y amabilidad), en el funcionamiento del eje HHA evaluado a través del patrón diurno de cortisol, en el cambio/declive cognitivo, y otros índices de salud objetivos y subjetivos, en adultos mayores relativamente sanos.

Objetivo específico 3.1: Estudiar la relación entre los rasgos de personalidad y el patrón diurno de cortisol (cortisol al despertar, respuesta matutina de cortisol [CAR, por sus siglas en inglés], DCS y cortisol antes de dormir).

Objetivo específico 3.2: Estudiar la relación entre los rasgos de personalidad y el cambio/declive cognitivo (atención y función ejecutiva, memoria de trabajo, y memoria declarativa verbal).

Objetivo específico 3.3: Estudiar el efecto mediador de la DCS y la reserva cognitiva, en la relación entre los rasgos de personalidad y el cambio/declive cognitivo.

Objetivo específico 3.4: Estudiar la relación entre los rasgos de personalidad y otras medidas objetivas de salud (IMC, ICC, HbA1c, LDL y HDL- colesterol y presión del pulso).

Objetivo específico 3.5: Estudiar la relación entre los rasgos de personalidad y la salud subjetiva física y mental.

Objetivo específico 3.6: Investigar el papel moderador del sexo en los objetivos específicos previamente mencionados.

En base a la literatura previa, se hipotetizó una relación entre una desregulación del funcionamiento del eje HHA (es decir, menores niveles de cortisol del despertar, un mayor CAR y DCS y mayores niveles de cortisol antes de dormir) y mayores

puntuaciones en neuroticismo (un rasgo de personalidad considerado como un factor de riesgo), y menores puntuaciones en los rasgos de personalidad considerados factores protectores (es decir, responsabilidad, extraversión, apertura a la experiencia y amabilidad) para la salud (Weston et al., 2015). Además, esperábamos observar que la asociación entre el neuroticismo y una desregulación del eje HHA, fuera más pronunciada en mujeres que en hombres (Puig-Perez et al., 2016a).

Con respecto a la asociación entre la personalidad y el cambio/declive cognitivo, se planteó la hipótesis de que mayores puntuaciones en neuroticismo y menores puntuaciones en responsabilidad, extraversión, apertura a la experiencia y amabilidad, estarían relacionadas con un mayor declive cognitivo (Terracciano et al., 2014).

También se hipotetizó que, mayores puntuaciones en neuroticismo estarían relacionadas con una mayor DCS (es decir, una curva de cortisol más aplanada), así como mayores puntuaciones en apertura a la experiencia estarían relacionadas con una mayor reserva cognitiva. Además, se esperó observar una asociación entre una mayor DCS y menores puntuaciones en reserva cognitiva, y un mayor declive cognitivo. Por último, se esperó encontrar un efecto mediador del DCS en la asociación entre el neuroticismo y cambio/declive cognitivo, así como un efecto mediador de la reserva cognitiva en la relación entre la apertura a la experiencia y el cambio/declive cognitivo.

Finalmente, planteamos la hipótesis de que, principalmente, con una mayor puntuación en neuroticismo y una menor puntuación en responsabilidad, y en menor grado, con menores puntuaciones en extraversión, apertura a la experiencia y amabilidad, se observaría una asociación con una peor salud objetiva y subjetiva.

Además, esperábamos que las asociaciones entre los rasgos de personalidad y la salud fueran más pronunciadas en mujeres que en hombres, al menos para el neuroticismo y la responsabilidad, como ha sido descrito en estudios previos con otros rangos de edad (Armon et al., 2013; Brumett et al., 2006; Faith et al., 2001; Sutin y Terracciano, 2016a; 2016b).

En los capítulos 5, 6 y 7 nos planteamos responder a estos objetivos. Concretamente, en el capítulo 5 se han abordado los objetivos 3.1 y 3.6, en el capítulo 6 los objetivos 3.2, 3.3 y 3.6, y en el capítulo 7 los objetivos 3.4, 3.5 y 3.6.

10.2. METODOLOGÍA

Con el fin de proporcionar una visión global de la metodología utilizada en la presente tesis, en esta sección se realiza un breve resumen sobre las características de los participantes, el procedimiento y las variables evaluadas.

10.2.1. Participantes

La muestra de la presente tesis proviene de un programa de estudios de la Universidad de Valencia para personas mayores de 55 años (NAU GRAN). Los criterios de exclusión en la primera fase en la línea base fueron: presencia de problemas importantes de visión o audición, fumar más de diez cigarrillos al día, abuso de alcohol u otras drogas, presencia de enfermedades neurológicas, psiquiátricas o endocrinas, uso de medicación relacionada con el rendimiento cognitivo o el control emocional, o que pueda afectar los niveles hormonales, tales como glucocorticoides, medicación para

diabetes, antidepresivos, anticoagulantes, benzodiacepinas o antipsicóticos, y haber estado bajo los efectos de anestesia general o haber vivido un evento vital estresante en el último año. Todas las mujeres eran postmenopáusicas y habían tenido su último periodo menstrual al menos un año antes, y ningún participante obtuvo una puntuación menor de 27 en el MEC (Mini-Examen Cognoscitivo; Lobo et al., 1999), indicando ausencia de deterioro cognitivo.

10.2.2. Procedimiento

Los participantes del estudio siempre fueron citados a las 10 o a las12 horas de la mañana en el Laboratorio de Neurociencia Social Cognitiva en la Facultad de Psicología de la Universitat de València. Se indicó a los participantes que, el día de antes de venir a la sesión, debían mantener sus hábitos generales, dormir el tiempo habitual, abstenerse de realizar actividad física intensa, y no consumir alcohol desde la noche anterior. También se les indicó que al menos 1 hora antes de la sesión debían beber solo agua, y no comer, fumar, tomar estimulantes (como café, cola, cafeína, té o chocolate) o cepillarse los dientes. Una vez en el laboratorio, los participantes realizaron una evaluación neuropsicológica con el fin de evaluar el rendimiento en cognición global (MEC), atención y función ejecutiva (Trail Making Test y test de Stroop), memoria de trabajo (test de dígitos y test de letras y números), y memoria declarativa verbal (Rey Auditory Verbal Learning Tests y Test de recuerdo de historias Rivermead). Además, los participantes proporcionaron muestras de saliva a partir de las cuales se determinaron los niveles de cortisol, y el genotipo ApoE. Cuatro años después, se contactó de nuevo con los participantes vía telefónica, y se les invitó a

continuar participando en el estudio. 87 participantes fueron citados nuevamente en el mismo laboratorio. Antes de venir a la sesión, se indicó a los participantes que debían seguir las mismas recomendaciones previamente mencionadas. Al llegar al laboratorio, se midió el peso, la altura, la cintura y la cadera de los participantes, así como la presión arterial durante tres veces consecutivas. Tras esto, se realizó la misma evaluación neuropsicológica que en la línea base. Al finalizar la evaluación neuropsicológica, se obtuvieron los niveles de LDL y HDL-colesterol, y la HbA1c, a través de una punción digital (muestra de sangre capilar). Los participantes también completaron diversos cuestionarios con el objetivo de evaluar la soledad percibida, la depresión, la reserva cognitiva y la salud subjetiva. Además, se pidió a los participantes que proporcionaron muestras de saliva en casa durante en días consecutivos (inmediatamente al despertarse, a los 15, 30, y 45 minutos del despertar y antes de irse a dormir) con el fin de evaluar diferentes componentes del patrón diurno de cortisol: niveles de cortisol al despertar, CAR, DCS, AUCg y niveles de cortisol antes de dormir.

10.2.3. Variables biológicas

Variables genéticas. La determinación de las tres variaciones alélicas del polimorfismo genético *ApoE* (ApoE-ε2, ApoE-ε3 y ApoE-ε4), se obtuvo a partir de muestras de saliva (KIT REALPURE "SSS"), mediante la técnica de la reacción en cadena de la polimerasa (PCR) y electroforesis.

Variables hormonales. La actividad del eje HHA fue evaluada analizando el *cortisol* en saliva. Los participantes proporcionaron muestras de saliva utilizando salivettes (Sarstedt, Nümbrecht, Germany). Los niveles de cortisol fueron analizados

con la técnica apropiada para este tipo de determinaciones utilizando kits comerciales, siempre por duplicado y todas las muestras proporcionadas de un mismo participante en el mismo kit.

Medidas antropométricas. El *índice de masa corporal (IMC)* se calculó como peso (kg) / altura $(m)^2$. El *índice cintura-cadera (ICC)* se calculó como circunferencia de la cintura/ circunferencia de la cadera.

Biomarcadores en sangre. La *hemoglobina glicosilada (HbA1c*) y la *Lipoproteína de baja densidad (LDL)* ("colesterol malo") y la *Lipoproteína de alta densidad (HDL)* ("colesterol bueno"), se determinaron a partir de muestras de sangre capilar a través de una punción digital, con el sistema Cobas b 101 (Roche Diagnostics, España).

Presión sanguínea. La presión arterial se midió mediante el monitor automático ORMON M6W (ORMON Healthcare, Japón) durante tres veces consecutivas. La *presión de pulso* se calculó como la diferencia entre la presión arterial sistólica (PAS) y presión arterial diastólica (PAD) (mm Hg).

10.2.4. Variables cognitivas

La *cognición global* fue evaluada con el Mini-Examen Cognoscitivo (MEC) (versión española del Mini-Mental State Examination; Lobo et al., 1999), que incluye 11 cuestiones que miden varias funciones cognitivas.

La *atención y la función ejecutiva* se evaluó con el test del Trazo o Trail Making Test (Reitan, 1992) y el Stroop test de colores y palabras (Golden, 1978). El TMT consiste en dos pruebas, TMT-A y TMT-B. La primera consiste en trazar una línea en

una hoja, uniendo de forma secuencial unos círculos numerados del 1 al 25. En la segunda, el participante tiene que trazar una línea uniendo números (del 1 al 13) y letras (de la A a la I) de forma alternante. La puntuación obtenida es la cantidad de segundos necesarios para finalizar cada prueba. El test de Stroop de palabras y colores se compone de tres ensayos. En cada ensayo, los participantes tienen que nombrar tantas palabras como sea posible en 45 segundos. En la primera prueba, los participantes deben leer la palabra escrita (P) (rojo, azul o verde). En la segunda prueba, deben nombrar el color impreso (C) (rojo, azul o verde) de las letras XXX. En la tercera prueba, deben nombrar el color de la palabra impresa (rojo, azul o verde), que difiere de la palabra escrita (rojo, azul o verde) (PC). Posteriormente, se calcula el índice PC' ((P x C) / (P + C)) y el índice de Interferencia Stroop (WC - WC ').

La *memoria de trabajo* se evaluó con el test de dígitos, y con el test de letras y números (LNS), de la versión española de la escala de memoria Wechsler III (Wechsler, 1997). En el test de dígitos, el experimentador lee una serie de dígitos, y el participante debe repetirlos en el mismo orden (dígitos directos, DD), y posteriormente en orden inverso (dígitos inversos, DI). En el test de LNS, el experimentador lee una serie mezclada de números y letras, y el participante debe repetirlos ordenando los números de forma ascendente, y las letras en orden alfabético.

La *memoria declarativa verbal* se evaluó con la versión española del Rey Auditory Verbal Learning Tests (RAVLT) (Miranda y Valencia, 1997), y con el subtest de recuerdo de historias de la versión española del Test de Memoria Conductual Rivermead (Wilson et al., 1985). El test de RAVLT consiste en la presentación de una lista de 15 palabras neutras que el experimentador lee en voz alta durante cinco

ensayos, y los participantes deben aprender (*aprendizaje total*). Tras esto, se presenta una lista de interferencia, e inmediatamente después (*recuerdo inmediato*) y tras 20 minutos (*recuerdo demorado*) el participante debe intentar recordar el máximo número de palabras. En el test Rivermead, el experimentador lee en voz alta dos historias cortas, y el participante debe recordar inmediatamente (*recuerdo inmediato*) y tras 20 minutos (*recuerdo demorado*) el máximo número de ideas.

10.2.5. Cuestionarios

La *soledad percibida* fue evaluada con la adaptación española (Vázquez y Jiménez, 1994) de la escala de Soledad UCLA revisada (UCLA-R) (Russell et al.,1980). Se trata de una escala de 20 items tipo Likert cuya puntuación va de uno (nunca) a cuatro (a menudo).

La *depresión* fue evaluada con la versión española (Fernández-San Martín et al., 2002) de la escala de Depresión geriátrica (GDS) (Yesavage et al., 1982). Se trata de una escala de 30 items formulados como preguntas con respuesta dicotómica (sí / no).

Los *cinco grandes factores de personalidad* se evaluaron con la versión española (Costa y McCrae, 1999) del NEO-Five Factor Inventory (Costa y McCrae, 1992). Incluye 60 items que se responden con una escala Likert de 5 puntos. El test evalúa neuroticismo, responsabilidad, extraversión, apertura a la experiencia y amabilidad.

La *salud subjetiva* se evaluó con la versión española (Alonso et al., 1995) del Cuestionario de Salud SF-36 (Short Form Health Survey-36) (Ware, & Sherbourne, 1992). Consta de 36 items distribuidos en 8 subescalas: función física, rol físico, dolor corporal, salud general, viltalidad, función social, rol emocional y salud mental. Estas subescalas se agrupan en dos componetes principales: salud física y salud mental.

La *reserva cognitiva* fue evaluada con el Cuestionario de Reserva Cognitiva (CRC) (Ramí et al., 2011). Este cuestionario consta de 8 items que miden actividad intelectual.

10.3. CONCLUSIONES

El primer objetivo de esta tesis fue determinar el impacto de las tres variaciones alélicas del polimorfismo ApoE (ApoE-ε2, ApoE-ε3, y ApoE-ε4) en la función cognitiva y en el funcionamiento del eje HHA evaluado a través de los niveles de cortisol durante la evaluación neuropsicológica. Los resultados, presentados en el capítulo 3**,** mostraron una asociación entre el genotipo ApoE y la memoria declarativa verbal, específicamente en la capacidad de aprendizaje. Concretamente, el grupo ApoE-ε2 obtuvo un mejor rendimiento cognitivo que los grupos ApoE-ε4 y ApoE-ε3, en la misma línea que estudios previous (Helkala et al., 1995; 1996; Wisdom et al., 2011). Además, no se observaron diferencias en el rendimiento cognitivo entre los grupos ApoE-ε4 y ApoE-ε3, como en una revisión meta-analítica en adultos de mediada edad (Lancaster et al., 2016). Tampoco se observaron diferencias en los niveles de cortisol entre los diferentes alelos de la ApoE, al igual que estudios previos

(Fiocco et al., 2008; Lara et al., 2013; Li et al., 2006). Por el contrario, mayores niveles medios de cortisol durante la evaluación neuropsicológica se relacionaron con un peor rendimiento en memoria declarativa verbal sólo en el grupo ApoE-ε4, apoyando la idea de que el alelo ApoE-ε4 puede ser un factor de vulnerabilidad para los efectos adversos de la desregulación del eje HHA en la cognición durante el envejecimiento, como se ha encontrado en otros estudios (Gerritsen et al., 2011; Lee et al., 2008; Singh - Manoux et al., 2014). Por el contrario, sólo en el grupo ApoE-ε3, un incremento en los niveles de cortisol durante la sesión neuropsicológica se asoció con un mejor rendimiento en la memoria declarativa verbal, y niveles medios de cortisol más altos se asociaron a un mejor rendimiento en atención, sugiriendo que el alelo ApoE-ε3 podría estar asociado a una respuesta más adaptativa del eje HHA.

El segundo objetivo de la presente tesis fue analizar el efecto mediador del funcionamiento del eje HHA en la relación entre la soledad percibida y el rendimiento cognitivo, tal y como han propuesto otros estudios (Boss et al., 2015; Cacioppo y Hawkley, 2009; Cacioppo et al., 2014; Ong et al., 2015). Los resultados, presentados en el capítulo 4, mostraron una asociación entre la soledad percibida y una desregulación del eje HHA (mayores niveles de cortisol antes de dormir). A su vez, mayores niveles de cortisol antes de dormir se asociaron con un peor rendimiento cognitivo (atención, función ejecutiva, memoria de trabajo y memoria declarativa verbal inmediata), en línea con otros estudios (Geerlings et al., 2015; Li et al., 2006; Tene et al., 2018). Además, aunque no se observó una relación directa entre la soledad percibida y la función cognitiva, se encontró una relación indirecta entre una mayor soledad percibida y un peor rendimiento en la atención, la función ejecutiva, y memoria

verbal inmediata, a través de los niveles de cortisol antes de dormir. Por lo tanto, nuestros resultados confirman que el funcionamiento del eje HHA podría ser uno de los mecanismos biológicos que subyace a la relación entre la soledad percibida y la función cognitiva. Además, no se observaron diferencias de sexo en la asociación entre la soledad, y el eje HHA y la función cognitiva, a pesar de que se hipotetizó que estas asociaciones fueran más pronunciadas en mujeres que en hombres (Christiansen et al., 2016).

El tercer objetivo de esta tesis fue explorar la relación entre los cinco grandes rasgos de la personalidad y el funcionamiento del eje HHA, así como el papel mediador del funcionamiento del eje HHA y la reserva cognitiva, en la relación entre la personalidad y el cambio/declive cognitivo. Además, se pretendió explorar la asociación entre los factores de personalidad y otros índices objetivos y subjetivos de salud, así como el papel moderador del sexo en estas asociaciones, en adultos mayores.

Los resultados, presentados en el capítulo 5, mostraron que mayores puntuaciones en neuroticismo, y menores puntuaciones en responsabilidad se asociaron con un perfil de cortisol diurno menos saludable (menores niveles de cortisol al despertar y un mayor CAR y DCS), de forma similar a estudios previos (Gerritsen et al., 2009; Puig-Perez et al., 2016a; Steptoe et al., 2017). Además, la extraversión se relacionó con una desregulación del eje HHA en hombres (mayores niveles de cortisol antes de dormir), y con un perfil más saludable en mujeres (menores niveles de cortisol antes de dormir).

Por otra parte, los resultados presentados en el capítulo 6 revelaron que mayores puntuaciones en amabilidad se asociaron a un menor declive en memoria verbal

demorada, en línea con el estudio de Aiken-Morgan et al. (2012). Sin embargo, mayores puntuaciones en extraversión se asociaron tanto con un mayor declive en memoria verbal inmediata, como con un menor declive en memoria verbal demorada. De forma similar, algunos estudios observaron que la extraversión se relacionó tanto de forma positiva como negativa con la memoria episódica (Allen et al., 2011; Luchetti et al., 2016; Meier et al., 2002). Además, mayores puntuaciones en extraversión se asociaron a una mayor reserva cognitiva, lo que sugiere que estos individuos tienden a realizar más actividades intelectuales. A pesar de que no se observó una relación directa entre la extraversión y la función ejecutiva, se observó que una relación indirecta entre una mayor puntuación en extraversión y un mejor rendimiento en función ejecutiva, a través de la reserva cognitiva. Además, los resultados mostraron que un mayor neuroticismo no estaba directamente relacionado con el cambio cognitivo, pero estaba indirectamente relacionado con un mayor declive en atención y en memoria de trabajo sólo en hombres, a través de DCS. Además, mayores puntuaciones en extraversión y apertura a la experiencia se relacionaron con un menor declive en función ejecutiva y en memoria verbal, respectivamente, a través de la reserva cognitiva. Por lo tanto, los resultados sugieren que el funcionamiento del eje HHA y la reserva cognitiva, median la relación entre el neuroticismo, y la apertura a la experiencia y la extraversión, con el cambio/declive cognitivo, respectivamente.

Por último, los resultados mostrados en el capítulo 7 indican que mayores puntuaciones en neuroticismo y menores puntuaciones en responsabilidad se asociaron a una peor salud objetiva (mayor IMC, ICC, HbA1c y LDL-colesterol), y, en menor medida, mayores puntuaciones en extraversión y amabilidad se relacionaron con una

mejor salud objetiva (menores niveles de LDL-colesterol y mayores niveles de HDL-colesterol, respectivamente). Además, mayores puntuaciones en neuroticismo y menores puntuaciones en responsabilidad y extraversión se relacionaron con una peor salud subjetiva. Al explorar las diferencias de sexo, sólo en las mujeres, mayores puntuaciones en neuroticismo se asociaron con una peor salud objetiva (mayor IMC y HbA1c) y subjetiva, mientras que una mayor extraversión se relacionó con una mejor salud subjetiva. Por el contrario, sólo en los hombres, mayores puntuaciones en responsabilidad y amabilidad se relacionaron con una peor salud objetiva (mayor presión del pulso y LDL-colesterol, respectivamente).

A modo de resumen, los resultados de esta tesis confirman que la responsabilidad y el neuroticismo son los rasgos de personalidad más consistentemente relacionados con la salud. Los individuos que puntúan alto en responsabilidad tienden a mostrar comportamientos más saludables (no fumar, consumo moderado de alcohol, ejercicio físico y dieta saludable), y tienen una mayor capacidad de regulación emocional (Friedman & Kern, 2014). Por el contrario, los individuos que puntúan alto en neuroticismo, tienden a mostrar comportamientos menos saludables y experimentan más eventos estresantes y respuestas emocionales menos reguladas (Friedman & Kern, 2014; Lahey, 2009). En línea con esta evidencia, los resultados de esta tesis mostraron que mayores puntuaciones en responsabilidad se relacionaron con un funcionamiento del eje HHA más saludable y con una mejor salud objetiva y subjetiva, tanto en hombres como en mujeres. Por lo tanto, la responsabilidad se considera un factor protector para la salud en hombres y mujeres mayores. Sin embargo, la responsabilidad no se relacionó con un menor declive cognitivo como se esperaba (Chapman et al.,

2012; Wilson et al., 2007b), posiblemente debido a que en un seguimiento de cuatro años, no ha transcurrido tiempo suficiente como para poder observar un deterioro cognitivo en personas mayores sanas.

Por el contrario, mayores puntuaciones en neuroticismo se asociaron a una desregulación del eje HHA, a una peor salud objetiva y subjetiva, y a un mayor declive cognitivo en atención mediado por una desregulación del eje HHA. Además, mayores puntuaciones en neuroticismo se relacionaron con un mayor declive cognitivo en memoria de trabajo mediado por una desregulación del eje HHA sólo en hombres, y a una peor salud objetiva (IMC y HbA1c) y subjetiva sólo en mujeres. Por lo tanto, el sexo es un importante moderador a tener en cuenta cuando se estudia la relación entre el neuroticismo y la salud y la cognición.

La extraversión se ha asociado con un mayor afecto positivo y bienestar, y salud percibida (Costa y McCrae, 1984; McCrae y Costa, 1991), pero también se ha relacionado con conductas tanto positivas (dieta saludable y ejercicio físico), como negativas (consumo de alcohol y tabaco) (Booth-Kewley y Vickers, 1994) para la salud. Los resultados de esta tesis mostraron que mayores puntuaciones en extraversión se relacionaron con una mejor salud subjetiva y objetiva en un solo índice (LDL - colesterol). Además, mayores puntuaciones en extraversión se relacionaron con un menor declive en función ejecutiva (a través de la reserva cognitiva) y memoria verbal demorada, pero también con un mayor declive en memoria verbal inmediata. Además, al considerar las diferencias de sexo, los resultados mostraron que una mayor extraversión estaba relacionada con una desregulación del eje HHA en los hombres,

mientras que en las mujeres se relacionó con un funcionamiento del eje HHA más saludable y con una mejor salud subjetiva. Por lo tanto, los resultados sugieren que la extraversión podría ser un factor de riesgo para la salud en hombres y tener efecto protector para las mujeres, lo que podría explicar los resultados inconsistentes encontrados por los estudios previos.

La apertura a la experiencia no se relacionó con ningún indicador de salud. Sin embargo, se observó una relación indirecta entre mayores puntuaciones en apertura a la experiencia y un menor declive en memoria verbal inmediata, a través de la reserva cognitiva. Los individuos con mayores puntuaciones en apertura a la experiencia tienden a participar en más actividades cognitivamente estimulantes, lo que contribuiría a una mayor reserva cognitiva y, por lo tanto, a enfrentar mejor los cambios cognitivos relacionados con la edad.

Por último, mayores puntuaciones en amabilidad sólo se asociaron a una mejor salud objetiva en un índice (mayores niveles de HDL-colesterol), por lo que parece que la amabilidad muestra poca relación con la salud en comparación con otros rasgos de personalidad. Por el contrario, mayores puntuaciones en amabilidad se relacionaron con un menor declive cognitivo en memoria declarativa verbal, respaldando la asociación observada entre la amabilidad y un menor riesgo de desarrollar EA (Terracciano et al., 2014).

En resumen, esta tesis muestra la importancia de abordar el estudio de los cambios cognitivos relacionados con la edad y, específicamente, de la relación entre el eje HHA y el funcionamiento cognitivo en personas mayores, considerando diferentes factores

genéticos (genotipo ApoE), situacionales (soledad) e individuales (personalidad). En líneas generales, esta tesis muestra que la posesión del alelo ApoE-ε4, la soledad percibida y el neuroticismo, son factores de riesgo para la salud y la función cognitiva en las personas mayores. Por el contrario, la posesión de los alelos ApoE-ε2 y ApoE-ε3, y la responsabilidad, apertura a la experiencia y amabilidad, pueden considerarse factores protectores para la salud y la función cognitiva. Finalmente, la extraversión parece tener tanto efectos beneficiosos y perjudiciales en la cognición, y puede considerarse un factor de riesgo para la salud en hombres, mientras que es un factor protector para la salud en mujeres mayores.

Finalmente, las conclusiones generales más importantes que se pueden extraer de esta tesis doctoral son las siguientes:

1. El alelo ApoE-ε2 allele puede considerarse un factor protector para la función cognitiva, concretamente para la capacidad de aprendizaje.
2. El alelo ApoE-ε3 está relacionado con una respuesta más adaptativa del eje HHA, con efectos beneficiosos sobre el rendimiento cognitivo (memoria declarativa verbal y atención). Por el contrario, el alelo ApoE-ε4 podría ser un factor de vulnerabilidad a los efectos adversos de la desregulación del eje HHA en la cognición (memoria declarativa verbal).
3. La soledad percibida está asociada con una desregulación del eje HHA (niveles más altos de cortisol antes de dormir), que, a su vez, se asocia con una peor función cognitiva.

4. La soledad percibida no está asociada directamente con la función cognitiva en adultos mayores sanos, sino que está indirectamente relacionada con un peor rendimiento cognitivo, a través de niveles más altos de cortisol antes de dormir. Por lo tanto, la desregulación del eje HHA aparece como uno de los mecanismos subyacentes que explican la relación entre la soledad percibida y el deterioro cognitivo.
5. El neuroticismo es un factor de riesgo importante para la salud en el envejecimiento, ya que se asocia con una desregulación del eje HHA, un deterioro de la atención a través de la desregulación del eje HHA, y una peor salud objetiva y subjetiva.
6. La responsabilidad es un factor protector importante para la salud en las personas mayores, ya que está asociado con un funcionamiento más saludable del eje HHA, y con una mejor salud objetiva y subjetiva. Sin embargo, no es un predictor de cambio cognitivo en adultos mayores sanos.
7. La extraversión podría ser un factor protector para el declive cognitivo de la función ejecutiva y del recuerdo demorado, pero favorece el declive de la memoria inmediata. La reserva cognitiva podría explicar parte de estos resultados.
8. La apertura a la experiencia y la amabilidad no son predictores importantes para la salud en las personas mayores, pero son factores protectores para el deterioro cognitivo en el recuerdo inmediato a través de la reserva cognitiva, y para el recuerdo demorado, respectivamente.

9. El sexo no modera la relación entre la soledad percibida y la desregulación del eje HHA y la función cognitiva.
10. El sexo es un moderador relevante de la relación entre el neuroticismo y la extraversión, y el declive cognitivo y/o la salud. Específicamente, el neuroticismo es un factor de riesgo mayor para el deterioro cognitivo en la memoria de trabajo en los hombres, y para la salud en las mujeres, mientras que la extraversión es un factor protector para la salud en las mujeres, pero un factor de riesgo en los hombres.

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