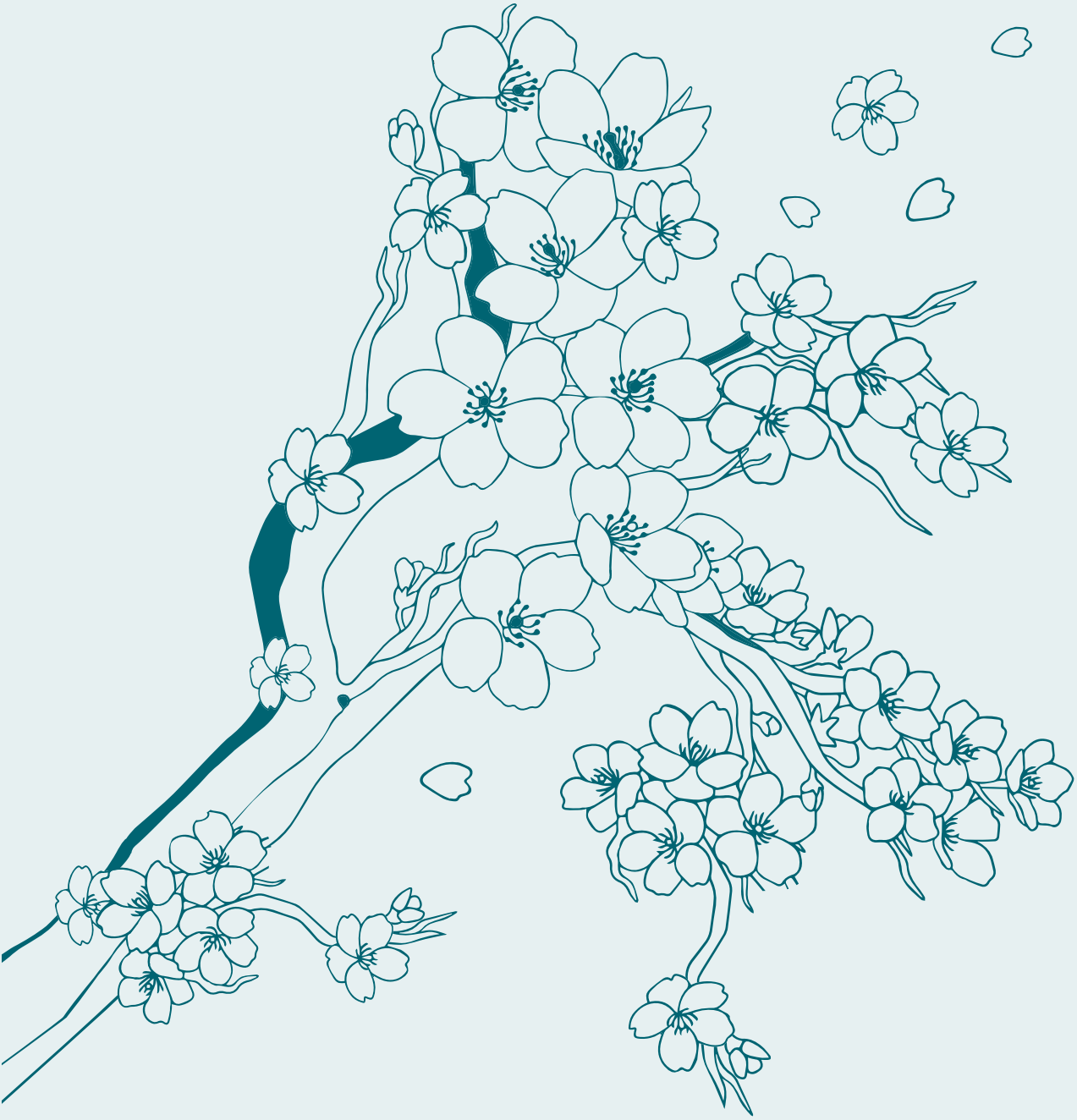
PhD Thesis by **Mariola Zapater Fajarí**

Importance of Psychological Factors in Adrenocortical Functioning and Subjective Memory Complaints

Promotors: Dra. Alicia Salvador Fernández-Montejo
and Dra. Vanesa Hidalgo Calvo

Valencia, December 2022
PhD Program in Neurosciences



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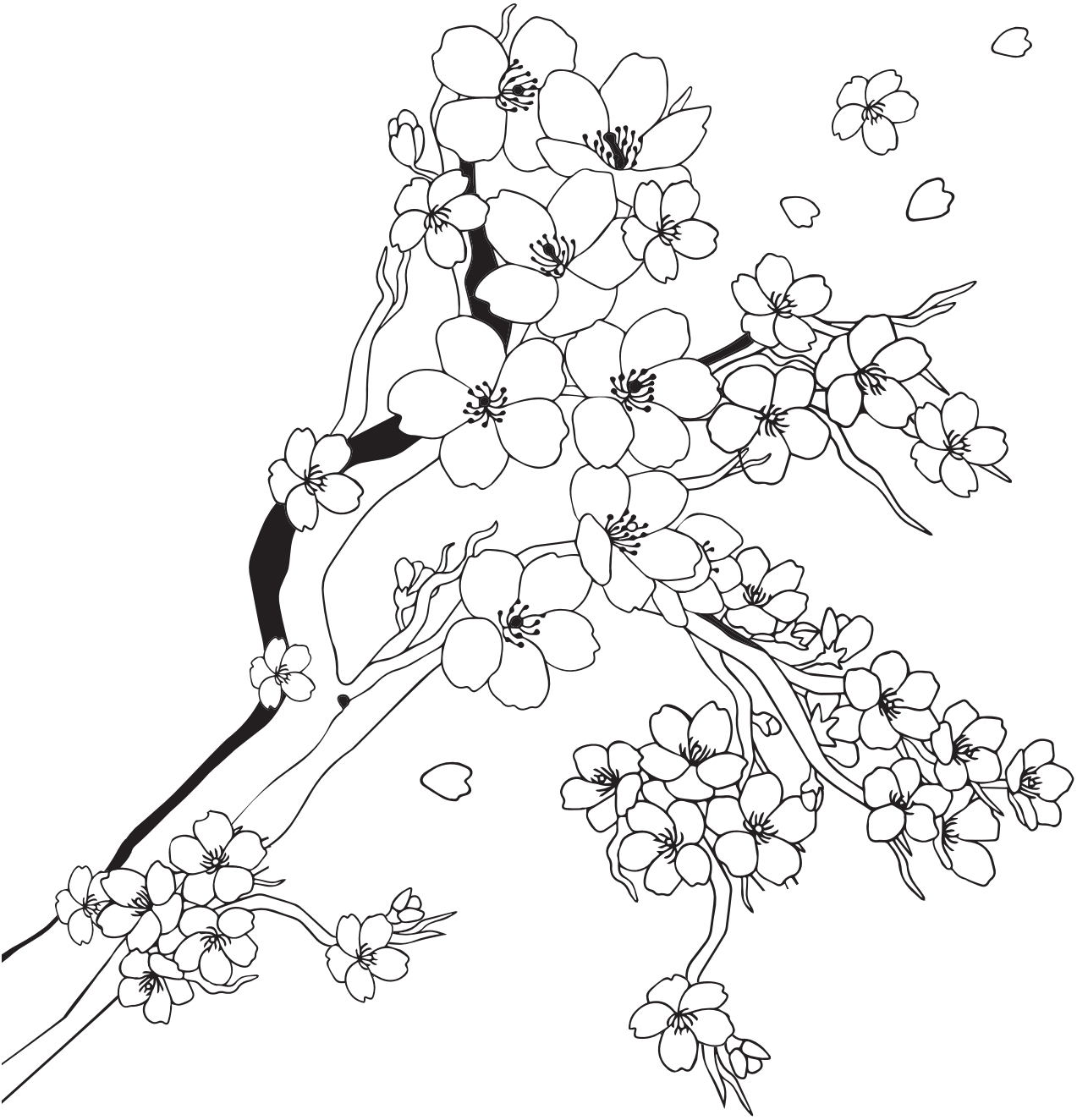

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Agradecimientos

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¡El camino no se acaba aquí!

Contents

Contents

Abbreviations

Abbreviations

ACC: Anterior Cingulate Cortex

AD: Alzheimer's Disease

AUCi: Area Under the Curve with respect to the increase

AUDIT: Alcohol Use Disorder Identification Test

Aβ: Amyloid-Beta

BAI: Beck Anxiety Inventory

BDI: Beck Depression Inventory

BMI: Body Mass Index

CAR: Cortisol Awakening Response

CDR: Clinical Dementia Rating

CD-Risc: Connor-Davidson Resilience Scale

CI: Confidence interval

COPE: Coping Orientations to Problems Experienced Inventory

CPRS: Comprehensive Psychopathological Rating Scale

CSF: Cerebrospinal Fluid

CVD: Cerebrovascular Disease

DCS: Diurnal Cortisol Slope

DHEA: Dehydroepiandrosterone

ELISA: Enzyme-Linked Immunosorbent Assay

FLAIR: Fluid-attenuated Inversion Recovery

GDS: Geriatric Depression Scale

HC: Hair Cortisol

HC:HDHEA$_{ratio}$: Cortisol:DHEA ratio in hair

HDHEA: Hair dehydroepiandrosterone

HPA axis: Hypothalamic Pituitary Adrenal axis

IAPS: International Affective Picture System

LLCI: Lower Level of Confidence Interval

LOT-R: Life Orientation Test Revised

LP: Lumbar Puncture

MADRS: Montgomery-Åsberg Depression Rating Scale

MCI: Mild Cognitive Impairment

MFE-30: Everyday Life Memory Failure Questionnaire

MMSE: Mini-Mental State Examination

MRI: Magnetic Resonance Imaging

OR: Odds Ratio

PCR: Polymerase Chain Reaction

POS: Positivity Scale

PSS: Perceived Stress Scale

p-tau: Posphorylated tau

SCD: Subjective Cognitive Decline

SCD-I: Subjective Cognitive Decline Initiative

SD: Standard Deviation

SE: Standard Error

SEM: Standard Error of the Mean

SES: Subjective Socioeconomic Status Scale

SMCs: Subjective Memory Complaints

SNP: Single Nucleotide Polymorphisms

SRB: Synonyms, Reasoning, and Block Design

STAI-S: State Anxiety Inventory

STAI-T: Trait Anxiety Inventory

TIA: Transient Ischemic Attack

TIV: Total Intracranial Volume

TSST: Trier Social Stress Test

UCLI: Upper Level of Confidence Interval

VIF: Variance Inflation Factor

WMSA: White Matter Signal Abnormalities

Thesis Outline

Thesis Outline

Stress has been considered one of the main problems in today's society, given that it has been related to several mental and physical health conditions, as well as age-related diseases and memory problems. However, there are individual differences in the way people face stressful situations. In this sense, some people, despite being exposed to the same levels of stress, do not develop pathologies, suggesting that certain psychological factors could act as protectors from the effects of stress. Resilience, which encompasses positive and optimistic thoughts, has been understood as the ability to face adversity and recover from stressful situations. More importantly, it has been proposed as an important factor associated with better health and successful aging. Among the age-related disorders, greater attention has been paid to cognitive decline and early dementia detection. The current priority is to study which factors could prevent or delay cognitive impairment. In this regard, Subjective Memory Complaints (SMCs) over time, or Subjective Cognitive Decline (SCD), have been indistinctly suggested as a proxy for early dementia detection in older people. However, these SMCs also appear in young individuals, increasing the need to determine which factors could be associated with this phenomenon at different ages. All of this highlights the importance of studying how certain psychological factors, especially protective factors, could be related to stress and SMCs across the lifespan, but especially in older people because it is a stage of greater vulnerability to both stress and memory decline.

In this doctoral thesis, the two main issues of concern in today's societies are addressed, that is, stress and early detection of dementia and how different psychological factors can affect them. For this purpose, psychological factors of resilience (positivity, optimism) and vulnerability (pessimism) have been related to different stress biomarkers (saliva and hair) and contexts (response to a stressor and

chronic stress) in healthy older people. Secondly, the relationship between SMCs and different vulnerability factors (anxiety and depression), protective factors (resilience and positivism), stress (basal functioning of the Hypothalamic Pituitary Adrenal axis [HPA axis]), and neurodegenerative biomarkers of Alzheimer's disease (AD) and Cerebrovascular Disease (CVD) have been addressed. This second general objective has also been addressed in different age groups (i.e., healthy young, middle-aged, and older people).

The *first chapter* is a general introduction that frames the studies contained in this doctoral thesis. It describes several psychological vulnerability (depression and anxiety) and protective (resilience, optimism, and positivity) factors and how they are related to stress. More precisely, it addresses the relationship between these psychological factors and the HPA axis, which is an important endocrine system related to stress and health. It points out the importance of studying how protective factors, little studied in the literature, can prevent stress-related disorders due to better HPA axis adjustment. The second part of this chapter describes the concept of SMCs and how they can be associated with non-degenerative causes such as stress and psychological factors in different periods of the lifespan. It reviews current literature on the association between SMCs and negative psychological factors of depression and anxiety in young, middle-aged, and older people. It also highlights the less studied association between stress and SMCs and the importance of studying positive psychological traits as protective of SMCs. Finally, it addresses the current discussion about the role of depressive symptomatology in SCD individuals and whether it is related to early brain pathology of AD or CVD in older people. Finally, in the last part, the aims and hypotheses of this doctoral thesis are presented.

In the following chapters, the five studies of this doctoral thesis are presented. Each study contains a brief introduction about the specific topic, the methodology used, the results, and a discussion of the main findings.

The *second and third chapters* describe the two studies in which we examine the role of psychological factors in the HPA axis measured in saliva and hair. Specifically, in the second chapter, we investigate how resilience is related to the psychobiological response (cortisol and anxiety reactivity) to a laboratory stressor, the Trier Social Stress Test (TSST), as well as the mediating role of coping strategies in this relationship. In the third chapter, we analyze the relationship between dispositional optimism and its subscales (optimism and pessimism) and chronic stress biomarkers measured in hair (cortisol and dehydroepiandrosterone [DHEA]).

In the *fourth and fifth chapters*, the two studies that focus on the relationship between psychological factors, the HPA axis, and SMCs in young, middle-aged, and older individuals are presented. The *fourth chapter* specifically addresses the relationship between depression, anxiety states, and the Cortisol Awakening Response (CAR) and SMCs. Moreover, it studies the relationship between resilience and SMCs through depression, anxiety, or the CAR. *The fifth chapter* investigates the relationship between trait anxiety, positivity, and basal HPA axis activity (awakening cortisol and Diurnal Cortisol Slope [DCS]) and SMCs in two different samples of young people and middle-aged and older people. It also tests whether the relationship between basal HPA axis activity and SMCs is moderated by psychological traits or age. The *sixth chapter* includes the fifth study, in which we investigate the relationship between depressive symptomatology, neurodegenerative biomarkers of AD and CVD, and SCD.

The *seventh chapter* includes a general discussion of the main findings found, limitations, strengths, and future directions. In addition, the main conclusions are presented in the *eighth chapter*. Finally, a brief summary of this thesis in Spanish is included in the last part of this dissertation, the *ninth chapter*.

Chapter I

General Introduction

General Introduction

Psychological Factors: From Vulnerability to Resilience to Stress

Depression, Anxiety, and Stress

Mental disorders are leading causes of the worldwide health-related burden, with approximately 500 million people living with any of these conditions (World Health Organization [WHO], 2022b; Global Health Data Exchange [GHDx], 2021). Therefore, increased importance has been given to the study of mental health and its repercussions on well-being.

Depression is characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration. Anxiety disorders, characterized by feelings of distress and fear, are common conditions that share comorbidity with depression (Diagnostic and Statistical Manual of Mental Disorders, DSM-5; American Psychiatric Association [APA], 2022). Current evidence suggests that depression and anxiety disorders are associated with several health problems and illnesses such as cardiovascular disease or diabetes mellitus, and they have an impact on physical and psychological well-being (Allgulander, 2016; Clarke & Currie, 2009; Engum, 2007). Given that depression and anxiety have increased dramatically since the world pandemic began, more than 25% since 2020, there is a need to deeply understand their causes and their relationship with health indicators in different contexts and ages, in order to create intervention programs to enhance the well-being of the population (COVID-19 Mental Disorders Collaborators, 2021).

Increased risk of depressive and anxiety symptomatology has often been associated with the hectic pace of life in the 21st century, commonly called stress

(Daviu et al., 2019; Hammen, 2005; McEwen, 2018). Stress is currently considered a main health problem due to its association with several mental and physical health conditions (O'Connor et al., 2021) and age-related diseases (Zsoldos et al., 2014). Stress can be interpreted as the noxious stimuli in the environment (stressor), as the extent in which a situation is appraised as threatening (stressful), or as the emotional or physiological response (stress response). The stress response entails the activation of cognitive, emotional, behavioral, and physiological systems (Campbell & Elhert, 2012). The first step entails the person's appraisal and perception of the situation (cognitive response). Thus, these perceptions predispose the individual to respond to the situation by performing specific tasks, strongly related to individual differences in coping behaviors (behavioral response). At an emotional level, people experience increases in state anxiety and negative affect (Campbell & Elhert, 2012; Villada 2014a; 2017). From a physiological perspective, the organism responds to environmental changes through the activation of physiological systems (i.e., the Autonomic Nervous System [ANS] and the Hypothalamic Pituitary Adrenal axis [HPA axis]) to adapt and respond to daily activities. This physiological stress response is adaptive, leading the individual to cope with the situation successfully and, thus, maintain internal homeostasis (Sterling & Eyer, 1988). However, as the *Allostatic Load Model* shows, when the resources the person needs to manage the situation exceed the energy the person has to deal with it, the pathology appears (McEwen, 1998). Thus, repeated or heightened activation due to chronic or acute stress leads to the allostatic load, and then to increased risk of physical and mental health problems, such as cardiovascular disease, cognitive problems, depression, or anxiety disorders (McEwen, 2018).

Resilience to Stress

Occasionally, some people do not perceive a situation that objectively seems stressful as threatening or dangerous due to some coping skills. In 2020, the WHO published a guide to help manage stress and face adversity, highlighting the importance of coping effectively with stressful situations (WHO, 2020). There is evidence that individual differences in the way people experience stressful situations can increase or reduce the chronic conditions associated with stress (Chida & Steptoe, 2009). Based on Lazarus and Folkman´s (1984) theory, the individual's appraisal of the situation and the resources to cope with it explains individual differences in the stress response. From this perspective, increased attention has been focused on the concept of resilience.

Resilience can be understood as a psychological trait where individuals understand stressful situations as a challenge, experiencing growth and adaptation rather than only recovery (Connor & Davidson, 2003). Resilient individuals recover from stressful experiences faster and more efficiently due to their characteristics, with this concept emerging from resilient materials that bend but do not break (Connor & Davidson, 2003; Fletcher & Sarkar, 2013; Reich et al., 2010). Resilient people are characterized by using active coping skills (Thompson et al., 2018) and having high levels of positivity, optimism, and positive emotions (Souri & Hasanirad, 2011; Tugade & Fredrickson, 2004). Based on the *Broaden and Build Theory of Positive Emotions* (Fredrickson, 1998), experiencing positive emotions during stressful situations can have an enhancing effect on the individual's personal resources, thus promoting health and well-being. Hence, positive emotions seem to help resilient individuals to bounce back from stress (Ong et al., 2006). Resilience has been related to higher life satisfaction and well-being (MacLeod et al., 2016), as well as a lower occurrence of depression and anxiety symptomatology (Bonanno, 2004; Hjemdal et al., 2006; Smith et al., 2016),

protecting people from adversity and, thus, promoting a successful aging process (Hildon et al., 2010; MacLeod et al., 2016). More work is needed on the relationship between positive psychological traits, rather than negative, and health or health-related behaviors, such as stress responsiveness, in order to enhance health and quality of life (MacLeod et al., 2016) and lower overall health care costs (Vieta et al., 2021).

With all of this in mind, people's individual differences in their psychological traits could explain differences in the stress response (e.g., physiological and emotional responses) and, thus, protect them from or prevent stress-related disorders. It has been suggested that a main mediating factor between stress and health is the HPA axis, and so the current need in this field is to determine which factors could modulate the HPA axis response and, thus, provide protection from diseases, including age-related diseases and/or cognitive decline.

The Hypothalamic Pituitary Adrenal Axis (HPA)

HPA axis functioning can be studied in the acute response to a stressor and in its basal activity (under normal or non-stress situations). Situations of acute stress trigger the stress response, overcoming the basal activity. However, the basal HPA activity can be used as an indicator of chronic stress exposure through two different measures: (i) in saliva using the diurnal cortisol pattern and specifically with two indexes: the Cortisol Awakening Response (CAR) and the Diurnal Cortisol Slope (DCS); (ii) and in hair with hair cortisol (HC) and dehydroepiandrosterone (HDHEA) (Adam & Kumari, 2009; Greff et al., 2019; Stalder et al., 2016; Ullmann et al., 2016). Hormonal levels in saliva represent a more momentary stress exposure, whereas hair samples cover a longer period of time (Stalder et al., 2012).

The Acute Stress Response

As mentioned above, one of the main components of the stress response, together with the ANS, is the HPA axis. When a situation is experienced as threatening or harmful, the paraventricular nucleus of the hypothalamus releases peptide hormones such as corticotrophin releasing hormone (CRH) and arginine vassopresin (AVP). These two hormones travel through the bloodstream via the portal system to the anterior pituitary gland, stimulating the release of the adrenocorticotropic hormone (ACTH). The ACTH arrives to the adrenal cortex through the circulatory system and stimulates the secretion of cortisol. Thus, the principal end-hormone secreted by the HPA axis is widely known as the stress hormone, cortisol (Nelson & Kriegsfeld, 2022). In laboratory situations, this HPA axis response is measured before and after acute stressors (typically expressed as a cortisol reactivity index). The most widely used psychosocial acute stressor is the Trier Social Stress Test (TSST) (Kirschbaum et al.,1993b), which induces a psychobiological stress response characterized by increased anxiety and negative affect as well as an increase in cortisol (Allen et al., 2014; Dickerson & Kemeny, 2004; Pulopulos et al., 2018). Following the *Cortisol Reactivity Threshold Model*, an adaptive stress response would be characterized by moderate rather than heightened levels of HPA axis reactivity, and moderate levels of negative emotions (Herman et al., 2016; Vrshek-Schallhorn et al., 2018).

In everyday life, we find a large number of situations related to work, relationships, and health that produce stress. In a moderate way, the HPA axis is adaptive, helping us to manage the stressful situation successfully (McEwen et al., 2019). However, a heightened and prolonged HPA axis response after acute stress over time has negative long-term consequences for memory (Allen et al., 2014; Lupien et al., 2018), well-being, and aging (McEwen et al., 2008), among others. Some studies

suggest that older people have a heightened and prolonged HPA axis response to stressors compared to young people, although more research is still needed (Pulopulos et al., 2018). In this line, higher acute HPA axis responsiveness has been found to predict future cognitive decline longitudinally in older people (de Souza-Talarico et al., 2020). Thus, it is interesting to delve into into possible individual differences among older people that could produce a more adjusted HPA axis response.

Circadian Cortisol Rhythm and Health

Cortisol secretion follows a circadian rhythm whose release increases during the night, with the highest concentration 30-45 minutes after waking; this increase is called the Cortisol Awakening Response (CAR). After peaking at 30-45 minutes, cortisol concentrations start to decrease until nighttime, with the lowest concentrations right before going to bed. This difference between wake-up and bedtime cortisol concentrations is called the Diurnal Cortisol Slope (DCS; Adam & Kumari, 2009; Fries et al., 2009; Stalder et al., 2016). These two cortisol indexes are studied better separately, given that the CAR has been highlighted as a distinct feature of HPA axis functioning related to different biological mechanisms and brain structures because it activates the organism through daytime action (Clow et al., 2010; Fries et al., 2009). Disruption of this cortisol circadian rhythm over time (typically lower CAR, flatter DCS, and higher overall cortisol during the day) has been associated with several health problems. Therefore, cortisol indexes have been considered objective health indicators (Adam et al., 2017; Dedovic & Ngiam, 2015; Lupien et al., 2009; Stalder et al., 2016). As occurs with the stress response, the basal cortisol function is altered in elderly individuals, showing heightened overall cortisol secretion, lower CAR, and flatter DCS (Heaney et al., 2012; Kudielka & Kirschbaum, 2003; Nater et al., 2013) compared to young individuals. These differences in cortisol secretion across the lifespan highlight

the importance of studying objective cortisol indicators of the HPA axis in different age populations, as well as their influence on health, given the relationship between basal HPA axis activity and stress-related disorders.

Measuring Steroid Hormones

Cortisol is released, in addition to its normal circadian rhythmicity, under situations of acute (e.g., TSST, acute work demand, public speech) and chronic (e.g., burnout, political conflicts, bullying) stress, and it is the main product of the HPA axis. There are different ways of measuring cortisol concentrations in different stress situations. Blood sampling offer the possibility of measuring cortisol levels at one time point, whereas urinary samples reflect overall cortisol secretion in no more than 24h. However, typically, saliva samples have been used to detect the day-to-day variations in cortisol concentrations by collecting saliva at different time points of the day, in order to detect both stress responses and circadian rhythmicity (e.g., the CAR or the DCS). Saliva sampling has mainly been chosen rather than blood or urinary samples, given that this sampling protocol is less invasive and easier to collect (Golden et al., 2011).

Although saliva sampling is good for capturing day-to-day variations in cortisol secretion, it has some limitations when information about more chronic stress exposure is needed. Thus, a large number of samples are required if the researcher wants to know the stress exposure over a one-month period. Another limitation of saliva sampling is that it is affected by several situational factors, such as food intake, exercising, smoking, etc. (Stalder & Kirschbaum, 2012). Taking all this into account, saliva samples are good at capturing acute cortisol changes in a less invasive way, but they are not a good sampling method when it comes to studying chronic stress exposure (Stalder et al., 2012).

In recent years, greater attention has been paid to the novel analysis of hair cortisol (HC) to measure long-term stress exposure (i.e., several months) (for a review see: Greff et al., 2019; Russell et al., 2012). Hair grows approximately 1cm/month; thus, the first 1cm segment from the scalp gives information about the cortisol production in the past month, and the next 1cm about the month before, allowing researchers to examine cortisol production retrospectively up to the past 6 months (Kirschbaum et al., 2009). The mechanism of cortisol incorporation into hair is not fully understood, although it has been suggested that cortisol enters the hair via diffusion from follicular blood capillaries to the medulla of the hair. Another explanation is that it can enter the hair via sweat and sebaceous glandular secretions or from a small synthesis in hair follicles from a local HPA-like pathway (Greff et al., 2019; Russell et al., 2012). However, the most widely supported hypothesis is the incorporation via the bloodstream (Stalder & Kirschbaum, 2012).

Another steroid hormone involved in stress regulation that has also been studied in hair is the dehydroepiandrosterone (DHEA). Although cortisol is the main end product secreted by the HPA axis, recently DHEA has also been incorporated as a reliable biomarker. The ACTH released by the pituitary gland not only stimulates the zona fasciculate of the adrenal cortex to release cortisol, but it also stimulates the zona reticularis to release DHEA. DHEA has an antagonist effect of cortisol, given that higher DHEA concentrations are translated into lower chronic cortisol secretion (Kalimi et al., 1994; Kamin & Kertes, 2017). Thus, DHEA has been related to better coping with stress due to its antiglucocorticoid effect (Kalimi et al., 1994). Although less studied, hair DHEA (HDHEA) has been related to lower physical stress and stress perception (Ullman et al., 2016), and together with HC, it is increasingly becoming a proxy of chronic stress (Bürgin et al., 2020; Hennessey et al., 2020).

Based on the above, HC and the newly studied HDHEA have been found to be good biomarkers of stress-related and chronic conditions and, therefore, long-term stress exposure (Bürgin et al., 2020; Manenschijn et al., 2011; Ullmann et al., 2016). Therefore, future research should aim to know to what extent HC and HDHEA can be changed or modulated, given the influence of chronic stress on health (Stalder et al., 2017), especially in older samples because older ages are at greater risk of developing stress-related disorders mainly associated with long-term stress exposure. In this regard, it has been found that HC is higher as participants' age, with older people being more prone to experiencing the negative consequences of chronic exposure (Feller et al., 2014). DHEA levels have mainly been investigated in blood and saliva, showing that DHEA concentrations also have an effect on aging (Kamin & Kertes, 2017), with an 80% decline in DHEA concentrations at about the age of 60 (Stárka et al., 2015). Given the beneficial effects of DHEA on neuroprotection, neurite growth, and anti-inflammatory and anti-glucocorticoid effects, it is of great importance to study the related factors in this age population (Maggio et al., 2015). The study of HDHEA will, therefore, expand current knowledge about DHEA in aging, focusing on chronic stress.

Resilience and HPA Axis

The relationship between negative psychological traits and the HPA axis has been widely studied. In this line, depression and anxiety have both been related to a dysregulation of the basal HPA axis activity (Adam et al., 2017; Chida & Steptoe, 2009). Specifically, depressive symptomatology has been related to a flatter DCS, whereas anxiety has been related to a steeper DCS (Adam et al., 2017). However, mixed results have been published regarding the CAR, with positive, negative, and null associations (Chida & Steptoe, 2009; Dedovic & Nigam, 2015; Steptoe & Serwinski, 2016). In addition to the associations between the HPA axis and negative psychological

traits, there is growing interest in the role of positive or protective psychological traits in HPA axis functioning, in order to determine which traits can reduce the negative consequences of stress on physical and psychological health (McEwen, 2018). Particularly, studying which factors could result in a more adaptive stress response in older individuals would help to slow down stress-related disorders in a population in which such the consequences could be more harmful. Stress in this period of the lifespan has been correlated with negative health outcomes, such as higher risk of mood, anxiety, or stress-related disorders, cardiovascular disease, hypertension, rheumatoid arthritis, decreased immunity, among other chronic conditions common in later life (Gaffey et al., 2016). It is important to keep in mind that the world's population over 60 years old is increasing dramatically and will reach approximately two billion by 2050, and so the United Nations declared 2021-2030 the UN Decade of Healthy Aging, seeking to reduce health inequities and improve the lives of older people (WHO, 2022a).

Resilience has been proposed as an important factor related to the HPA axis due to its characteristics and definition. However, research is scarce and has focused on a wide range of populations and ages. Regarding basal HPA axis activity, resilience has been related to higher CAR and steeper DCS in undergraduate students (Lai et al., 2020), a pattern related to better coping with chronic stress. Lower waking levels and lower overall cortisol levels 60 min after waking were found in resilient autism caregivers (Ruiz-Robledillo et al., 2014). In children with parents with Human Immunodeficiency Virus (HIV), higher resilience was associated with higher waking and diurnal levels and a steeper DCS (Chi et al., 2015). However, in the case of acute stress exposure, to date only two studies have investigated the relationship between resilience and the cortisol response to stress (Mikolajczak et al: 2008; Ruiz-Robledillo

et al., 2017). They found that more highly resilient individuals secreted less cortisol in anticipation of a stressor compared to less resilient healthy young men (Mikolajczak et al., 2008). In adult men and women who were autism caregivers, highly resilient autism caregivers showed lower cortisol secretion during a stressor (Ruiz-Robledillo et al., 2017). However, no study has specifically investigated the relationship between resilience and the psychobiological response to stress in older people. Therefore, the first study of this thesis was proposed to answer this question.

An ambitious goal in the study of resilience is to investigate the possible mechanisms through which resilient individuals face adversity (Thomson et al., 2018). It has been suggested that resilient individuals experience positive emotions to bounce back from emotional experiences, as stated in the *Broaden and Build Theory of Positive Emotions* (Fredrickson et al., 1998). The concept of resilience involves the use of positive emotions in stressful situations, which encourages these individuals to develop more active and less avoidant coping strategies (Gloria & Steinhardt, 2016). Active coping corresponds to the use of adaptive coping strategies (i.e., active coping, planning and suppression of competing activities) in response to stress. In contrast, avoidance coping entails the use of maladaptive or passive coping strategies (i.e., behavioral disengagement, mental disengagement, denial, and religious coping), often resulting in undesirable consequences (Carver et al., 1989; Hasking & Oei, 2002). Whereas active coping results in better physiological and psychological adjustment in stressful situations (Villada et al., 2016; 2017), avoidant coping strategies predict anxiety and depressive symptoms (Mahmoud et al., 2012), psychological distress (Wu et al., 2013), and poorer cortisol recovery after a stressor (Villada et al., 2017). Thus, resilience seems to influence the appraisal of the situation, whereas coping is defined as the active or passive mobilization of sources (behavioral and cognitive) to cope with the adversity

after this appraisal (Fletcher & Sarkar, 2013). Resilient individuals seem to employ active coping strategies to deal with stress demands (Li et al., 2018; Smith et al., 2016; Thompson et al., 2018), which may be a mechanism through which they reach well-being at older ages (Mayordomo et al., 2016; Tomás et al., 2012).

More active and less avoidant coping strategies have been related to a better psychobiological response to stress (Villada et al., 2016; Wu et al., 2013) and higher resilience (MacLeod et al., 2016; Thomson et al., 2018). However, the study of the relationship between resilience and the psychobiological response to a stressor in older people who use more active and less passive coping strategies remains understudied. This issue was addressed in the first study of this doctoral thesis.

Positivity, Optimism, and HPA axis: Chronic Stress Biomarkers

Positivity and optimism have arisen as two important components of resilience (Milioni et al., 2016; Souri & Hasanirad, 2011; Yu & Zhang, 2007). Positivity, or a positive perception about oneself, one's life, one's future, and one's confidence in others, also encompasses optimistic thoughts, given that optimism is defined as positive thinking about the future (Caprara et al., 2010). Together, positivity and optimism have been defined as psychological traits, given their relatively stability, and they have been related to the HPA axis (Endrighi et al., 2011; Lai et al., 2005; Pasquali et al., 2020). The *Positive Orientation Theory* states that the internal characteristics of positive individuals make them more likely to cope successfully with stressful situations (Caprara et al., 2010). More precisely, it seems that positive expectancies about the future (i.e., high dispositional optimism) play a key role in stress responsiveness (Puig-Perez et al., 2021) and may protect one from disease (Carver et al., 2010). Traditionally, dispositional optimism has been considered a psychological trait with two opposite

poles (optimism vs. pessimism). However, it has also been argued that rather than being a unidimensional construct, it might be two separate dimensions (optimism vs. pessimism) (Carver et al., 2010; Ferrando et al., 2002; Mroczek et al., 1993; Robinson-Whelen et al., 1997). Specifically, in older people, some authors have recommended understanding optimism and pessimism separately (Puig-Perez et al., 2015; 2018; Rasmussen et al., 2009) because older people, given the meta-cognitive changes in adulthood, can use optimism or pessimism perspectives adaptatively depending on the situation (Puig-Perez et al., 2021). Following the *Self-Regulatory Behavior Theory* (Scheier & Carver, 2000), these positive and negative expectancies about the future may influence the way they cope with stress (Nes & Segerstrom, 2006). Highly optimistic people tend to be more confident when facing life challenges and more persistent in achieving their goals due to their expectations of success, making a greater use of adaptive coping strategies. In contrast, pessimistic people tend to be more hesitant about success and have negative expectations about the future (Carver et al., 2010; Scheier & Carver, 1992; Scheier et al., 1994; Nes & Segerstrom, 2006). Thus, optimism has been related to greater physical and psychological health (Puig-Perez et al., 2021), possibly due to greater resilience to stress (Gallagher et al., 2020).

Low positivity has been found to be associated with higher awakening cortisol levels in healthy young people (Pasquali et al., 2020). Additionally, optimism has been related to a lower CAR in healthy young people (Lai et al., 2005) and older people (Endrighi et al., 2011). However, Ebrecht et al. (2014) did not find a significant relationship between optimism and the CAR in healthy young people. In contrast, pessimism has not been related to HPA axis functioning (Endrighi et al., 2011; Lai et al., 2005). Our group previously tested the relationship between optimism, pessimism, and the stress response to the TSST and the circadian rhythm. We found that optimism

was related to faster recovery after a stressor (Puig-Perez et al., 2015). Our results also showed a negative relationship between the CAR and positive emotions and cognitions, although a significant relationship with optimism was not found (Puig-Perez et al., 2018). In addition, pessimism did not appear to be significantly related to any HPA axis index (Puig-Perez et al., 2015; 2018). Taking all this into account, based on the inconclusive results about the association between optimism and pessimism with stable cortisol measures, a current gap in the field requires studying more chronic stress biomarkers and the extent to which they can be related to more positive psychological traits in order to achieve resilience to stress and, therefore, health (Rasmussen et al., 2009). In this regard, hair sampling seems to be a good method to capture adrenal activity in the previous months in healthy participants (Stadler et al., 2012).

Two recent studies have investigated the relationship between HC, HDHEA, or the HC:HDHEAratio and resilience and factors related to resilience (Bürgin et al., 2020; García-León et al., 2019). On the one hand, these authors found that resilience was related to lower HC in young people (García-León et al., 2019). On the other hand, measures related to resilience, which were sense of coherence and self-care, were both positively related to HDHEA levels and a lower HC:HDHEAratio in young and older people (Bürgin et al., 2020). As far as we know, only one study investigated the relationship between optimism and HC, showing that high optimism was associated with lower HC levels (Milam et al., 2014).

It is important to study what factors could be related to lower HC and higher HDHEA, especially in the elderly population, due to the effects of chronic stress on health and aging (Feller et al., 2014; Maggio et al., 2015). In fact, due to the positive effects of the DHEA and the negative effect of cortisol on health and wellness, higher levels of HDHEA and lower levels of HC at these ages may be associated with healthy

aging (Kamin & Kertes, 2017; Maggio et al., 2015). To our knowledge, no previous study has investigated the relationship between optimism and pessimism and chronic stress biomarkers measured in hair samples in healthy older people. This issue was covered in the second study of this doctoral thesis.

HPA axis and Subjective Memory Complaints (SMCs)

Repeated stress can affect brain function, especially memory processes, due to the hyper activation of the HPA axis and the subsequent effect of cortisol on the hippocampus, among other brain regions. This brain structure, which has a high concentration of cortisol receptors, is one of the main areas involved in memory processing and emotional regulation (Lupien et al., 2018; McEwen et al., 2008), along with the amygdala and prefrontal cortex. Dementia is currently the seventh cause of death among all diseases and one of the major causes of disability in today's society, with an estimated 55 million people living with this condition (WHO, 2022a). Thus, a current priority is to identify strategies to prevent or delay cognitive decline or dementia, given its impact on health and, therefore, on society and economy (Anstey et al., 2013). Perceptions of repeated forgetfulness about everyday aspects, known as Subjective Memory Complaints (SMCs), have been suggested as an important proxy for early dementia detection and cognitive health (Sunderland et al., 1986). The perception of SMCs over time, or a perception of cognitive decline that is not accompanied by objective impairment, is currently defined as Subjective Cognitive Decline (SCD) (Jessen et al., 2014). Both concepts have been widely and indistinctly used in older samples. The leading international SCD Initiative (SCD-I) has developed a conceptual framework in which they address several issues related to the complexity of the concept. They suggest that at older ages (around 60 years old and above), SCD is more associated with Alzheimer's Disease (AD) and neurodegenerative processes, whereas at

younger ages, other different factors (e.g., medical conditions, psychiatric conditions, personality traits, and exposure to stress) might be more important (Jessen et al., 2014; 2020). SMCs can be viewed as a vicious circle in which the perception of memory loss can cause persistent distress, which leads to HPA axis dysfunction, resulting in brain changes and memory problems (Peavy et al., 2013). Only a few studies have addressed the correspondence between SMCs and HPA axis dysregulation (Fiocco et al., 2006; Peavy et al., 2013; Wolf et al., 2005). They found that SMCs were associated with a flatter DCS (Fiocco et al., 2006), higher diurnal cortisol concentrations, a lower CAR (Peavy et al., 2013), and higher 12h urinary cortisol concentrations (Wolf et al., 2005) in healthy older people. To note, it has been found that SMCs are common in the elderly, but also in younger adults (Derouensé et al., 1999; Ginó et al., 2010; Mendes et al., 2008). Given the change in HPA axis functioning across age (Nater et al., 2013), it is also important to study how HPA axis indicators could be related to SMCs in different age populations. To date, no study has investigated whether the HPA axis is related to SMCs in young people. Hence, the third study in this doctoral thesis was proposed to answer this question. The fourth study in this doctoral thesis also covered this issue in different age groups. Additionally, given the possible different meanings of SMCs in middle-aged and older people before and after 65 years of age, the fourth study in this doctoral thesis also investigated the relationship between HPA axis functioning and SMCs in people under and over the age of 65.

Psychological Factors and Subjective Memory Complaints (SMCs)

Negative Affective States and SMCs

Psychological factors related to stress have also been associated with the appearance of SMCs. The relationship between negative affective states, such as depression and anxiety, and the presence of SMCs has been highly studied in mixed-aged samples (Derouesné et al., 1999; Montenegro et al., 2013; Pellicer-Porcar et al., 2014; Söğütlü & Alaca, 2019). All these studies concluded that a greater number of complaints are related to higher levels of depression and anxiety (Derouesné et al., 1999; Montenegro et al., 2013; Pellicer-Porcar et al., 2014; Söğütlü & Alaca, 2019). The low motivation and energy shown by depressed individuals, as well as the attentional problems exhibited by anxiety states, could explain this relationship (Weaver Cargin et al., 2008). However, a current gap remains in elucidating which affective state could be the most important for SMCs. Some studies have suggested that depression is a better predictor than anxiety (Derouesné et al., 1999; Montenegro et al., 2013), although Pellicer-Porcar et al. (2014) stated the opposite. Taking into account that SMCs also appear in young people, few studies have been carried out to clarify the relationship between affective states and SMCs in younger samples (Loprinzi, 2019; Pearman, 2009). Shedding light on which factors are related to the presence of SMCs in younger populations could help to manage interventions to slow down their appearance. The third study in this doctoral thesis investigated the relationship between negative affective states (i.e., depression and anxiety state) and their weight in predicting SMCs in a sample of young individuals.

Negative Traits and SMCs

Stable psychological factors have also been found to be important predictors of SMCs (Carrigan & Barkus, 2016; Pearman & Storandt, 2004; Pearman et al., 2009). Trait anxiety is considered a potential stable factor related to more SMCs at all ages (Balash et al., 2013; Mahoney et al., 1998; Mecacci et al., 2004; Norman et al., 2020; Pavisic et al., 2021; Sabatini et al., 2022). Different from state anxiety, which could be viewed as a transitory emotional state caused by a threatening situation, trait anxiety is defined as a stable anxious propensity that causes people and situations to be perceived as more threatening (Daviu et al., 2019). Thus, trait anxiety makes people more vulnerable to stress conditions, and it also affects attention and memory processes (Eysenck, 2007). Based on the *Attentional Control Theory* (Eysenck et al, 2007), anxious people are more prone to being distracted and put their attentional focus on internal and external stimuli such as worrisome thoughts or threatening task-irrelevant distractors (Eysenck et al., 2007). A current limitation in the field is that it is still necessary to study the relationship between trait anxiety and SMCs across the life span, given the possible different explanations for SMCs depending on age. To date, only one study has addressed this issue in young (18-39 years), early middle-aged (40-54 years), middle-aged (55-64 years), older (65-74 years), and oldest-old (75-99 years) people (Rowell et al., 2016). The fourth study in this doctoral thesis will add evidence about the relationship between trait anxiety and SMCs considering the age factor. Additionally, attention has been focused on elucidating to what extent psychological variables could interact with stress biomarkers, in order to slow down their effects on health. Given the relationship between anxiety and the HPA axis (Adam et al., 2017), and between the HPA axis and SMCs (Fiocco et al., 2006; Peavy et al., 2013; Wolf et

al., 2005), the fourth study included in this doctoral thesis also looked into the moderation effect of trait anxiety in the relationship between HPA axis and SMCs.

Positive Traits and SMCs

The study of positive psychological traits in relation to SMCs and how they could act as protectors against their appearance or development is still incipient. Taking the aforementioned important relationship between stress and SMCs into account (Fiocco et al., 2006; Peavy et al., 2013; Wolf et al., 2005), resilience emerges as an important factor related to coping with stress and adversity that could be negatively related to SMCs (VanMeter & Cicchetti, 2020). Positivity also appears as a factor associated with resilience that facilitates coping with stress (Caprara et al., 2010; 2012). Given the positive association between stress and stress perception and SMCs, and the negative association between resilience and positivity and stress, it makes sense to study these positive psychological traits in relation to SMCs. Only one study has addressed the relationship between resilience and SMCs and found that one resilience factor in particular (trust in one's own instincts) was negatively associated with SMCs (Montejo-Carrasco et al., 2013). Other resilience related factors have been studied in relation to SMCs. Thus, higher positive coping, positive affect, extraversion, and optimism have been related to fewer SMCs (Fastame, 2022; Lee et al., 2016; Molina-Rodriguez et al., 2016; Sutin et al., 2020). More interestingly, no study has investigated whether these positive psychological traits could be related to SMCs through the mediation or moderation of stress-related factors such as negative affective states or the HPA axis. Considering the scarce literature on the association between positive traits and SMCs and the need to corroborate whether these psychological traits can interact with other factors associated with SMCs (i.e., affective states and HPA axis), the third and fourth studies in this doctoral thesis covered this gap on the literature. More precisely, the third

study investigated the relationship between resilience and SMCs through depressive symptoms, state anxiety, and the HPA axis in a young population. The fourth study investigated the relationship between positivity and SMCs, as well as the moderation of positivity in the relationship between the HPA axis and SMCs, in two different samples of young and older individuals.

Depression, Subjective Cognitive Decline, and Brain Pathology in Aging

The appearance of SMCs after 60 years of age is an important risk factor to take into account in the relationship between SCD and cognitive decline, given that at these ages SCD is greatly associated with brain pathology and neurodegenerative causes (Jessen et al., 2020). The National Institute on Aging-Alzheimer's Association (NIA-AA) classification system places SCD at the second stage of the preclinical phase of AD. At this stage, there is positivity in AD biomarkers, but the cognitive impairment is still not present (Jessen et al., 2020; Sperling et al., 2011). Thus, SCD in later stages of life has been related to AD pathology biomarkers, such as amyloid beta (Aβ) and tau biomarkers (Amariglio et al., 2012; Buckley et al., 2017). However, SCD can also be associated with other neurodegenerative causes, such as Cerebrovascular Disease (CVD) (Cedres et al., 2019; 2021; Diaz-Galvan et al., 2021a; Diniz et al., 2013; Minett et al., 2005). As mentioned above, depressive symptomatology has been strongly related to SCD (Hill et al., 2016). Currently, the SCD-I has raised a main concern regarding the role of depressive symptomatology in patients with SCD (Jessen et al., 2020). A few studies have also found a relationship between depression and neurodegenerative factors (Diniz et al., 2013; Pomara et al., 2016; Taylor et al., 2013). Therefore, the current question is to clarify whether the depressive symptomatology found in SCD individuals is due to neurodegenerative factors or not. Previously, a study investigated the relationship between depressive symptomatology and CVD in SCD, and the results

showed that SCD was independently related to depressive symptomatology and CVD. Depressive symptomatology was not associated with CVD, but biomarkers of AD pathology were not included (Diaz-Galvan et al., 2021a). The fifth and last study in this doctoral thesis extended this work by investigating the relationship between SCD, depressive symptomatology, and CVD and AD pathology biomarkers. We also tested whether depressive symptomatology found in SCD was associated with brain pathology (CVD, Aβ, and tau). Additionally, some studies have differentiated types of SCD: (i) SCD in memory (SCD-memory), by using the presence of SMCs and (ii) SCD in concentration (SCD-concentration), by using Subjective Concentration Complaints (Grambaite et al., 2013; Topiwala et al., 2021). A recent study showed that different complaints were associated with different Magnetic Resonance Imaging (MRI) profiles and depressive symptomatology, but they did not include cerebrospinal biomarkers of AD (Aβ and tau) (Diaz-Galvan et al., 2021b). The fifth study in this thesis tested the aforementioned associations for both memory and concentration complaints.

Aims and Hypotheses

Overall, the literature described in this first chapter highlights the current gaps in the study of several psychological factors and the HPA axis and how they could be associated with SMCs. The central aim of this doctoral thesis was to clarify the role of different psychological factors of vulnerability and resilience in two important contexts of today's society, that is, stress and early dementia detection or cognitive health.

The general aims of this doctoral thesis were twofold:

1. To study the relationship between positive psychological traits (resilience and optimism) in both the acute and chronic stress response of the HPA axis in the

older population. This aim was addressed specifically in the first and second studies in this thesis.

2. To investigate the role of positive (resilience and optimism) and negative psychological traits (anxiety and depressive symptomatology), the HPA axis and neurodegenerative biomarkers in SMCs in different age populations. The third, fourth, and fifth studies in this thesis were designed to cover this aim.

To do so, we designed and carried out five studies with the following specific aims and hypotheses:

Study 1

The aim of this study was to examine the relationship between resilience and the psychobiological stress response (cortisol and anxiety reactivity) in healthy older people. Moreover, we wanted to investigate whether coping strategies (active and passive) mediated the association between resilience and endocrine and psychological responses to acute stress.

We expected a negative relationship between resilience and both cortisol and anxiety reactivity. Additionally, we hypothesized that the negative relationship between resilience and the psychobiological stress response would be mediated by more active and less passive coping strategies. That is, higher resilience would be related to more active and less passive coping strategies, and this would be translated into a more adaptive stress response (moderate cortisol and lower anxiety reactivity).

Study 2

The main objective was to analyze the relationship between dispositional optimism and its subscales of optimism and pessimism, and chronic stress biomarkers measured

in hair (HC, HDHEA, and their ratio HC:$HDHEA_{ratio}$) in healthy older people. Finally, we wanted to explore sex differences in HC, HDHEA, and HC:$HDHEA_{ratio}$ concentrations, as well as in their relationship with psychological traits (optimism and pessimism).

We expected that people who are higher in optimism and lower in pessimism would show a lower HC and HC:$HDHEA_{ratio}$, and higher HDHEA. Moreover, we hypothesized that men would have a higher HC and HC:$HDHEA_{ratio}$ and lower HDHEA compared to women. We also expected to find a positive association between optimism and HDHEA only in women.

Study 3

The purpose of this study was to focus on the role of resilience, affective states, and HPA axis in SMCs of young healthy people. First, we wanted to confirm the relationship between some affective states (depression and anxiety) and SMCs, and clarify which affective state had the greatest weight in predicting SMCs. Additionally, we wanted to explore the relationship between basal HPA axis functioning (CAR) and SMCs. And finally, the main goal was to find out whether resilience was negatively related to SMCs through the mediation of depression, anxiety, and the CAR.

According to the available evidence, we expected that depression and anxiety would be positively related to SMCs, with depression being the factor the most strongly related to SMCs. As no previous study had assessed the relationship between the HPA axis and SMCs in young people, and given the age differences in the HPA axis, we did not make any hypotheses. Finally, we hypothesized that the negative association between resilience and SMCs would be due to lower depression and anxiety and a greater CAR experienced by resilient individuals.

Study 4

The aim of the fourth study was to test, in two independent samples of young and older people, the relationship between stable psychological traits, such as trait anxiety and positivity, and SMCs. We also wanted to study whether the relationship between basal HPA axis functioning (awakening cortisol and DCS) and SMCs was direct or moderated by these psychological traits.

We expected to find that greater anxiety and lower positivity would be related to more SMCs in both young and older people. Regarding the relationship between the HPA axis and SMCs, in young people we could not establish any hypotheses about the direct association due to the lack of studies. We expected that in individuals with higher anxiety and lower positivity, higher awakening cortisol and steeper DCS would be related to more SMCs. In older people, we expected a direct negative relationship between DCS and SMCs. Moreover, as no previous study had investigated the relationship between awakening cortisol and SMCs in young people, as we hypothesized in young people, higher awakening cortisol would be associated with more SMCs in people with high anxiety and low positivity. Additionally, given the impact of aging on HPA functioning, as well as the suggested differences in SMCs in people under and over 65 years of age, we explored the relationship between psychological traits, HPA axis biomarkers, and SMCs in these two age groups (aged 55 to 64 vs 65 to 75).

Study 5

The main objective of this study was to test the role of depressive symptomatology and biomarkers of brain pathology (CVD, Aβ42/40 and p-tau) in SCD individuals. We wanted to find out whether depressive symptomatology in SCD was due to brain

pathology. Additionally, recent findings suggest that different complaints are associated with different MRI-based biomarker profiles and depressive symptomatology. Thus, we wanted to confirm these differences by including CSF biomarkers of AD pathology in two types of complaints, memory and concentration complaints. Therefore, our second aim was to investigate memory and concentration complaints separately in relation to brain pathology (CVD, Aβ42/40 and p-tau) and depressive symptomatology.

Based on the results of previous studies, we hypothesized that SCD would be related to both depressive symptomatology and to biomarkers of AD and CVD. Moreover, depressive symptomatology would also be related to AD and CVD biomarkers. Additionally, these relationships would be different depending on the type of complaint. According to previous literature, memory complaints would be more strongly associated with AD biomarkers, whereas concentration complaints would be more strongly associated with CVD biomarkers. Finally, based on the strong relationship between depressive symptomatology and SCD, we expected that memory and concentration complaints would both be related to depressive symptomatology.

Chapter II

Study 1: Impact of resilience and active coping strategies on the psychobiological response to stress in healthy older people

The main results of this study have been published in:

Zapater-Fajarí, M., Crespo-Sanmiguel, I., Pulopulos, M. M., Hidalgo, V., & Salvador, A. (2021). Resilience and Psychobiological Response to Stress in Older People: The Mediating Role of Coping Strategies. *Frontiers in aging neuroscience*, *13*, 632141. https://doi.org/10.3389/fnagi.2021.632141

Introduction

Stress is considered one of the most significant health problems of the 21st century (World Health Organization [WHO], 2022c), due to its contribution to numerous disorders, such as depression and sleep problems (Vos et al., 2016), and several age-related diseases (Zsoldos et al., 2014). Current research has focused on which personality or coping factors could explain individual differences in stress responsiveness, and thus provide an explanatory approach of the protective or vulnerability factors to the psychobiological effects of the stress response.

At a physiological level, the stress response is mainly characterized by the activation of two physiological systems: the autonomic nervous system, which increases blood pressure and heart rate (Allen et al., 2011), and the Hypothalamic-Pituitary-Adrenal axis (HPA axis), with the consequent release of cortisol (McEwen, 2008). Furthermore, when exposed to stress, people experience emotional changes, such as increases in anxiety (Campbell & Ehlert, 2012; Fan et al., 2015; Villada et al., 2014a) and negative affect and decreases in positive affect (Schmaus et al., 2008; Villada et al., 2017). Moreover, over-reactivity of the HPA axis and heightened psychological reactivity (i.e.,increased negative affect and anxiety) have been associated with increased risk of several health problems, such as cardiovascular disease, Type 2 diabetes, reduced immune function, and cognitive impairment (Kiecolt-Glaser et al., 2003; Lundberg, 2005). The *Allostatic Load Model* explains the physiological effects underlying these conditions, given that a repeated or heightened HPA axis activation could led to the activation of the fight or flight response systems (i.e., cardiovascular, muscular, among others) resulting in an allostatic load, and thus multiple health problems (McEwen, 1998; Sterling & Eyer, 1988). Therefore, following the *Cortisol Reactivity Threshold Model* (Vrshek-Schallhorn et al., 2018), the more adaptive stress

response would be characterized by moderated HPA axis activation, rather than increased HPA axis reactivity (Herman et al., 2016), and stable levels of negative emotions such as anxiety (Kiecolt-Glaser et al., 2002).

Much of this research, however, has focused on stress reactivity in young adults, even though older adults are more vulnerable to the chronic conditions associated with stress (for reviews, see: Kudielka et al., 2009; Pulopulos et al., 2018; Uchino et al., 2005). Studies with older adults have found that they tend to show a stronger cortisol response to stress and worse regulation of the HPA axis under stressful conditions (Pulopulos et al., 2018). Furthermore,most of the aforementioned health problems associated with stress have also been found to be related to the aging process (e.g., Type 2 diabetes or cognitive impairment) (Kiecolt-Glaser et al., 2003; Lundberg, 2005). Therefore, it is important to investigate the factors that can explain individual differences in the stress response of older people, in order to develop prevention programmes and intervention targets, and consider the role of the stress response in the development of health problems in this population (MacLeod et al., 2016).

There are important individual differences in the way individuals face stressful situations, which could determine their psychobiological response and, therefore, their risk of stress-related diseases. As Lazarus and Folkman (1984) indicated in their stress and coping model, individual differences in the stress response depend on both the subject's appraisal of the situation and the resources available to manage the stress. In this line, according to the broaden and build theory of positive emotions (Fredrickson, 1998), having a positive appraisal of the situation, by experiencing positive emotions even in stressful situations, broadens an individual's thought-action repertoire and may have the effect of enhancing his or her personal resources, including physical, intellectual, and social resources, thus promoting health and well-being.

In recent years, research has shown that resilience is strongly associated with an aging process characterized by low risk of disease and high mental and physical functioning (for a review on this topic, see MacLeod et al., 2016). Resilience is also considered a key factor in the optimal development of health and quality of life (Hjemdal et al., 2006; Souri & Hasanirad, 2011; Tomás et al., 2012). Resilience can be understood as an approach to managing stress that allows an individual to perceive a stressful situation as a challenge and engage in overcoming it (Connor & Davidson, 2003); resilient people experience growth and adaptation as well as recovery (Richardson, 2002). At a neurophysiological level, resilience promotes better regulation of the associations between the prefrontal cortex and hippocampus (Montes-Rodríguez & Urteaga-Urías, 2018) and enhanced control of the neural mechanisms of reward and motivation, fear responsiveness, and adaptive social behavior (Charney, 2004; Ryff, 2012).

With regard to the relationship between resilience and the stress response in older people, resilience has been suggested to modulate the association between diurnal cortisol and health by reducing alterations in daily cortisol patterns and, through this, promoting health (for review, see Gaffey et al., 2016). At a psychological level, resilience has been found to be related to positive emotions (Ong et al., 2006) and to reporting fewer adversities related to health and stress (Hildon et al., 2010). In contrast, low resilience has been associated with greater difficulties in regulating negative emotions and higher stress reactivity to daily stressors (Ong et al., 2006). To the best of our knowledge, no previous study has investigated the relationship between resilience and the psychobiological response to stressors in healthy older people. In this context, investigating the mechanisms that explain why resilient individuals have a more

adaptive stress response may offer critical information to better understand inter-individual differences in stress regulation (Wu et al., 2013).

Coping strategies may be a mechanism through which resilient individuals face stressful demands and bounce back from negative experiences (Smith et al., 2016). However, several differences between resilience and coping should be acknowledged. Whereas, resilience influences how a stressful event is appraised, coping can be defined as the strategies (behavioral and cognitive) used to manage stressful demands after this appraisal. In addition, resilience seems to result in a positive response after a stressful situation, whereas coping can trigger both positive responses (e.g., active mobilization of sources) and negative responses (e.g., substance use) (Fletcher & Sarkar, 2013). In this regard, some authors have proposed that resilience promotes the active mobilization of resources under conditions of adversity. In other words, resilient individuals seem to employ adaptive coping strategies in stressful situations (Thompson et al., 2018). On the one hand, several studies have found that resilient older people use a more active coping style to manage adversity (for a review, see Southwick et al., 2005), and that resilience and active coping strategies play an important role in achieving well-being (Mayordomo et al., 2016; Tomás et al., 2012). In this regard, a study recently observed that postmenopausal women who actively coped with their condition (including engagement in planning strategies, acceptance or positive reinterpretation, and growth) showed better autonomic stress regulation (Villada et al., 2017). On the other hand, resilience also seems to be negatively related to avoidant (maladaptive) coping strategies (Hildon et al., 2010), and social withdrawal (an avoidant coping strategy) has been found to fully mediate the relationship between resilience and posttraumatic symptoms (Thompson et al., 2018). Given the strong relationship between resilience and coping and between coping and stress regulation, we expect older people with high resilience to

develop active coping (adaptive) behaviors while reducing avoidance coping (maladaptive) behaviors in stressful situations, which in turn would enhance the regulation of the psychophysiological stress response.

Previous research shows the importance of resilience and coping strategies for health and well-being. The present study advances this research by systematically exploring the role of resilience and coping strategies in the stress response of older adults. The aims of this study were twofold: (1) to analyse the association between resilience and endocrine and psychological responses to acute stress in healthy older people; and (2) to investigate whether coping strategies mediate the relationship between resilience and the psychobiological response to stress. To examine this, we exposed 66 older adults to either the Trier Social Stress Test (TSST; Kirschbaum et al., 1993b) or a control situation, and measured their psychobiological stress responses in addition to resilience, coping strategies, and perceived stress.

Based on previous studies, we expected to find a negative association between resilience and cortisol and anxiety reactivity to a stressor (Mikolajczak et al., 2008; Ruiz-Robledillo et al., 2017) and a positive relationship between resilience and active coping (Thompson et al., 2018). At the same time, we expected that active coping would predict a more adaptive stress response characterized by moderated cortisol reactivity (Chida & Hamer, 2008; Villada et al., 2014b, 2017) and lower anxiety reactivity (Mahmoud et al., 2012; Villada et al., 2014b). In contrast, we expected that there would be a negative relationship between resilience and avoidance coping, and that less use of avoidance coping would predict a more adaptive stress response (Thompson et al., 2018; Wu et al., 2013). Finally, we expected that the effect of coping strategies on the relationship between resilience and the stress response would only be observed among participants in the stress condition, but not in the control condition.

Materials and Methods

Participants

Participants recruited for this study were part of a study programme for people aged over 55 years at the university. They were interviewed to determine whether they met the study prerequisites. The exclusion criteria were as follows: smoking more than 10 cigarettes per day (following Kirschbaum et al., 1993a, 1994); abuse of alcohol (no more than 20 g/days for women and 30 g/day for men) or other drugs; having been under general anesthesia in the past 3 months; having experienced a stressful life event in the past year (e.g., death of a relative, divorce, or separation, having been fired, severe personal illness, serious personal accident or injury, serious illness in the family); presenting severe sight or hearing problems or a neurological, cardiovascular, psychiatric, endocrine, or HPA axis disease; or using drugs that can affect cognitive or emotional functioning or hormone levels (e.g., glucocorticoids, antidiabetics, antidepressants, anticoagulants, b-blockers, benzodiazepines, or hypnotics). All the women had their last period at least 2 years before the study, and none were receiving hormone replacement therapy.

The final sample consisted of 66 participants (53% women) between 56 and 75 years old (see Table I.1), with a medium subjective socioeconomic status (measured using the nine-rung 'social ladder', cf., Adler & Stewart, 2007; SES; where 1 was the lowest education and income and the worst jobs, and 10 was the best education, income, and jobs). Of all the participants, 53.1% had an educational level above secondary school, and 82.8% were retired. The mean body mass index was 26.522 (*SEM* = 0.531). The stress perceived by the participants during the previous month was low (*M* = 17.110, *SEM* = 0.732), according to the scores (ranging from 0 to 56). They were all non-smokers, except one men in the stress condition who smoked four cigarettes per

day. Given that he met the criteria for inclusion in the study, and that the statistical conclusions did not change after excluding this participant, we kept him in the statistical analyses.

The study was designed and carried out in accordance with the Declaration of Helsinki, and the experimental protocol was approved by the university's Ethics Committee. Participants were informed both verbally and in writing about the study content and the measures that would be taken, and each participant gave his/her written informed consent before participating in the study. To avoid anticipatory responses, they were not informed about the stress task until they received the instructions.

Table I.1 Means and standard errors for the study measures, in the total sample and by experimental condition and sex

	Total sample (N=66)	**Stress (N=30)**	**Control (N=36)**	***t(p)/ χ^2 (p)***	**Men (N=31)**	**Women (N=35)**	***t(p)/ χ^2 (p)***
Age (years)	64.24 (.573)	65.07 (.894)	63.56 (.732)	−1.321 (.191)	64.97 (704)	63.60 (.878)	1.195 (.236)
SES	5.49 (.150)	5.40 (.265)	5.58 (0.157)	.583 (.562)	5.32 (.236)	5.63 (.193)	−1.019 (.312)
BMI (kg/m^2)	26.522 (.531)	26.334 (.762)	26.687 (.749)	.330 (.743)	26.815 (.780)	26.283 (.732)	.496 (.622)
Educational level (%)	-	-	-	3.662 (.599)	-	-	4.468 (.484)
No studies	1.6	0	2.8	-	0	2.9	-

(Continue on next page)

Continuation of **Table I.1**

	Total sample (N=66)	**Stress (N=30)**	**Control (N=36)**	***t(p)/ χ^2 (p)***	**Men (N=31)**	**Women (N=35)**	***t(p)/ χ^2 (p)***
Basic studies	20.3	26.7	13.9	-	10.3	28.6	-
High school	25	23.3	25	-	27.6	22.9	-
College or higher	53.1	49.9	53.2	-	62	45.8	-
CD-Risc	30.85 (.585)	30.73 (.796)	30.94 (.855)	.177 (.860)	30.87 (.885)	30.83 (.789)	.032 (.974)
Active coping	2.934 (.054)	2.944 (.088)	2.936 (.067)	−.166 (.868)	2.917 (.076)	2.95 (.077)	−.304 (.762)
Emotional coping	2.722 (.051)	2.644 (.066)	2.788 (.075)	1.415 (.162)	2.658 (.078)	2.776 (.066)	−1.155 (.253)
Avoidance coping	1.804 (.045)	1.773 (.070)	1.832 (.059)	.646 (.521)	1.713 (.055)	1.884 (.067)	−1.917 (.060)
Cognitive coping	2.972 (.034)	2.975 (.054)	2.969 (.043)	−.087 (.931)	2.964 (.051)	2.978 (.045)	−.215 (.830)
PSS-14	17.11 (.732)	16.10 (1.045)	17.97 (1.012)	1.282 (.205)	17.83 (1.137)	16.49 (.949)	.917 (.363)

Note: BMI = body mass index; CD-Risc = Connor-Davidson Resilience Scale; PSS = Perceived Stress Scale; SES = subjective socioeconomic status scale. Data represent means (standard errors), t (*p*) are presented for the differences between stress and control condition, and for men and women. Data for educational level is presented in percentages and χ^2 (p) are presented for the differences between stress and control condition and for men and women.

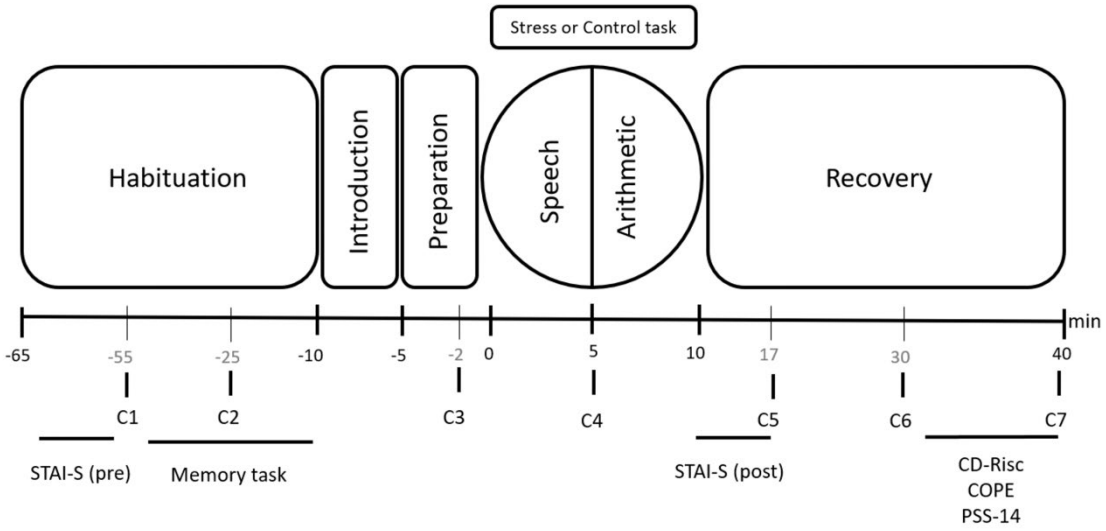
Procedure

Participants were invited to take part in a 2-h experimental session. They were randomly assigned to the stress (14 men and 16 women) or control (17 men and 19 women) condition. To control the circadian rhythm of cortisol secretion and sex differences in the cortisol response to stress (Allen et al., 2014; Pulopulos et al., 2018), the sessions were held in the afternoon (16–18 h or 18–20 h), and both the time when the participants started the session and sex were counterbalanced across conditions.

Participants were asked to sleep their usual number of hours, avoid intense physical exercise and the consumption of alcoholic drinks for 1 day prior to the study, and not eat or drink, smoke, or consume any type of stimulant (e.g., caffeine) for 2 h prior to the study. When participants arrived at the laboratory, the experimenter verified that they had followed these instructions. After consenting to take part in the study, participants had 55min of habituation before performing the task. During the first 10min, participants completed the state anxiety subscale of the State-Trait Anxiety Inventory (STAI), in order to obtain a baseline measure of their state anxiety (STAI-S pre). Then, the first saliva sample (C1) was taken 10min after the beginning of this habituation phase to obtain the basal cortisol level. This was followed by completion of the memory task, which is not part of the current research question and consisted of viewing 60 emotional and neutral images extracted from the International Affective Picture System (IAPS; Lang et al., 2005). In the middle of this task, participants provided the second saliva sample (C2). Next, participants received instructions for the stress or control task. Participants were then informed that they had 5min to prepare a speech (preparation phase). They provided the third saliva sample (C3) while preparing the speech. Between the Speech and Arithmetic tasks, the fourth saliva sample was collected (C4). After the stress or control task, participants were asked to remain calm

for 30min. During this period, they completed the post-task state anxiety subscale (STAI-S post) and provided the last three saliva samples (C5, C6, and C7). Finally, during the last 10min, participants filled out the remaining questionnaires (perceived stress, coping styles, and resilience [Figure I.1]), following the procedure described by Zoccola et al. (2010).

Figure I.1 Timeline of the experimental session



Note: Salivary cortisol samples: C1, C2, C3, C4, C5, C6 and C7. Psychological tests: State anxiety (STAI-S, pre and post); Resilience (CD-Risc); Coping strategies (COPE) and Perceived stress (PSS-14).

Stress and Control Tasks

The TSST (Kirschbaum et al., 1993b) was used to provoke psychological and cortisol responses to stress in the stress condition. The TSST consists of two tasks that last 5min each: a free speech task (job interview) and an arithmetic task. During both tasks, participants had to stand at a distance of 1.5m from a committee composed of a woman and a man. Similar to our previous studies, in order to control for other

confounding variables, a member of the committee who was of the opposite sex to the participant carried out all the interactions, which also elicits increased levels of cortisol (Duchesne et al., 2012), anxiety, and discomfort (Chorney & Morris, 2008; Dodge et al., 1987; Martinson & Zerface, 1970; McCubbin et al., 1991). In addition, both tasks were recorded using a video camera and amicrophone, both of which were visible to the participant. Several previous studies have shown that this task provokes robust psychological and endocrine responses to stress in young and older people (for a review, see: Pulopulos et al., 2018).

Salivary samples were collected before the introduction to the TSST during the habituation phase (−55 and −25min prestress), during preparation for the TSST (−2min pre-stress), immediately after the speech (+5min), and after the TSST (+17, +30, and +40min). Measurements of state anxiety were taken during the habituation and the post-task phases.

Participants assigned to the control condition performed a non-stressful task with similar mental workload and physical effort to the TSST, but without stressful components (Dickerson & Kemeny, 2004) such as social evaluation and lack of control. The control task consisted of 5min of reading aloud, followed by 5min of counting backward by fives (as previously used in Almela et al., 2011; Hidalgo et al., 2012). The control task was not performed in front of an audience. Saliva sampling, questionnaire administration, and phase durations were similar to those of the stress condition.

Measures

Stress Response

Salivary Cortisol

Throughout the experimental session, participants provided seven saliva samples with Salivettes (SARSTEDT, Nümbrecht, Germany). After the samples were collected, they were centrifuged at 4,000 rpm for 15min to obtain a transparent supernatant that was stored at −80◦C until the analyses were carried out. The cortisol concentrations were determined using the Salimetrics commercial salivary cortisol enzyme-linked immunosorbent assay kit (Newmarket, UK). The sensitivity of the assay was 0.007µg/dL, and the intra- and inter-assay coefficients of variation were all below 10%. The samples from each subject were analyzed in the same assay and in duplicate.

Anxiety State

It was evaluated using the Spanish version (Seisdedos, 1988) of the STAI-S (Spielberger et al., 1970). Participants had to rate how they felt in general at the time of completing the questionnaire. The STAI-S consists of 20 items answered on a Likert scale ranging from 0 (“nothing”) to 3 (“a lot”). Cronbach’s alpha for this study was $\alpha = 0.90$.

Perceived Stress

To evaluate perceived stress, we used the Spanish version (Remor, 2006) of the Perceived Stress Scale (Cohen et al., 1983). The Perceived Stress Scale is a self-report questionnaire that assesses perceived stress in the past month. We intended to determine whether there were differences in perceived stress between groups before they performed the TSST or control tasks. This scale consists of 14 items with a 5-point

response format (from 0 “never” to 4 “very often”), with 56 being the highest score and 0 the lowest. The higher the score, the greater the perceived stress. The Cronbach’s alpha for this questionnaire in the current sample was 0.70.

Resilience

Resilience was assessed using the short version (Campbell-Sills & Stein, 2007) of the Connor-Davidson Resilience Scale (CDRisc) (Connor & Davidson, 2003). The 10-item CD-Risc scale assesses one’s ability to evaluate and face adversity and stress during the past month (e.g., able to adapt to change, face adversities, and bounce back, and belief that one can deal with whatever comes). The Spanish version of the scale (Notario- Pacheco et al., 2011) consists of 10 items rated on a 5-point Likert scale (0 = “rarely”, 4 = “almost always”). The results obtained range from 0 to 40, and higher scores indicate higher levels of resilience. The CD-Risc is considered a good measure of resilience, given that it has been related to lower post-traumatic symptoms, increased social support, active coping strategies, and quality of life, less perceived stress and depression, and fewer avoidant coping strategies (Serrano-Parra et al., 2013; Thompson et al., 2018). Cronbach’s alpha for this study was $\alpha = 0.80$.

Coping Strategies

Coping strategies were examined using the Spanish version of the Coping Orientations to Problems Experienced Inventory (COPE; Crespo & Cruzado, 1997). The COPE is a self-report questionnaire on which subjects have to indicate what they usually feel and do in a stressful situation. The items are rated on a 4-point Likert scale from 1 (“I do not usually do it”) to 4 (“I usually do this a lot”). The questionnaire consists of 60 items grouped into 15 subscales (social support, religion, humor, alcohol or drugs, planning and active coping, avoiding coping, focusing on emotions, acceptance, denial, restraint coping, concentrating efforts to solve the situation, personal

growth, positive reinterpretation, behavioral disengagement, and escape). These 15 subscales can be grouped into secondorder factors with a four-factor structure that includes active, cognitive, and emotional coping (i.e., active coping strategies), and avoidance (i.e., passive coping strategies) (Carver et al., 1989; Hasking & Oei, 2002). Because the majority of the studies have related resilience to active and avoidance coping (for review, see Southwick et al., 2005), we used these two secondorder factors to determine whether resilience leads to more adaptive and less maladaptive coping strategies. We employed the active (planning, active coping, and suppression of competing activities) and avoidance (behavioral disengagement, mental disengagement, denial, and religious coping) factors. In our data, Cronbach's alpha for all the items was a = 0.74. Cronbach's alphas for active coping and avoidance coping were $\alpha = 0.76$ and $\alpha = 0.70$, respectively.

There is no consensus about the relationship between resilience and emotional coping. This may be due to the nature of the scale itself, which includes different responses ranging from the adaptive mobilization of sources (i.e., seeking emotional support) to the expression of negative emotions (i.e., venting emotions), suggesting that emotional coping is not essentially adaptive or maladaptive (Carver & Connor-Smith, 2010). In the current study, Cronbach's alpha for emotional coping was $\alpha = 0.75$; however, cognitive coping showed a low Cronbach's alpha that was not adequate ($\alpha = 0.42$). In addition, the analyses carried out with these factors confirmed they do not mediate the relationship between resilience and stress outcomes (cortisol and anxiety). Therefore, these results have not been reported.

Statistical Analyses

Cortisol values were not normally distributed and, therefore, were log transformed. Cortisol reactivity was calculated by subtracting pre-stress levels (mean −55min, −25min, and −2min) from the highest cortisol indexes reached for each participant. Anxiety reactivity was calculated as the difference between the pre- and post-task measures for each condition (Almela et al., 2012; Villada et al., 2014a, 2018). The area under the curve with respect to the increase (AUCi) for cortisol values was computed as an index of the cortisol response to TSST (for the formula see: Pruessner et al., 2003).

The Student's t-test for independent samples and the χ^2 test were performed to evaluate the differences between groups in psychological variables and educational level, respectively. A mixed analysis of variance (ANOVA) was used to investigate the differences between conditions in the changes in cortisol and anxiety levels during the session, with time (cortisol: −55, −25, −2, +5, +17, +30, +40min; anxiety: pre-task vs. post-task) as the within-subject factor and condition (stress vs. control) and sex (mens vs. women) as the between-subject factors. The statistical conclusion remains the same if the analyses are repeated usingmixed-effects regression. Two-way ANOVAs were used to study condition and sex differences in cortisol reactivity and AUCi indexes. Pearson correlations were used to investigate the relationship between resilience and delta changes in cortisol and anxiety.

Following Preacher et al. (2007), we performed moderated mediation analyses to investigate whether coping strategies mediate the relationship between resilience and cortisol and anxiety responses in the stress condition. It is generally agreed that there should only be one requirement to establish mediation: the indirect effect (a*b) has to

be significant (Hayes, 2017; Zhao et al., 2010). For cortisol, we entered resilience as the independent variable, active coping as the mediator variable, cortisol reactivity as the dependent variable, and condition (stress or control) as the moderator variable. Standardized values were used to perform the moderated mediation analysis. Bias corrected bootstrapping was conducted to assess the mediating effect of active coping on the relationship between resilience and cortisol reactivity. We also observed the moderator effect of the condition in the relationships between resilience and cortisol reactivity and between active coping and cortisol reactivity. The same methods were employed with avoidance coping as a mediator. When anxiety reactivity was examined as the dependent variable, we carried out the same analyses, with active and avoidance coping as mediators and condition (stress or control) as moderator. Bootstrap data resampling procedures establish confidence intervals (CIs) to test the statistical significance of an indirect effect (Shrout & Bolger, 2002). Confidence intervals are considered statistically significant when they do not include zero. The analysis was based on 10,000 bootstrap iterations, and the CI was set at 95%, as recommended by Mallinckrodt et al. (2006). Post-hoc power analysis showed that all the relationships included in the mediations presented an adequate power >0.80, with an alpha level of $p = 0.05$. Only the relationship between resilience and avoidance coping showed a statistical power of 0.247. However, these power analyses are based on linear regression analyses. Although the sample size can be considered relatively small, the bootstrap technique draws random samples of a fixed sample size with replacement from the dataset, which increases the statistical power. This type of statistical approach takes the real sample size into consideration and controls for this factor in the analyses (Hayes, 2017). Therefore, the use of bootstrap-corrected confidence intervals solves the issues of a relatively small sample size. We used Hayes' PROCESS macro (Hayes, 2017),

specifically model number 15, with SPSS (version 26; IBM Corporation, Armonk, NY, USA). One outlier in the cortisol data (one men in the control condition) and four outliers in the anxiety data (two women: one in the stress condition and one in the control condition; two men: one in the stress condition and one in the control condition) were winsorized by replacing extreme values that differed by more than three standard deviations (*SD*) from the mean with the value corresponding to ±3 *SD*. No differences were found in the analyses of cortisol, anxiety, or moderated mediations after excluding the outliers. The different numbers of participants included in the analyses of cortisol and the psychological variables are explained by missing values.

Post-hoc planned comparisons were performed using Bonferroni adjustments for the p-values. The level of significance was set at 0.05. When not otherwise specified, the results are presented as means ± *SEM*. All statistical analyses were performed with SPSS 26.0. For an easy interpretation of the figures, the values shown represent raw values and not log-transformed values.

Results

Preliminary Results

Sample Characteristics

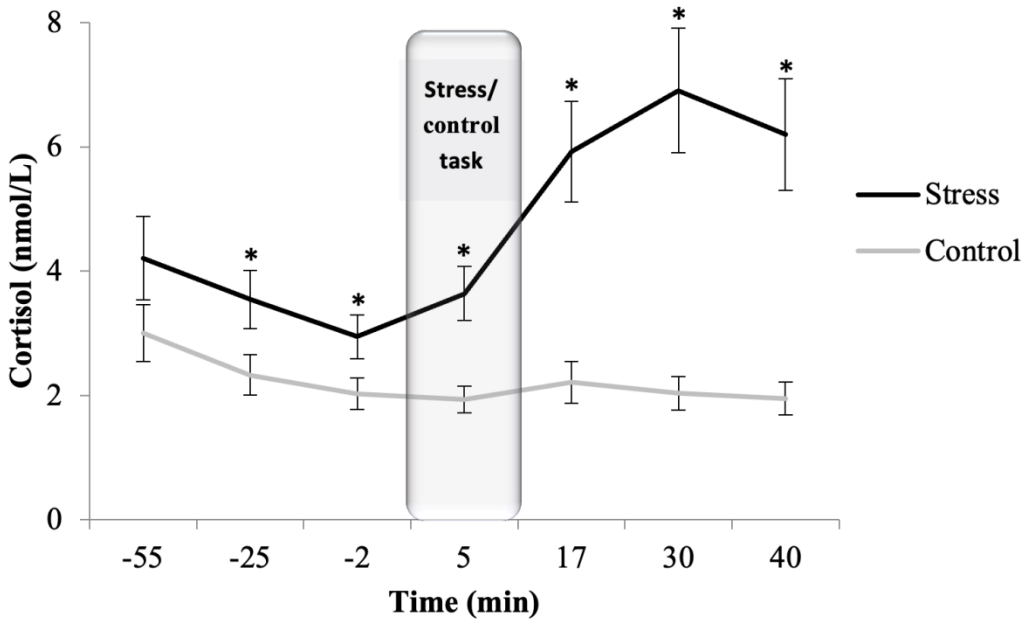
Table I.1 shows sample characteristics. No significant differences between conditions were found for age ($t = -1.321$, $p = 0.191$), SES ($t = 0.583$, $p = 0.562$), BMI ($t = 0.330$, $p = 0.743$), educational level ($\chi^2 = 3.662$, $p = 0.599$), resilience ($t = 0.177$, $p = 0.860$), coping strategies (all $p > 0.05$), or perceived stress ($t = 1.282$, $p = 0.205$).

Cortisol Response[1]

The mixed ANOVA showed effects of time ($F_{(1.99, 109.92)} = 9.884$, $p < 0.001$, $\eta^2_p = 0.152$), sex ($F_{(1, 55)} = 9.140$, $p = 0.004$, $\eta^2_p = 0.143$), condition ($F_{(1, 55)} = 21.299$, $p < 0.001$, $\eta^2_p = 0.279$), and the time and condition interaction ($F_{(1.99, 109.92)} = 17.092$, $p < 0.001$, $\eta^2_p = 0.237$). Post-hoc analyses revealed that cortisol levels were higher in men than in women. Specifically, cortisol levels were significantly higher in the stress condition than in the control condition in all the samples (all $p < 0.020$), except −55min ($p = 0.087$). In the stress condition, there were no significant differences between the first four salivary samples (all $p > 0.100$).However, cortisol levels were significantly higher after the stress task than during the stress task (+5min vs. +17 min: $p < 0.001$). After peaking (+30min), cortisol concentrations decreased until they showed no statistically significant differences from those of the habituation period (+40min vs. −55 min: $p = 0.235$). In the control condition, there was a significant decrease in cortisol levels from the −55-min to −25-min saliva samples ($p = 0.003$). No other differences in cortisol levels were observed during the control session (all $p > 0.05$) (see Figure I.2).

[1] As BMI is clearly associated to HPA-axis functioning, we tested the relationship between the BMI and cortisol changes for the two conditions but no significant correlations were found (both $p > 0.05$).

Figure I.2 Salivary cortisol concentrations for stress (N = 27) and control (N = 32) conditions.



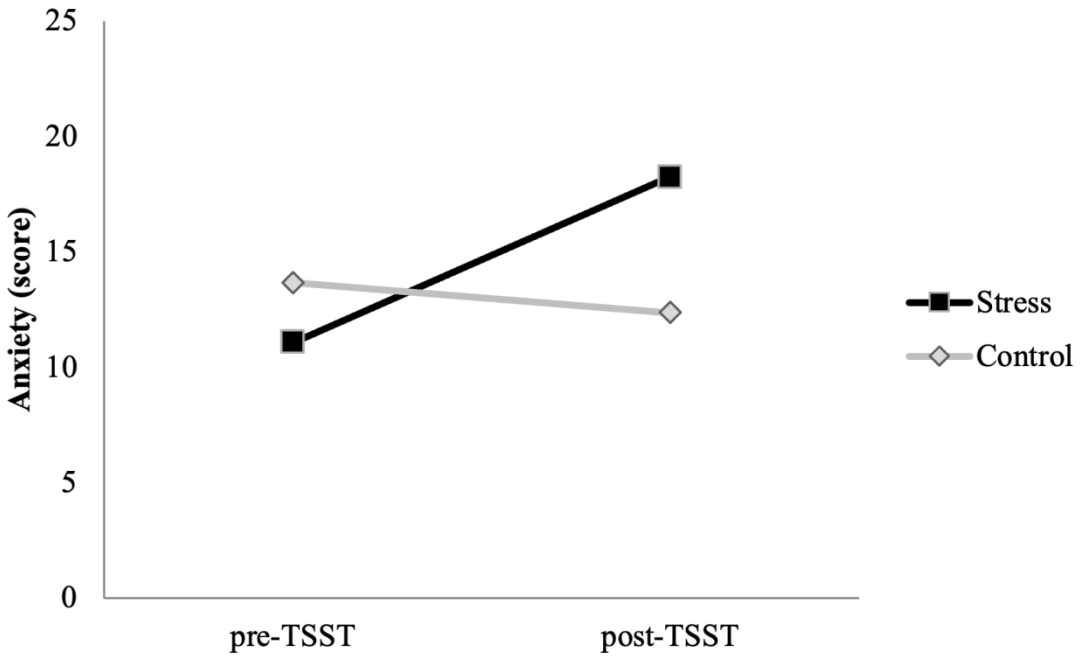
Note: Depicted values are means and error bars represent the SEM of raw cortisol values. (*p<.020).

There were also differences in cortisol reactivity between conditions ($p < 0.01$). Participants in the stress condition demonstrated a response to the task, whereas those in the control condition did not (stress: $M = 0.250$, $SEM = 0.044$; control: $M = 0.007$, $SEM = 0.038$). Cortisol differences between conditions were also found in the AUCi ($F_{(1, 58)} = 15.102$, $\eta^2_p = 0.207$, $p < 0.001$); participants in the stress condition showed higher AUCi values than participants in the control condition ($p < 0.001$). No differences were found between sexes in AUCi or cortisol reactivity levels (all $p > 0.500$).

Anxiety Response

The mixed ANOVA showed significant effects of time ($F_{(1,62)} = 19.005$, $p < 0.001$, $\eta^2_p = 0.235$) and the time × condition interaction ($F_{(1,62)} = 38.123$, $p < 0.001$, $\eta^2_p = 0.381$). No baseline differences between conditions were found ($p = 0.098$).However, participants in the stress condition showed higher levels of anxiety after the task than participants in the control condition ($p = 0.001$). Moreover, participants in the stress condition showed higher levels of anxiety after the task than before it ($p < 0.001$). Anxiety levels in the control participants did not change significantly ($p = 0.183$) (Figure I.3).

Figure I.3 Pre-and post-task anxiety for stress (n = 30) and control (n = 36) conditions.



Role of Resilience in Psychobiological Stress Response

Relationship Between Resilience and Stress Indicators

Pearson correlations showed no significant relationships between resilience and cortisol reactivity (stress: $r = 0.226$, $p > 0.05$; control: $r = -0.101$, $p > 0.05$) or anxiety reactivity (stress: $r = -0.034$, $p > 0.05$; control: $r = -0.152$, $p > 0.05$) in either condition.

Testing the Moderated Mediation Model: Cortisol Response

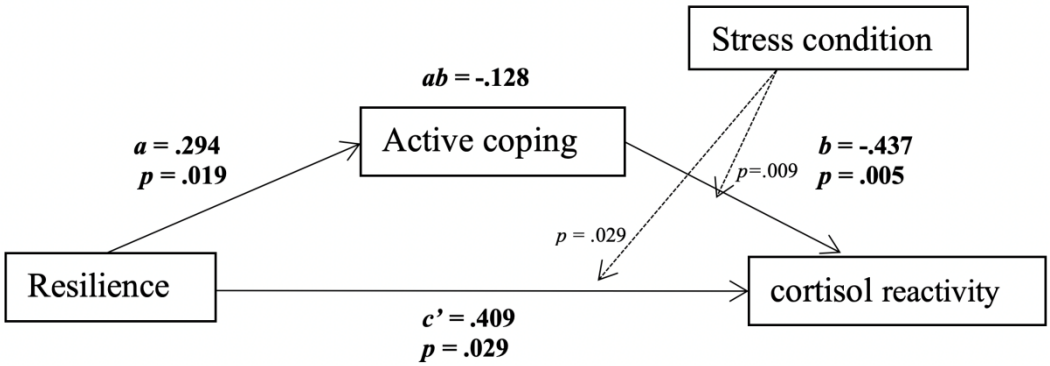
Active coping, understood as a second-order factor, was tested as a mediator in the association between resilience and cortisol reactivity. Moderated mediation analysis showed that higher resilience was associated with higher active coping (path *a*: $B = 0.294$, $SE = 0.122$, $p = 0.019$).

With regard to the moderating effect of condition in the relationship between resilience and cortisol reactivity and between active coping and cortisol reactivity, we observed that the interaction effects between resilience and the stress condition ($p = 0.029$) and between active coping and the stress condition ($p = 0.009$) were significant.

The relationship between active coping and cortisol reactivity was negative and significant in the stress condition (path *b*: $B = -0.437$, $SE = 0.151$, $t = -2.891$, $p = 0.005$), but not in the control condition (path *b*: $B = 0.165$, $SE = 0.163$, $t = 1.008$, $p = 0.317$). Analysis of the conditional direct effect of the relationship between resilience and cortisol reactivity, controlling for active coping, showed a significant direct effect in the stress condition (path *c'*: $B = 0.409$, $SE = 0.182$, $p = 0.029$), but not in the control condition (path *c'*: $B = -0.103$, $SE = 0.139$, $p = 0.460$). The conditional indirect effect of resilience on cortisol reactivity through active coping was examined for both conditions. The results showed an indirect effect of resilience on cortisol reactivity through active coping in the stress condition (path *ab*: $B = -0.128$, 95% CI = −0.329 to −0.008), but

not in the control condition (path *ab*: *B* = 0.048, 95% CI = −0.013 to 0.152) (Figure I. 4).

Figure I.4 Moderation and mediation analysis with stress condition, using bias-corrected bootstrapping in conjunction with multiple regression analysis.



Note: Numbers on the lines show B and *p* values. Solid lines indicate direct and indirect effects; dashed lines indicate moderations. Results are reported only for the stress condition. Resilience was positively related to active coping (path *a*: *B* = .294, *SE*= .122, *t* = 2.401, *p* = .019). Stress condition moderated the relationship between active coping and cortisol reactivity (active coping x condition: *p* = .009), and it also moderated the relationship between resilience and cortisol reactivity (resilience x condition: *p* = .029). The relationship between active coping and cortisol reactivity was significant for the stress condition (path *b*: *B* = -.437, *SE* = .151, *t* = -2.891, *p* = .005). The conditional direct effect of the relationship between resilience and cortisol reactivity was also significant for the stress condition (path *c'*: *B* = .409, *SE* = .182, *p* = .029, 95% confidence interval = .043 to .775). There was an indirect effect of resilience on cortisol reactivity through active coping in the stress condition (path *ab*: *B* = -.128, 95% confidence interval = -.329 to -.008).

Regarding the moderated mediation analysis between resilience and cortisol reactivity through avoidance coping, Table I.2 shows that the relationship between resilience and avoidance coping was not significant (path *a*: *B* = −0.055, *SE* = 0.130, *p* = 0.672). Moreover, neither the interaction effect between resilience and the stress

condition (p = 0.268) nor the interaction effect between avoidance coping and the stress condition (p = 0.461) was significant. Analysis of the conditional direct effect of the relationship between resilience and cortisol reactivity, controlling for avoidance coping, did not show a significant direct effect in the stress condition (path *c'*: B = 0.231, SE = 0.188, p = 0.225) or the control condition (path *c'*: B = −0.030, SE = 0.139, p = 0.828). Finally, the conditional indirect effect of resilience on cortisol reactivity through avoidance coping was not significant in the stress condition (path *ab*: B = −0.016, 95% CI = −0.117 to 0.086) or the control condition (path *ab*: B = −0.007, 95% CI = −0.066 to 0.019). The findings observed with cortisol reactivity are not replicated with AUCi. However, we would like to note that this is not uncommon in cortisol research because these two indexes reflect different information (see Pulopulos et al., 2018, 2020).

Table I.2 Moderated mediation between resilience and cortisol reactivity through avoidance coping

Dependent variable (Y): cortisol reactivity
Mediator (M): Avoidance coping
Moderator (W): Condition

		Effect	SE	*t*	*p*	LLCI	ULCI
a		−.055	.130	−.426	.672	−.316	.205
c'	Stress	.231	.188	1.227	.225	−.146	.609
	Control	−.030	.139	−.219	.828	−.308	.247
ab	Stress	−.016	.047	-	-	−.117	.086
	Control	−.007	.022	-	-	−.066	.019

Note: Letters represent the relationship between resilience and avoidance coping (a), the direct effect (c'), and the indirect effect (ab) for each condition. LLCI = lower level of confidence interval; SE = standard error; UCLI = upper level of confidence interval.

Testing the Moderated Mediation Model: Anxiety Response

Table I.3 presents the results of the moderated mediation analysis of resilience and anxiety reactivity through active coping. The relationship between resilience and active coping was significant (path *a*: $B = 0.343$, $SE = 0.118$, $p = 0.005$). However, neither the interaction effect between resilience and the stress condition ($p = 0.573$) nor the interaction effect between active coping and the stress condition ($p = 0.346$) was significant. Therefore, analysis of the conditional direct effect of the relationship between resilience and anxiety reactivity, controlling for active coping, did not show a significant direct effect in the stress condition (path *c'*: $B = 0.049$, $SE = 0.144$, $p = 0.734$) or the control condition (path *c'*: $B = -0.055$, $SE = 0.116$, $p = 0.633$). Finally, the conditional indirect effect of resilience on anxiety reactivity through active coping was not significant in the stress condition (path *ab*: $B = -0.085$, 95% CI = −0.275 to 0.017) or the control condition (path *ab*: $B = -0.026$, 95% CI = −0.112 to 0.045). As Table I.3 indicates, the moderated mediation analysis of resilience and anxiety reactivity through avoidance coping showed that the relationship between resilience and avoidance coping was not significant (path *a*: $B = -0.024$, $SE = 0.126$, $p = 0.847$). Moreover, neither the interaction effect between resilience and the stress condition ($p = 0.933$) nor the interaction effect between avoidance coping and the stress condition ($p = 0.942$) was significant. Therefore, the conditional direct effect of the relationship between resilience and anxiety reactivity, controlling for avoidance coping, did not show a significant direct effect in the stress condition (path *c'*: $B = 0.057$, $SE = 0.144$, $p = 0.694$) or the control condition (path *c'*: $B = -0.072$, $SE = 0.115$, $p = 0.531$). Moreover, the conditional indirect effect of resilience on anxiety reactivity through avoidance coping was not significant in the stress condition (*ab*: $B = -0.001$, 95% CI = −0.032 to 0.033) or the control condition (*ab*: $B = -0.001$, 95% CI = −0.034 to 0.028).

Table I.3 Moderated mediation between resilience and anxiety reactivity through active or avoidance coping

Dependent variable (Y): Anxiety reactivity
Mediator (M): Active coping
Moderator (W): Condition

		Effect	SE	*t*	*p*	LLCI	ULCI
a		.343	.118	2.898	.005	.106	.579
c'	Stress	.049	.144	.342	.734	−.240	.339
	Control	−.055	.116	−.479	.633	−.287	.176
ab	Stress	−.085	.074	-	-	−.275	.017
	Control	−.026	.034	-	-	−.112	.045

Dependent variable (Y): Anxiety reactivity
Mediator (M): Avoidance coping
Moderator (W): Condition

		Effect	SE	*t*	*p*	LLCI	ULCI
a		−.024	.126	−.194	.847	−.276	.227
c'	Stress	.057	.144	−.394	.694	−.345	.232
	Control	−.072	.115	−.628	.531	−.302	.158
ab	Stress	−.001	.015	-	-	−.032	.033
	Control	−.001	.014	-	-	−.034	.028

Note: Letters represent the relationship between resilience and active or avoidance coping (a), the direct effect (c'), and the indirect effect (ab) for each condition. LLCI = lower level of confidence interval; SE = standard error; UCLI = upper level of confidence interval.

Discussion

The general purpose of the present study was to examine the role of resilience in health and well-being in older people by analyzing several components of their stress response. First, we investigated how resilience is related to the psychobiological response to an acute psychosocial stressor (i.e., the TSST). Second, we examined the mediating role of coping strategies in the relationship between resilience and the endocrine and psychological responses to this stressor. Our results showed that resilience was not directly related to cortisol or anxiety responses to the stressor. However, active coping strategies mediated resilience's relationship with cortisol, but not with anxiety. In contrast, passive coping strategies did not mediate the relationship between resilience and psychobiological components of the stress response. The current findings highlight the importance of resilience and coping in the regulation of the stress response, suggesting that these are factors that may prevent the development of stress-related pathologies associated with aging and facilitate healthy and satisfactory aging. Following the allostatic load model, the benefits of resilience and active coping on health could be due to a better regulation of the HPA axis in these people, as observed in this study. It should be noted that, although some participants in our sample were relatively young (the ages ranged from 56 to 75, and the mean age was nearly 65 years), our results are consistent with those from studies employing older samples (Hildon et al., 2010; Tomás et al., 2012).

Resilience and the Psychobiological Response

In agreement with previous findings in older people, the stress task used in our study provoked an acute increase in cortisol and anxiety levels, compared to the control task (for reviews, see Allen et al., 2014; Pulopulos et al., 2018). Although there is an existing relationship between increased general fat tissue and altered HPA axis

(Champaneri et al., 2013), little is known about the relationship between BMI and cortisol stress reactivity (Therrien et al., 2010). We did not find an association between BMI and cortisol reactivity in the stress nor in the control group. These results are in line with other studies, which do not find differences between obese and non-obese participants in the cortisol reactivity to the TSST in people aged from 50 to 70 years old (Jayasinghe et al., 2014) and in young people (Herhaus & Petrowski, 2018; Therrien et al., 2010; but see Cano-López et al., 2019 as an exception), suggesting that an increase in body mass is not associated to cortisol reactivity to an acute stressor. Moreover, contrary to our hypothesis, resilience was not correlated with cortisol or anxiety responses to the stressor. Two previous studies found that highly resilient individuals had lower overall cortisol secretion during acute stress than their less resilient peers (Mikolajczak et al., 2008; Ruiz-Robledillo et al., 2017). However, two other studies found that, consistent with our findings, resilience was not related to cortisol reactivity to the TSST (García-León et al., 2019; Simeon et al., 2007). With regard to anxiety, some studies found that resilience was associated with fewer anxiety symptoms (Hjemdal et al., 2011; Shi et al., 2015) and attenuated anxiety after a stressor (Ruiz-Robledillo et al., 2017), whereas others only found this association in women (Carvalho et al., 2016). Nevertheless, in our study, resilience was analyzed as a continuous variable, with no differentiation between individuals with high and low resilience. In addition, past research has focused on young people and people in chronic stress conditions, such as caregivers of people with autism, patients with cardiovascular diseases, and medical students, which could already have an altered HPA response. It has been found that whereas acute stress causes transient effects on the HPA response, chronic stress produces prolonged HPA activity, which led to an impaired negative feedback and both high and low long-term cortisol levels (Marković et al., 2011; Miller

et al., 2007). Our results suggest that resilience alone is not related to the psychobiological response to an acute stressor in healthy older people.

The Mediating Role of Active Coping Strategies in the Relationship Between Resilience and Cortisol

Despite failing to confirm our first hypothesis, we found that coping plays an important role in the relationship between resilience and stress regulation. It has been suggested that resilience is related to active coping strategies, which, in turn, influence well-being (Smith et al., 2016; Tomás et al., 2012). In our study, we observed that resilience was positively related to active coping and that more active coping led to lower cortisol reactivity (i.e., partial mediation). Moreover, resilience was positively related to cortisol reactivity when controlling for active coping, but only in the stress condition. These results suggest that the relationships among resilience, coping, and stress reactivity may be explained by a competitive mediation (i.e., with a mediated effect [ab] and a direct effect [c] both existing and pointing in different directions; Zhao et al., 2010). The positive direct association between resilience and cortisol reactivity suggests the possible existence of secondary mediators that we did not examine. This positive direct association indicates that in future mediations, the sign of the indirect mediation will be positive. That is, resilience will be positively related to the mediator, and the mediator will be positively related to cortisol reactivity (Zhao et al., 2010). Future research should include other physiological variables in order to explore other resilience biomarkers. Heart rate variability is one possible biomarker which has been proposed as an objective measure of cognitive flexibility, the ability to adapt to stress, and resilience (for a review, see Perna et al., 2019).

It is also possible that only resilient individuals who use active (adaptive) coping strategies are able to have a more adaptive stress response (Gloria & Steinhardt, 2016).

Our finding showing a positive relationship between resilience and cortisol reactivity after controlling for active coping might support this idea. This relationship was not found in the previous correlations when we did not control for active coping. These results are consistent with studies showing that resilience is positively related to active coping strategies (Mayordomo et al., 2016; Thompson et al., 2018; Tomás et al., 2012), and that active coping has a positive influence on the physiological regulatory functions in situations of stress (Villada et al., 2017). Together, following Lazarus and Folkman model (1984) the current evidence suggests that resilience enhances the use of effective coping strategies as a resource to manage stress (Connor & Davidson, 2003), and it supports the idea that resilience and coping strategies are different constructs. In this line, according to the *Broaden-and-Build Theory of Positive Emotions* (Fredrickson, 1998), resilient individuals may be aware of the benefits of positive emotions in stressful situations, appraising the situation positively and developing more effective and adaptive strategies to manage stress (Feder et al., 2009; Gloria & Steinhardt, 2016), leading them to deal with and recover from these situations more easily (Tugade & Fredrickson, 2004).

We can also speculate that these findings may reflect the neurobiological processes underlying resilience. Studies have shown that, in resilient individuals, greater gray matter volumes in the ventral medial prefrontal cortex, the rostral anterior cingulate cortex (ACC), and the subgenual ACC modulate the emotional responsiveness of the amygdala and its subsequent effective stress response (Feder et al., 2009; van der Werff et al., 2013). More precisely, the cortical thickness of the ACC has been positively associated with resilience and positive coping styles (Gupta et al., 2016; Holz et al., 2016). Therefore, the cortical thickness and volume of the ACC may be responsible for the feedback inhibition of the amygdala and, thus, explain individual

differences in the extent and duration of stress circuit activations in resilient individuals (Carnevali et al., 2018). However, we did not measure the activity and connectivity of these areas, and so further studies are warranted.

No Mediating Role of Active Coping Strategies in the Relationship Between Resilience and Anxiety

With regard to the anxiety response, and in contrast with our hypothesis, active coping did not mediate the relationship between resilience and the psychological response to a stressor. These results could be due to the lack of precision in our anxiety measure. The cortisol response is evaluated through seven measures during the entire stressful situation, whereas the anxiety response is only evaluated through two measures, before and after the stressful situation. Therefore, using only a pre- and post-anxiety measure could produce misleading results because emotional states have been found to change rapidly and interact with other emotional processes preceding the endocrine response, which is slower (Schlotz et al., 2008). Moreover, the baseline anxiety measure may not reflect a neutral anxiety state due to expectations about the experiment. Therefore, it would be informative to collect the measures while people experience stress (not before or after it) (Campbell & Ehlert, 2012). Additional explanations could be related to the concept of emotion regulation. In adults, emotion regulation increases with age (Charles & Piazza, 2009), and this capacity may facilitate control over their emotional arousal (Nielsen et al., 2008). Therefore, in the present study, anxiety reactivity in the participants in the stress condition might have been more dependent on their age and less influenced by resilience or active coping strategies. Overall, the mixed results obtained for the influence of resilience on physiological and psychological variables are consistent with psychoendocrinological studies that show no correspondence between physiological and affective responses to laboratory stress tasks

(Campbell & Ehlert, 2012; Villada et al., 2014b). Our results suggest that physiological and psychological reactions to a stressor apparently work in different ways, with precise and different data collection being necessary in each case (Campbell & Ehlert, 2012).

The Role of Avoidance Coping Strategies

Unexpectedly, in the present study, resilience did not predict avoidance coping strategies, and avoidance coping was not related to cortisol or anxiety reactivity. Although this result did not support our hypothesis, our findings agree with a recent study showing that avoidance coping was not associated with or dependent on resilience (Smith et al., 2016). One explanation could be that a decrease in passive (maladaptive) coping strategies (i.e., mental disengagement and denial) would influence the way the individual evaluates the event and have a growing influence on resilience (Gloria & Steinhardt, 2016), and perhaps, on his or her engagement in active coping strategies (Thompson et al., 2018). Therefore, less use of maladaptive coping strategies would predict resilience, in contrast to our primary explanation that resilience triggers less use of maladaptive strategies, which was our hypothesis. As explained above, resilient individuals seem to experience positive emotions when a stressful situation occurs, rather than negative emotions, as in avoidance coping (Mayordomo et al., 2016). Thus, the fact that resilient people tend to exhibit lower levels of denial and experience more positive emotions during stressful situations triggers an upward spiral toward active coping and enhanced well-being (Fredrickson, 2001).

Limitations

Despite this study's novel findings, some limitations should be considered. First, the results of this study should be replicated in larger samples with different ages and/or age-related diseases (i.e., diabetic or hypertensive older people) to ensure its generalization to the whole population given that our participants were selected based

on their good physical and psychological health. Second, due to small sample size and in order to do not reduce the power of our statistical analyses, we did not study the role of sex on the relationships among resilience, coping, and cortisol. So, further studies are needed to investigate the influence of sex on these relationships. Third, the timing of the measurements is an important aspect to consider. In our study, we measured stable psychological aspects at the end of the session. Although no differences between conditions were observed with these measures, the stress task might have affected the responses. Future studies may benefit from asking the participants to complete the questionnaire on a different day and in non-stressful conditions. In addition, future research on the psychological response to stress exposure should add repeated real-time emotional reports, and not just pre- and post-task measures, in order to more adequately represent the stress experience. Finally, given the cross-sectional nature of this study, causality and directionality could not be determined.

Conclusions and future directions

In conclusion, the results of this study suggest that greater resilience is associated with active coping strategies, which, in turn, are related to a lower cortisol response to stress in healthy older people. These results differ from those found for young individuals, in whom resilience was directly related to the psychological and physiological response to an acute stressor. Our results highlight the importance of the relationship between resilience and active coping strategies and are in line with other studies investigating this relationship in older samples. However, to the best of our knowledge, this is the first study to investigate the relationships between resilience, coping, and psychobiological factors such as cortisol and anxiety, using a laboratory-based stress paradigm in healthy older people.

Future research on stress protective factors should integrate resilience and coping into a theoretical framework where environmental and social support agents, such as community programmes or support groups, may play an important role in overcoming obstacles and enhancing personal growth (Fletcher & Sarkar, 2013; MacLeod et al., 2016). It is also important to investigate the moderating effects of resilience on other situational factors, such as adverse childhood experiences or chronic stressors (Connor et al., 2003). Overall, we encourage future studies to further examine the relationship between resilience and physiological and emotional stress responses in order to advance in this area. These studies would help to design interventions that consider the central role of resilience in the coping process and in overcoming stress-related pathologies, in an effort to improve the well-being of older people.

Chapter III

Study 2: Importance of Optimism and Pessimism in chronic stress biomarkers in healthy older people

The main results of this study are being prepared for submission:

Zapater-Fajarí, M., Crespo-Sanmiguel, I., Montoliu, T., Hidalgo, V., Salvador, A.The impact of Optimism and Pessimism on chronic stress: relationship with hair cortisol and dehydroepiandrosterone.

Introduction

The number of older people around the world is increasing dramatically, with an anticipated elderly population of two billion in 2050. This is a problem in today's society because this age group faces various physical and mental health problems. However, older people show differences in the way they age. Whereas some older adults maintain good health until advanced ages (healthy/normal aging), others experience health problems (pathological/unsatisfactory aging) (World Health Organization, [WHO], 2022a). In this regard, several personality traits have been proposed as important factors in health, given that they can affect the evolution or maintenance of many age-related disorders in older people, including stress pathologies and diseases (Mroczek et al., 2006). Thus, individual differences in the stress response could be one of the mechanisms explaining these differences in aging in the elderly, given that stress is an important problem in the 21st century (WHO, 2022c).

Along these lines, cortisol is a product of Hypothalamic-Pituitary-Adrenal axis (HPA axis) activation and, therefore, one of the most important components of the stress response. It has been related to several health problems linked to aging, such as cardiovascular disease, Type 2 diabetes, and reduced immune function (Kiecolt-Glaser et al., 2003; Lundberg, 2005). Generally, the regulation/functioning of the HPA axis has been studied through traditional cortisol measures (plasma or saliva) that are good at capturing the impact of acute stressors, but fail to reflect long-term stress exposure (Stadler & Kirschbaum, 2012). In this regard, hair cortisol (HC) has been found to be a good biomarker of chronic stress exposure (Russell et al., 2012; Stadler & Kirschbaum, 2012). Some studies have suggested that there are sex differences in HC secretion, reporting higher HC levels in men than in women (for a review see: Stadler et al., 2017), whereas other studies have found inconclusive results (Dettenborn et al., 2012;

Gao et al., 2010; Manenschijn et al., 2011; Raul et al. 2004; Thomson et al., 2010). Thus, this gap in the field makes it necessary to address sex differences in chronic stress biomarkers such as HC in the elderly population, which could increase the knowledge about sex differences in the evolution of several age-related diseases associated with stress.

Additionally, dehydroepiandrosterone (DHEA), another adrenal hormone involved in stress regulation, has shown an antagonistic effect to cortisol (Buoso et al., 2011) and is a biomarker of neuroprotective and anti-inflammatory processes (Kamin et al., 2017). Interestingly, DHEA concentrations decrease with age, and so it has been suggested that an increase in DHEA may be involved in longevity and aging processes (Barrou et al., 1997; Maggio et al., 2015). DHEA levels have mostly been investigated in blood and saliva, but their measurement in hair (HDHEA), as a proxy for chronic stress, is increasing (Bürgin et al., 2020; Hennessey et al., 2020). In addition, it has been suggested that the Cortisol:DHEA ratio (Cort:$DHEA_{ratio}$), which represents the interaction between these two hormones, might be a more accurate and physiological reflection of adrenocortical activity (Kamin et al., 2017; Sollberger & Elhert, 2016). In this regard, a higher Cort:$DHEA_{ratio}$ would be associated with increased chronic stress and poorer health (Sollberger & Elhert, 2016). Therefore, studies that incorporate the two biomarkers and their interaction in the Cort:$DHEA_{ratio}$ would be of interest (Kamin et al., 2017).

Another current question is the extent to which these biomarkers could be related to psychological traits, thus explaining possible differences in the stress response of the elderly population (Russell et al., 2012). Determining the factors associated with HC and HDHEA concentrations would improve our understanding of the role of these adrenal hormones in normal development and psychopathology associated with the

aging process (Kamin et al., 2017). In this context, optimism is a psychological trait that has been found to be involved in the healthy aging process, mainly due to its association with HPA axis regulation (Puig-Perez et al., 2021). Some authors have proposed that the type of expectations we hold affects our physical and mental health (Carver et al., 2010). In this line, the *Self-Regulatory Behavior Theory* (Scheier & Carver, 2000) deals with the psychological traits of optimism and its opposite, pessimism. According to this theory, optimistic individuals are confident and persistent in their expectations of success in achieving their goals, whereas pessimists are more hesitant about success and have negative expectations (Carver et al., 2010; Scheier & Carver, 1992; Scheier et al., 1994). Positive expectations about future events make people less reactive to stress in life, which translates into more adaptive physical and psychological stress-related responses (Brydon et al., 2009; Carver et al., 2010). Optimistic individuals show greater resilience to stress, and optimistic thoughts about the future in stressful situations have been proposed as an important aspect of resilience (Souri et al., 2011; Yu & Zhang, 2007). Several studies have highlighted the roles of optimism and pessimism in the stress response (Baumgartner et al., 2018; Salzmann et al., 2018; Nes et al., 2005). Optimism has also been linked to more stable cortisol measures, such as the cortisol awakening response (CAR), an index that reflects the dynamic of morning cortisol levels (Endrighi et al., 2011; Lai et al., 2005). Thus, on the one hand, higher optimism has been related to lower CAR in both healthy young (Lai et al., 2005) and older (Endrighi et al., 2011) people, whereas other studies failed to confirm this association in young people (Ebrecht et al., 2004). On the other hand, pessimism has not been related to cortisol secretion after a stressor (Endrighi et al., 2011; Puig-Perez et al., 2015) or to CAR (Lai et al., 2005).

In two previous studies by our group, we demonstrated that optimism was more related to physiological components of the stress response, whereas pessimism was more related to psychological aspects of stress, but we could not test for associations between optimism and more chronic stress biomarkers such as HC or HDHEA. We found that optimism was related to faster recovery after a stressor (Puig-Perez et al., 2015). Although we did not find an association between optimism and CAR, a healthier CAR was related to a higher percentage of positive cognitions and emotions and a lower percentage of negative ones in healthy older people (Puig-Perez et al., 2018). Pessimism was not related to the physiological stress response or CAR, but it was associated with worse psychological adjustment to stress (Puig-Perez et al., 2015) and more negative affect and negative cognitions and emotions (Puig-Perez et al., 2018). Hence, the question of how optimism and pessimism are related to biomarkers of chronic stress remains unanswered. Thus, optimism and pessimism could be potential factors related to HPA axis activity and, thus, long-term HC and HDHEA concentrations.

To date, only one study has investigated the relationship between optimism and HC. Specifically, optimism was associated with lower levels of HC in adolescents (Milam et al., 2014). However, in young samples, optimism-related factors such as resilience have been negatively related to HC (García-León et al., 2019). Bürgin et al. (2020) investigated whether some measures of resilience were related to HC, HDHEA, or the $HC:HDHEA_{ratio.}$ in young and older adults. They found that sense of coherence and self-care were associated with higher HDHEA levels and a lower $HC:HDHEA_{ratio}$. This study also looked for sex differences and confirmed a positive relationship between sense of coherence and HDHEA only in women (Bürgin et al., 2020). Thus, resilience and optimism, which are strongly related factors (Souri et al.,2011), have both been associated with chronic stress biomarkers (Bürgin et al., 2020; García-León et al., 2019;

Milam et al., 2014). However, no study has investigated the relationship between optimism and pessimism and chronic stress biomarkers (HC, HDHEA, and $HC:HDHEA_{ratio}$) in healthy older people. Studying the relationship between protective/ vulnerability factors and chronic stress biomarkers can have important implications for health, especially in older people, given that chronic stress has been related to a maladaptive aging process and age-related diseases (Yegorov et al., 2020).

Therefore, the aim of this study was to test the relationship between the protective and vulnerability factors of optimism and pessimism, respectively, and chronic stress biomarkers measured in hair (i.e. HC and HDHEA). Additionally, we explored sex differences in HC and HDHEA concentrations and in their relationship with optimism and pessimism. We expected that optimism would be negatively related to HC and the $HC:HDHEA_{ratio}$ (Bürgin et al., 2020; García-León et al., 2019; Milam et al., 2014) and positively associated with HDHEA (Bürgin et al., 2020). In the case of pessimism, we expected to find the opposite direction in these relationships. Finally, we expected higher HC and HDHEA and a lower $HC:HDHEA_{ratio}$ in men (Bürgin et al., 2020; Stalder et al., 2017). We also expected to find a positive association between optimism and HDHEA only in women (Bürgin et al., 2020).

Materials and Methods

Participants

Participants belonged to a study program at the University of Valencia for people over 55 years of age. We recruited participants from the classes of this study program and through informative talks and posters at the faculties of the University campus. Volunteers were interviewed by telephone to determine whether they met the study prerequisites. Inclusion criteria were: smoking less than 10 cigarettes a day, no alcohol or other drug abuse (we asked the participants how many glasses and what kind of

beverages they drank per week; we included those who drank less than 20 g/day for women and 30 g/day for men), not having been under anesthesia in the past three months, and no presence of a stressful life event during the past year (volunteers were asked about any important event considered stressful that had changed their life; e.g. widowhood, retirement, etc.).

Volunteers were also asked if they had diabetes or a neurological or psychiatric disease, or if they were taking any medication directly related to emotional or cognitive functioning or able to influence hormonal levels, such as glucocorticoids, psychotropic substances, or sleep medications. Six participants had Type II diabetes, although medically treated, which has not been found to affect the HPA axis stress response (Vallejo et al., 2020), sixteen participants were taking medication directly related to the central nervous system (i.e. sleep medication), and three participants were taking β-blockers. Thus, medication/disease was included as covariate in the analyses to control it .Socioeconomic status (SES) was measured with the MacArthur Scale of Subjective Social Status (Adler et al., 2000). Participants rated themselves from 1 (people with the lowest education and income and the worst jobs) to 10 points (people with the best education, income, and jobs). All women participants were postmenopausal and had their last menstrual period more than two years prior to the testing time, and none of them were taking estrogen replacement therapy.

One hundred twenty-four participants were assessed in this study; however, three men had to be excluded because they did not have enough hair for biochemical analyses (3 cm). Hence, the final sample was composed of 121 participants (46 men and 75 women) between 56 and 81 years old.

Procedure

Participants were asked to visit the laboratory, where they filled out the psychological tests. Additionally, their height and weight and waist and hips were measured to calculate their Body Mass Index (BMI) and waist hip ratio (waist: hip), respectively. At the end of the session, 3-cm hair samples (~ 3mm) were collected from the posterior vertex region on the head. Hair was collected by the researcher using stylist's scissors, and it was cut as close to the scalp as possible (recommended by the Society of Hair Testing, 1997). The samples were wrapped in aluminum foil individually for storage.

All the participants provided their 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.

Instruments

Perceived Stress

The Spanish version (Remor, 2006) of the 14-item Perceived Stress Scale (Cohen et al., 1983) was used to evaluate the degree to which people perceived their lives as stressful, uncontrollable, and unpredictable in the past month. The respondents had to answer using a 5-point Likert scale (from 0 = never to 4 = very often), where higher scores indicate higher perceived stress. Internal consistency of this scale had a Cronbach's $\alpha = 0.81$.

Life Orientation Test Revised

The Spanish version (Otero et al., 1998) of the Life Orientation Test Revised (LOT-R) (Scheier et al., 1994) was used to address optimism and pessimism. This is a 10-item scale rated on a 5-point Likert scale. It provides a measure of optimism and pessimism

or a total score of dispositional optimism, depending on whether it is considered a one-dimensional or two-dimensional measure, respectively. Three items measure optimism (i.e. 'In uncertain times, I usually expect the best'), three other items measure pessimism (i.e. 'If something can go wrong for me, it will'), and the last four items are distractors. Several studies have confirmed the two-dimensional measure (Carver et al., 2010; Ferrando et al., 2002; Mroczek et al., 1993; Robinson-Whelen et al., 1997), and the use of the separate scores in addition to the total score has been recommended, especially in older people (Rasmussen et al., 2009; Puig-Perez et al., 2015; 2018). Internal consistency for this scale was α =.75.

Hair Cortisol and DHEA

Hair samples were prepared and analyzed in the laboratory of Prof. Kirschbaum (Department of Psychology, Technische Universität Dresden, Germany), following the protocol described in detail in Kirschbaum et al. (2009). Analysis was performed on the proximal 3 cm of hair, which, based on a hair growth rate of 1 cm/month, represents the average cortisol accumulation over a period of 3 months (Russell et al., 2012). Hair samples were incubated in 1800µl methanol for 18h at 45ºC (see Stalder et al., 2012, for more detail) and then analyzed by immunoassay technique. Results are in fluid units and were converted to pg/mg hair.

Statistical analyses and data management

Because HC, HDHEA, and HC:HDHEA$_{ratio}$ values did not show normal distributions, they were log transformed. The HC:HDHEA$_{ratio}$ was calculated by dividing HC by HDHEA values.

As mentioned above, the current issue in research on optimism is whether to address optimism and pessimism as two strongly correlated bipolar constructs or as two separate

dimensions (Carver et al., 2010; Herzberg et al., 2006; Rasmussen et al., 2009; Scheier & Carver, 2018). In this regard, it has been argued that, after the age of 50, these constructs show low shared variance and are better studied separately (Mroczek et al., 1993; Plomin et al., 1992; Puig-Perez et al., 2015; Robinson-Whelen et al., 1997). Some theories (Diener et al., 1999; Ryff & Singer, 1998) defend the independence of positive and negative mental states, based on previous studies (e.g. Lai, 1994, 1997; Lai et al., 2005). For these reasons, following the recommendations of Rasmussen et al. (2009), we explored both dispositional optimism and the optimism and pessimism subscales separately.

Student's *t* tests for independent samples and the χ^2 test were performed to evaluate the differences between the sexes in the sociodemographic variables, perceived stress, dispositional optimism, optimism and pessimism subscales, HC, HDHEA, and $HC:HDHEA_{ratio}$.

Correlation analyses were performed to investigate the relationships between the sociodemographic variables, perceived stress, dispositional optimism, optimism and pessimism subscales, HC, HDHEA, and $HC:HDHEA_{ratio}$. To investigate the association between chronic stress biomarkers and psychological traits, first, separate hierarchical linear regression analyses were performed, with each trait as independent variable and HC, HDHEA, or the $HC:HDHEA_{ratio}$ as dependent variables. In addition, several covariates were included to control for possible confounder effects. Age, sex, SES, BMI, waist:hip ratio, and PSS were included because of their effects on HPA axis activity (Bürgin et al., 2020; Stalder et al., 2017). Thus, we conducted hierarchical analyses, including the covariates in Step one and each psychological trait (dispositional optimism or its optimism or pessimism subscale) in Step two. Additionally, all the regression analyses were performed with medication/disease included as a covariate.

To investigate whether the relationship between psychological traits and chronic stress biomarkers was moderated by sex, moderation analyses were performed following Preacher et al. (2007). We included the psychological traits (dispositional optimism or its optimism or pessimism subscale) as independent variables, each chronic stress biomarker (HC, HDHEA, or HC:$HDHEA_{ratio}$) as dependent variable separately, and sex as a moderator. We used standardized values to perform the moderation analysis. Bias-corrected bootstrapping 95% intervals were conducted to assess the mediating effects using 5,000 bootstrap iterations. Confidence intervals that do not contain zero are considered statistically significant. We used Hayes' PROCESS macro model number 1 (Hayes, 2017) with SPSS (version 26; IBM Corporation, Armonk, NY, USA).

Standardized residuals were used to detect multivariate outliers (± 3 SD). Specifically, one man was excluded because he was an outlier in the relationship between chronic stress biomarkers and psychological traits. Two women had missing data on the optimism and pessimism scales. Tolerance and variance inflation factor values indicated that there were no collinearity issues for the variables included in the regressions.

To perform the statistical analyses, version 26.0 of SPSS was used (IBM Statistics, Chicago, IL, USA). All *p* values were two-tailed, and the level of significance was taken as $p<.05$. *Post hoc* planned comparisons were performed using Bonferroni adjustments for the *p*-values.

Results

General sample characteristics

Descriptive data for the complete sample and for men and women are reported in Table II.1 Sex differences in age, waist:hip index, and perceived stress were found (*all* $p < .035$). Men were older, with a higher waist:hip index and lower perceived stress. No differences were found between men and women in SES, BMI, educational level, dispositional optimism, optimism, pessimism, HC, HDHEA, or the HC:$HDHEA_{ratio}$ (all $p > .05$).

Table II.1 Sample characteristics for the total sample and for men and women

	Total sample (n=121)	**Men (n=46)**	**Women (n=75)**	***t*/ χ^2**	***p* value**
Age (years)	67.36	68.74	66.51	2.29	.024
	(5.30)	(4.94)	(5.37)		
SES	6.00	6.27	5.84	1.79	.076
	(1.28)	(1.21)	(1.30)		
BMI *(kg/m2)*	26.59	27.10	26.28	1.24	.217
	(3.81)	(2.99)	(4.23)		
Waist: hip	.89	.96	.85	8.18	<.001
	(.09)	(.06)	(.08)		
Educational Level (%)				9.52	.090
Without studies	.8	0	1.3		
Primary school	16.5	6.5	22.7		
Secondary school	25.6	23.9	26.7		
Graduate (3 years)	27.3	28.3	26.7		
Graduate (5 years)	28.9	39.1	22.7		

(Continue on next page)

Continuation of **Table II.1**

	Total sample (n=121)	**Men (n=46)**	**Women (n=75)**	***t*/ χ^2**	***p* value**
PhD	.8	2.2	0		
PSS	17.50	15.91	18.48	−2.14	.035
	(6.51)	(5.45)	(6.94)		
LOT-R					
Dispositional Optimism	22.45	22.67	22.31	.58	.562
	(3.27)	(2.87)	(3.51)		
Optimism	11.72	11.70	11.74	−.12	.905
	(1.95)	(1.82)	(2.04)		
Pessimism	7.28	7.04	7.42	−.99	.325
	(2.20)	(1.76)	(2.44)		
HC (pg/mg)	4.03	4.44	3.78	.07	.947
	(7.35)	(10.66)	(4.28)		
HDHEA (pg/mg)	15.92	14.88	16.56	.46	.646
	(18.15)	(14.30)	(20.21)		
HC:HDHEA$_{ratio}$	.41	.37	.43	−.34	.735
	(.56)	(.60)	(.53)		

Note: N=121 except for SES (N = 118), BMI (N = 120), ICC and LOT-R (N = 119). SES = Subjective Socioeconomic Status; BMI = Body Mass Index; Waist: hip= Waist hip index; PSS = Perceived Stress; LOT = Life oriented test; HC = Hair cortisol concentration; HDHEA = Hair dehydroepiandrosterone concentration; HC:HDHEA$_{ratio}$ = Cortisol to DHEA ratio in hair. Data represent means (standard deviations), except for educational level (%). *t* are presented for the differences between men and women, and χ^2 are presented for the differences between men and women in educational level.

Unadjusted correlation analyses

Table II.2 shows unadjusted correlations among all the variables included in the study. The dispositional optimism scale was negatively related to perceived stress (p<.001), HC (p =.009), and the HC:HDHEA$_{ratio}$ (p = .001). The optimism subscale was negatively related to perceived stress (p=.005) and the HC:HDHEA$_{ratio}$(p = .029). In contrast, pessimism was positively related to perceived stress (p = .004), HC (p = .020), and the HC:HDHEA$_{ratio}$ (p = .006).

Table II.2 Pearson's correlation values between all the factors used

	BMI	Waist:hip	SES	HC	HDHEA	HC:HDHEA$_{ratio}$	PSS	D-OP	OP	PES
Age	.116	.223*	−.026	−.093	−.050	−.037	−.191*	.011	.042	.020
BMI		.366**	−.213*	.178	−.001	.149	.059	.057	.095	−.001
Waist:hip			−.143	.008	.026	−.014	.058	.016	.003	−.017
SES				−.063	.150	−.176	−.176	−.004	−.114	−.094
HC					.274**	.616**	.069	−.239**	−.164	.213*
HDHEA						−.589**	−.035	.101	.072	−.082
HC:HDHEA$_{ratio}$							.087	−.289**	−.200*	.250**
PSS								−.331**	−.255**	.263**
D-OP									.756**	−.813**
OP										−.234*

Note: SES= Subjective socioeconomic status; BMI= Body Mass Index; Waist: hip= Waist hip index; PSS= Perceived Stress; D-OP=Dispositional Optimism; OP= Optimism; PES= Pessimism; HC= Hair cortisol concentration; HDHEA= Hair dehydroepiandrosterone concentration; HC:HDHEA$_{ratio}$ = Cortisol to DHEA ratio in hair . *p<.05. **p<.01. N=121, except for SES (N= 118), BMI (N= 120), ICC and LOT-R (N= 119).

Regression analyses

Relationship Between Dispositional Optimism and Chronic Stress Biomarkers

Linear regression analyses indicated that dispositional optimism was negatively associated with HC ($B = -.278$; $p = .005$) and the HC:HDHEA$_{ratio}$ ($B = -.279$; $p = .005$), but it was not related to HDHEA ($B = .065$; $p = .527$) (Table II.3). No effect of sex was found in all the aforementioned relationships (all $p > .05$). Results did not change after including medication/disease as covariate, confirming that optimism was negatively associated with HC ($B = -.251$; $p = .013$) and the HC:HDHEA$_{ratio}$ ($B = -.273$; $p = .007$) (Table II.4).

Relationship Between Optimism and Chronic Stress Biomarkers

The results show that optimism was negatively associated with the HC:HDHEA$_{ratio}$ ($B = -.198$; $p = .044$). Optimism was not significantly related to HC ($B = -.163$; $p = .099$) or HDHEA ($B = .056$; $p = .585$) (Table II.3). No effect of sex was found in all the aforementioned relationships (all $p > .05$). Results after including medication/disease as covariate showed similar results, except that the relationship between optimism and the HC:HDHEA$_{ratio}$ did not remain significant ($B = -.183$; $p = .064$) (Table II.4).

Relationship Between Pessimism and Chronic Stress Biomarkers

The results showed that pessimism was positively associated with HC ($B = .246$; $p = .010$) and the HC:HDHEA$_{ratio}$ ($\beta = .239$; $p = .012$), but not with HDHEA ($B = -.037$; $p = .714$) (Table II.3). No effect of sex was found in the aforementioned relationships (all $p > .05$). Results did not change when adding medication/disease as covariate, confirming that pessimism was positively associated with HC ($B = .222$; $p = .021$) and the HC:HDHEA$_{ratio}$ ($B = .260$; $p = .007$)(Table II.4).

Table II.3 Linear regression analyses with stress biomarkers (HC, HDHEA, and HC:HDHEA$_{ratio}$) as dependent factors and Dispositional Optimism, Optimism, or Pessimism as independent factors, including age, sex, SES, BMI, and PSS as covariates

HC				
	R^2	Adj R^2	R^2 change	Beta
D-Optimism	.152	.096	.067	−.278*
Optimism	.109	.050	.023	−.163
Pessimism	.141	.084	.056	.246*
HDHEA				
	R^2	Adj R^2	R^2 change	Beta
D-Optimism	.031	−.033	.004	.065
Optimism	.030	−.034	.003	.056
Pessimism	.029	−.035	.001	−.037
HC:HDHEA$_{ratio}$				
	R^2	Adj R^2	R^2 change	Beta
D-Optimism	.147	.091	.067	−.279*
Optimism	.115	.057	.035	−.198*
Pessimism	.159	.103	.052	.239*

Note: D-Optimism=Dispositional Optimism; HC= Hair cortisol concentration; HDHEA= Hair dehydroepiandrosterone concentration; HC:HDHEA$_{ratio}$ = Cortisol to DHEA ratio in hair; Waist: hip= Waist hip index. Covariates: age, sex, BMI, Waist: hip and PSS. *p<.05. n= 117, except for regressions between Optimism with HC, and Pessimism with HC or HC:HDHEA$_{ratio}$ (N= 116).

Table II.4 Linear regression analyses with stress biomarkers (HC, HDHEA, and HC:HDHEA$_{ratio}$) as dependent factors and Dispositional Optimism, Optimism, or Pessimism as independent factors, including medication/disease, age, sex, SES, BMI, and PSS as covariates

HC				
	R^2	Adj R^2	R^2 change	Beta
D-Optimism	.165	.092	.052	-.251*
Optimism	.132	.056	.019	-.145
Pessimism	.154	.091	.044	.222*
HDHEA				
	R^2	Adj R^2	R^2 change	Beta
D-Optimism	.058	-.023	.006	.088
Optimism	.054	-.027	.003	.055
Pessimism	.053	-.017	.007	-.090
HC:HDHEA$_{ratio}$				
	R^2	Adj R^2	R^2 change	Beta
D-Optimism	.167	.095	.061	-.273*
Optimism	.135	.060	.029	-.183
Pessimism	.164	.101	.060	.260**

Note: D-Optimism=Dispositional Optimism; HC= Hair cortisol concentration; HDHEA= Hair dehydroepiandrosterone concentration; HC:HDHEA$_{ratio}$ = Cortisol to HDHEA ratio in hair; Waist: hip= Waist hip index. Covariates: age, sex, BMI, Waist: hip, PSS and Medication/disease. *p<.05. **p<.01. n= 117, except for regressions between Optimism and HC, and between Pessimism and HC or HC:HDHEA$_{ratio}$ (N= 116).

Discussion

In this study, we investigated the relationship between optimism and pessimism and the hair stress biomarkers HC, HDHEA, and HC:$HDHEA_{ratio}$. To date, this is the first study to investigate psychological traits and chronic stress biomarkers in a sample of healthy older individuals. We observed that high dispositional optimism and low pessimism were related to lower HC and a lower HC:$HDHEA_{ratio}$. Higher scores on the optimism subscale were related to a lower HC:$HDHEA_{ratio}$. No sex interactions were found.

Linear regressions showed that higher dispositional optimism was related to less long-term cortisol exposure (i.e. the previous three months) and lower HC:$HDHEA_{ratio}$ concentrations measured in scalp hair. In contrast, higher pessimism was associated with greater HC and HC:$HDHEA_{ratio}$ concentrations. As previously mentioned, following the *Self-Regulatory Behavior Theory* (Scheier & Carver, 2000), anticipating that good things are going to happen leads to engaging in different coping strategies and sources to cope with stress (Carver et al., 2010). Thus, optimistic people have lower perceived stress (Endrighi et al., 2011; Milam et al., 2014), and they use positive or active coping strategies, which are considered more adaptive, to deal with stressors (Nes & Segerstorm, 2006). Positive or adaptive coping strategies include a wide range of sources, such as dealing with the stressor or the emotions stemming from it (Carver et al., 2010). Thus, optimism can lead to better adjustment to stressful situations and, in turn, more adaptive HPA regulation. In contrast, pessimistic individuals, given their greater doubts about achieving their goals and lower expectancies, tend to withdraw their efforts in stressful situations and use avoidant coping strategies. These avoidant coping strategies are also understood as maladaptive coping strategies, which make people more vulnerable to stress and health problems such as stress-related diseases

(Carver et al., 2010). Additionally, optimistic people tend to have larger social networks and attend more social activities than pessimists (Scheier & Carver, 2018), all of which have been related to better coping with stress and greater well-being (Taylor, 2007).

The results for dispositional optimism were consistent with Milam et al. (2014), who found that higher dispositional optimism was related to lower HC in young adults. Likewise, García-León et al. (2019) and Bürgin et al. (2020) found that resilience was negatively related to HC in young people (García-León et al., 2019), and that resilience factors (sense of coherence and self-care) were negatively related to HC:HDHEA $_{ratio}$ concentrations, due to the positive association with HDHEA, in mixed-aged samples (Bürgin et al. 2020). Overall, optimism can be understood as a resilience-related factor because these two traits have been strongly correlated with each other, and optimism is a factor included in the definition of resilience (Souri et al., 2011; Yu & Zhang, 2007). Sense of coherence can be understood as one's perception of life as comprehensible, manageable, and meaningful, whereas self-caring behavior can be described as specific health behaviors (e.g., participating in sports, sleeping enough, balancing nutrition). In this regard, based on their definitions, these two variables can be understood as components of optimism, given the close relationship between positive expectancies and health and healthy behaviors found in optimistic individuals (Carver et al., 2010). In contrast, no previous study has analyzed the relationship between pessimism and chronic stress biomarkers (HC or HC:HDHEA$_{ratio}$ concentrations) in young or older people. However, previous studies have suggested that pessimism is related to a higher stress perception (Endrighi et al., 2011), and that people who score high on pessimism perceive more effort and difficulty after a laboratory induced stressful situation (Puig-Perez et al., 2015).

The stronger effect of the dispositional optimism and pessimism subscale on chronic stress biomarkers compared to the effect of the optimism subscale in our study supports studying the optimism and pessimism subscales as two separate dimensions in people over 50 years of age, given that, although negatively and strongly related, multicollinearity tests showed that these variables were independent (Herzberg et al., 2006; Puig-Perez et al., 2015; Rasmussen et al., 2009). The greater effect of pessimism on chronic stress biomarkers is consistent with a previous study by our group showing that pessimists tended to report more negative events, cognitions, and emotions than positive ones, whereas optimism was not related to past life review. Thus, pessimism, compared to optimism, increases the focus on negative aspects of life and, thus, may have greater involvement in pathological aging processes and HPA axis regulation (Puig-Perez et al., 2018). Our results may strengthen the notion that the negative effects of pessimism have a greater weight than the protective effects of optimism in the relationship with HPA axis regulation (Scheier & Carver, 2018; Robinson-Whelen et al., 1997). Overall, the results of our study could suggest that the effects found between dispositional optimism and the HPA axis could be mainly due to the pessimism subscale, given its stronger association when studying the subscales separately.

We failed to find associations between the optimism and pessimism measures and DHEA hair cortisol concentrations. To our knowledge, this is the first study to investigate the associations between these psychological traits and HDHEA concentrations. Our results differ from Bürgin et al. (2020), who found associations between resilience factors and HDHEA levels. However, their sample was younger than ours (*M*=35.20 vs. *M*=67.36, respectively), and they did not study optimism per se. Further studies need to determine whether the relationship between optimism and

HDHEA levels differs depending on age and study their relationship with pessimism in populations of young and older people.

We also failed to find sex differences in the relationships between these protective/vulnerability factors and chronic stress biomarkers (HC, HDHEA, HC:$HDHEA_{ratio}$). Again, only one study looked for sex differences, and it found a positive relationship between sense of coherence and HDHEA only in women (Bürgin et al., 2020). However, the only study that looked for associations between optimism and HC did not address sex differences. Our results are supported by several studies that failed to find differences between men and women in HC concentrations (Dettenborn et al., 2012; Gao et al., 2010; Manenschijn et al., 2011; Raul et al. 2004; Thomson et al., 2010), although sex differences have also been found (for a meta-analysis see: Stalder et al., 2017). In fact, the question of whether the sex variable is associated with HC is still unclear, and future studies should address sex differences in the relationship between optimism or pessimism and chronic stress biomarkers.

Our study has some strengths, such as using HC and HDHEA concentrations as well as the ratio, which seem to be potential indicators of chronic stress exposure that would be better than saliva samples, which need rigorous methodological control (e.g., adherence problems). Thus, it may be interesting to take HC and HDHEA measures into consideration when studying the relationship between vulnerability/protective factors and long-term stress exposure. However, our results should be viewed with some limitations in mind. It would be advisable to replicate these results in larger samples and in longitudinal studies in order to shed light on the causality of the relationships. Moreover, it would be useful to include other variables, such as coping or social networks, to study the moderators between optimism/pessimism and the HPA axis more in depth. Although we controlled for several covariates, such as medication/disease,

waist: hip circumference, and BMI, among others, we did not have a strictly healthy older sample. This allowed us to obtain results that are more generalizable to the general population, but a more homogenous sample could have provided a clearer picture of the relationships studied.

Conclusions

In sum, our results contribute to clarifying the role of optimism and pessimism in relation to chronic stress biomarkers. Our results show the importance of dispositional optimism in the association with the HPA axis through lower levels of HC and the HC:HDHEA ratio. Moreover, they highlight the role of pessimism as a negative trait related to a maladaptive HPA axis response. It is important to study the psychological factors of vulnerability and coping in relation to physiological variables related to health, especially in the elderly population, in order to promote healthy aging. Other studies have highlighted the role of optimism in the aging process. More interventions should focus on the weight of positive and negative expectations about the future in health outcomes.

Chapter IV

Study 3: The role of resilience, affective states, and the HPA axis in Subjective Memory Complaints in healthy young people

The main results of this study have been published in:

Zapater-Fajarí, M., Crespo-Sanmiguel, I., Perez, V., Hidalgo, V., & Salvador, A. (2022). Subjective memory complaints in young people: the role of resilience. *Psychology & health*, 1–20. Advance online publication. https://doi.org/10.1080/08870446.2022.2141240

Introduction

Subjective memory complaints (SMCs) are understood as the perception of repeated forgetfulness about everyday aspects that decreases individuals' trust in their own cognitive capabilities (Lozoya-Delgado, et al., 2012; Sunderland et al., 1986). SMCs are frequent in the elderly, but they are also present in young people (Molina-Rodríguez et al., 2016; Pearman, 2009; Pellicer-Porcar et al., 2014). However, apart from this evidence, little is known about their origin and associated factors in the young population. In fact, the Subjective Cognitive Decline Initiative (SCD-I) working group defended the need to specifically explore the different age populations. This working group highlighted that at older ages SMCs would be greater associated with Alzheimer's Disease (AD) and neurodegeneration, whereas at younger ages, other different factors might be more important, such as medical conditions (i.e., epilepsy, head trauma, etc.) and substance misuse (Jessen et al., 2014). Furthermore, in healthy young people, some personality traits and the exposure to stress and its effects have also been pointed out (Jessen et al., 2020; Molina-Rodríguez et al., 2016). Because SMCs appear to be a complex and multifactorial construct, a better conceptualization and more investigation are necessary, given their impact on people's well-being and self-confidence (Montejo-Carrasco et al., 2013; Pearman, 2009).

Some studies have investigated the role of negative affective states such as depression and anxiety in SMCs. Specifically, several studies have been carried out in mixed-aged samples (Derouesné et al., 1999; Montenegro et al., 2013; Pellicer-Porcar et al., 2014; Söğütlü & Alaca, 2019) and a few in young people (Loprinzi, 2019; Pearman, 2009). In addition, some studies have also tried to know the relative importance of each affective state in the presence of SMCs. Along these lines, some results suggest that the best predictor of SMCs is depression, followed by anxiety (Derouesné et al., 1999;

Montenegro et al., 2013), whereas another study states the opposite (Pellicer-Porcar et al., 2014). In contrast, Pearman (2009) reported that the presence of SMCs in young people was better explained by personality traits.

Currently, information is available about some traits that are positively associated with SMCs, but there is a lack of knowledge about traits that are negatively related to them and can act as protectors. Among the stable traits, resilience seems to be a good resource for coping with stress and adversity (VanMeter & Cicchetti, 2020), and, consequently, it could be negatively related to SMCs. Resilience has been defined as the ability to successfully adapt to stressful life events, maintain physical and psychological functioning, and even experience growth beyond recovery (Wu et al., 2013). To date, only one study found that resilience was partially associated with SMCs (Montejo-Carrasco et al., 2013). However, greater resilience has been associated with lower depression, anxiety, and negative affect and more positive affect and life satisfaction in young individuals (Gloria & Steinhardt, 2016; Shi et al., 2015; Smith et al., 2016). Hence, resilience could be negatively linked to SMCs because of its negative association with depression and anxiety, given that it has been suggested that resilient individuals show better adjustment to stress (Ruiz-Robledillo et al., 2017). Thus, based on the proposed relationship between stress and SMCs, the perception of memory impairment could be associated with depressive and/or anxious symptomatology (Reid & MacLullich, 2006; Schweizer et al., 2018).

One of the main components of the stress response is the Hypothalamic-Pituitary-Adrenal axis (HPA axis), and its functioning is a good index of exposure to stress. The role of HPA axis functioning in negative affective states (Pariante & Lightman, 2008) and neurodegeneration and memory processes has been clearly recognized (Ouanes & Popp, 2019). The importance of this axis in SMCs has been recognized in older people

(Fiocco et al., 2006; Peavy et al., 2013), but this relationship has not yet been studied in young people.

In healthy young subjects, depression and anxiety have been associated with a general hyperactivation of the HPA axis (Heaney et al., 2010; Pruessner et al., 2003). HPA axis hyperactivation due to chronic stress has been related to structural alterations in brain regions, such as the hippocampus, that are crucial in memory processes (Lupien et al., 2018). However, subclinical depression has been related to an attenuated Cortisol Awakening Response (CAR), an index that reflects the dynamic of morning cortisol (Dedovic & Ngiam, 2015), whereas resilience to stress has been related to a higher CAR (Lai et al., 2020). Inconclusive results have been found for the relationship between the CAR and memory performance, with most of them suggesting that a greater, rather than lower, CAR is associated with better memory and executive function performance in young and older adults (Law & Clow, 2020). Given previous evidence about the relationship between psychological states and the HPA axis in young people, as well as the correspondence between the HPA axis and memory performance in older people, understanding the factors associated with SMCs, such as physiological stress indexes at young ages, could help to better understand this concept and its meaning at this age.

As far as we know, only three studies have investigated the role of cortisol secretion in SMCs, and all of them were carried out in older populations (Fiocco et al., 2006; Peavy et al., 2013; Wolf et al., 2005). However, only Peavy et al. (2013) found that older adults with SMCs had lower CAR compared to subjects without SMCs, and that the CAR contributed to the prediction of SMCs. No previous study has investigated the relationship between cortisol and SMCs in young people, although there is strong evidence about age differences in cortisol secretion. Older people exhibit a lower CAR

than younger people (Heaney et al., 2012; Kudielka & Kirschbaum, 2003). Thus, it is possible that, given the age differences in the CAR, the relationship between the CAR and SMCs could be different in young vs older people.

Hence, we aimed to confirm the importance of some negative affective states in SMCs in young people, further clarify which affective state has the greatest weight in predicting SMCs, and find out whether resilience is negatively related to SMCs in a sample of young people. Because no previous studies have addressed whether HPA axis functioning is related to SMCs in young people, we wanted to explore this association. More importantly, our ultimate goal was to test whether resilience was related to SMCs through the mediation of depression, anxiety, and CAR. First, we expected that depression and anxiety (Loprinzi, 2019; Pellicer-Porcar et al., 2014), would be related to more SMCs in young adults. Second, given that only one study has addressed the relationship between the CAR and SMCs in older adults (Peavy et al., 2013), and no studies have investigated this association in young individuals, and due to the age-related differences in the CAR, no hypotheses were proposed about the association between the HPA axis and SMCs. Moreover, we hypothesized that depression would be more strongly related to SMCs (Derouesné et al., 1999; Montenegro et al., 2013) when investigating all of these variables together to determine which one is the best predictor of SMCs. Finally, we hypothesized that the negative association between resilience and SMCs would be due to lower depression and anxiety and a greater CAR. Thus, resilience would be negatively related to depression and anxiety (Hjemdal et al., 2011) and in turn, depression and anxiety would be positively associated with SMCs. Finally, resilience would be positively related to the CAR (Lai et al., 2020), although, as mentioned above, no hypotheses were proposed for the relationship between the CAR and SMCs.

Materials and Methods

Participants

Participants were college students recruited through informative talks and posters at the faculties of the University campus. They were interviewed by phone to find out whether they met the study prerequisites. The exclusion criteria were age below 18 years old, smoking more than 10 cigarettes a day, abuse of alcohol (more than 20g/day for women and 30 g/day for men) or drug consumption, non-corrected visual or hearing problems, metabolic, neurological, or psychiatric diseases, using any medication directly related to emotional or cognitive functioning or able to influence hormonal levels, such as glucocorticoids, psychotropic substances, or sleep medications, and having been under anesthesia once in the past three months. Participants were also asked a question about the presence of a stressful life event or change in their habits during the past year. The following examples were given to guide them (e.g., death of a relative, serious personal illness, serious personal accident or injury, serious illness in the family, breaking up a relationship, change in work/educational activities, change in residence, and so on), and participants who reported any event they subjectively felt was stressful for them were excluded from the study. Given that the use of oral contraceptives in women has been associated with increased free cortisol levels and low HPA axis reactivity (Boisseau et al., 2013; Kajantie & Phillips, 2006; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005), we selected women who did not take oral contraceptives in order to reduce the effect of sex hormones on HPA axis activity. All the women were regular and free-cycling and participated in the study during the follicular phase (6th to 10th day) of their menstrual cycle. The menstrual cycle phase was determined by asking over the phone about the regularity and length of the menstrual cycle. Then, we estimated and verified by phone the day of onset of the next

menstruation. Thus, the day of the appointment was between six and 10 days after the start of menstruation.

Initially, 82 healthy young adults participated in the study (42 men, 40 women), and five participants were excluded from the analyses due to missing data (4 from depression and anxiety, and 1 from resilience). The final sample was composed of 77 participants (40 men and 37 women) between 18 and 34 years old (Total: M = 22.250, SD= 3.826) with a medium subjective socioeconomic status (SES; measured using the nine-rung 'social ladder', cf., Adler & Stewart, 2007; considering that 1 was the lowest salary and lowest educational level, and 10 was the highest salary and the highest educational degree; Total: M = 5.910, SD = 1.183). Of the total sample, 70.1% were undergraduates. Finally, the mean body mass index (BMI) was 22.720 (SD= 3.687). Given that the criterion for SMCs in the healthy population excludes psychiatric or neurological disease, medical disorders, or medications directly related to emotional functioning (Jessen et al., 2014), we controlled that our participants did not meet severe subclinical symptoms of depression and anxiety. To do so, we checked that none of the participants had severe symptoms of depression or anxiety, based on the clinical thresholds for depression (Beck et al., 1996) and anxiety (Beck et al., 1988). Participants' characteristics are presented in Table III.1.

Table III.1 Means and standard errors for the study measures in the total sample and by sex.

	Total sample (N=77)	**Men (N=40)**	**Women (N=37)**
Age (years)	22.25	23.00	21.43
	(3.826)	(3.948)	(3.536)
SES	5.91	6.05	5.76
	(1.183)	(1.154)	(1.211)
BMI (kg/m^2)	22.72	23.23	22.17
	(3.687)	(3.993)	(3.290)
MFE-30	24.38	22.28	26.65
	(15.814)	(15.555)	(15.988)
Educational level (%)	-	-	-
No studies	68.80	62.5	75.7
Basic studies	1.30	2.5	0
High school	18.20	20.0	16.2
College or higher	11.70	15.0	8.1
PSS	20.21	19.80	20.65
	(8.428)	(9.576)	(7.088)
BDI	7.36	7.80	6.78
	(6.220)	(7.108)	(5.138)
BAI	7.49	7.03	7.65
	(6.668)	(7.156)	(6.179)
CD-Risc	28.65	28.23	29.30
	(6.129)	(6.375)	(5.887)
CAR	.449	.441	.458
	(.505)	(.581)	(.410)

Note: Data are presented for the total sample (n=77) except for CAR analyses (n=73; 39 men and 34 women). SES = Subjective socioeconomic status; MFE-30 = Everyday Life Memory Failure Questionnaire; BDI = Beck Depression Scale; BAI = Beck Anxiety Inventory; CD-Risc = Connor-Davidson Resilience; CAR = cortisol awakening response. Data represent means (standard errors).

Procedure

Participants who met the criteria were contacted by telephone and asked to maintain their general habits, sleep the usual number of hours, and avoid doing intense physical exercise or consuming alcoholic drinks from the day before the session. When participants arrived at the laboratory, the experimenter verified whether they had followed these instructions. All the participants signed the informed consent to participate in the study, which was designed and carried out in accordance with the Declaration of Helsinki, and the Ethics Committee of the University of Valencia previously approved the experimental protocol (Code: 1034878). Data collection took place between April 2017 and April 2018.

In the session, the psychological tests were administered. Additionally, participants were asked to provide ten saliva samples at home on two consecutive days in order to determine cortisol levels immediately after waking (awakening cortisol) and 15, 30, and 45 min post-awakening. After a demonstration by the experimenter in the lab about how to collect saliva samples, participants were given written instructions and advised to drink only water and not eat, smoke, or brush their teeth at least 1h prior to each saliva sample. To check adherence to the sampling times, participants were asked to write down the exact time they collected the saliva samples in a diary. Participants were instructed to store their samples in the fridge and bring them to the University as soon as possible, never more than three days after completion. As compensation for participating in the study, participants were given a pen drive.

Instruments

Subjective Memory Complaints (SMCs)

SMCs were measured with the Spanish version (Lozoya-Delgado, et al., 2012) of the Everyday Life Memory Failure Questionnaire (Sunderland et al., 1986). The MFE-

30 explores SMCs through 30 items (e.g., “My memory failures cause me problems in everyday life”, “I forget something that was told to me yesterday or a few days ago”, “I forget where I keep things or I look for them in the wrong place”, etc.). Items are rated on a 5-point Likert scale from 0 (never) to 4 (always), where high scores indicate a higher perception of memory impairment. The Cronbach’s alpha for this study was α = 0.928.

Psychological tests

Perceived stress: To evaluate the degree to which people perceived their lives as stressful, uncontrollable, and unpredictable in the past month, we used the Spanish version (Remor, 2006) of the 14-item Perceived Stress Scale (Cohen et al., 1983). To characterize the sample, we intended to determine how high their perceived stress was, given that previous studies found that the level of stress perception could affect depressive and anxiety states (Bergdahl & Bergdahl, 2002), as well as HPA-axis activity (Miller et al., 2007). Participants had to answer using a 5-point Likert scale (from 0 = never to 4 = very often), with 56 being the highest score and 0 the lowest. Higher scores indicate higher perceived stress. The Cronbach’s alpha for this questionnaire for both studies was α =0.866.

Depression: Depressive state was evaluated using the Spanish version (Sanz et al., 2003) of the Beck Depression Inventory-Second Edition (BDI-II) (Beck et al., 1996). It is a 21-item questionnaire scored from 0 (e.g., I don’t feel sad; I am not discouraged about my future, etc.) to 3 (e.g., I feel so sad or miserable that I can't bear it; I feel like my future is hopeless and will only get worse, etc.). The BDI-II measures cognitive, somatic, and behavioral symptoms of depression in the previous two weeks, with high scores indicating more severe depression. The Cronbach's alpha for this study was α = 0.836.

Anxiety: Anxiety state was evaluated using the Spanish version (Sanz & Navarro, 2003) of the Beck Anxiety Inventory (BAI) (Beck et al., 1988). It is a 21-item questionnaire that addresses anxiety symptoms in the previous week that are shared less with depression (e.g., nervousness, inability to relax, trembling legs, etc.). Items range from 0 (not at all) to 3 (seriously). The Cronbach's alpha for this study was $\alpha = 0.882$.

Resilience: Resilience was assessed using the Spanish adaptation (Notario-Pacheco et al., 2011) of the 10-item version (Campbell-Sills & Stein, 2007) of the Connor-Davidson Resilience Scale (CD-Risc) (Connor & Davidson, 2003). The CD-Risc scale assesses one's ability to evaluate and face adversity and stress in the past month (e.g., able to adapt to change, face adversities, and bounce back; belief that one can deal with whatever comes, etc.). Items are rated on a 5-point Likert scale (0 = rarely, 4 = almost always). The results obtained range from 0 to 40, and higher scores indicate higher levels of resilience. The Cronbach's alpha for this study was $\alpha = 0.839$.

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. Once in the laboratory, the samples were kept in the refrigerator until they were centrifuged at 4000 rpm for 15 min to obtain a transparent supernatant that was stored at -80° C until the analyses were subsequently carried out. The cortisol concentrations were determined by the Salimetrics commercial salivary cortisol enzyme-linked immunosorbent assay kit (Newmarket, UK). The sensitivity of the assay was 0.007 ug / dL, and the intra- and inter-assay coefficients of variation were all below 10%. The samples from each subject were analyzed in the same assay and in duplicate. Cortisol levels were expressed in nmol/L.

Statistical analyses

Student's t test for independent samples and the χ^2 test were performed to evaluate the differences between sexes in sociodemographic variables, perceived stress, SMCs, depression, anxiety, resilience, and the CAR. ANOVAs for repeated measures with Time (Awakening, 30 min, 45 min) as within-subject factors, and Sex (men, women) as a between-subject factor were performed to investigate changes in cortisol levels from awakening to 45 min and between men and women.

Saliva was collected on two consecutive days. Given that cortisol concentrations correlated across days (for awakening cortisol: $r = 0.459$, 15 min: $r = 0.262$, 30 min: $r = 0.383$, 45 min: $r = 0.234$, all $p < 0.05$), we worked with the average cortisol levels for both days. The CAR was calculated as the cortisol area under the curve with respect to increase (AUCi, see Pruessner et al., 2003) from the 0-, 15-, 30-, and 45-min cortisol samples. In some cases, we had data from only one day (three men and three women), and they were included in the analyses instead of the average. The CAR was missing for four participants (three women and one man). Cortisol values were log transformed because they did not follow a normal distribution.

Correlation analyses were performed to investigate the relationships between the sociodemographic variables, SMCs, depression, anxiety, resilience, and the CAR. According to the results of Pearson's bivariate correlation analyses (see Table III.2), age and level of studies were included as covariates in all the analyses because these variables correlated with SMCs.

Linear regression analyses were performed to study the associations between SMCs and depression and anxiety and the CAR. When analyzing the CAR, we included the mean awakening time as covariate.

First, to investigate the association between depression, anxiety, the CAR, and SMCs, separate linear regression analyses were performed with depression, anxiety, or the CAR as independent variable and SMCs (MFE-30) as dependent variable. We conducted hierarchical analyses, including the covariates in Step one, and each affective state (depression or anxiety) or the CAR in Step two. Second, hierarchical multiple regression analyses were performed to study the association between SMCs and the two affective states, considering the specific effect of each variable. In Step one, we added age and level of studies as covariates. Step two contained the affective states. In Step three, we added the CAR.

Following Preacher et al. (2007), we performed mediation analyses to investigate whether depression, anxiety, or the CAR mediates the relationship between resilience and SMCs. We included resilience as independent variable, SMCs as dependent variable, and depression, anxiety, or the CAR as mediator, respectively. Standardized values were used to perform the mediation analysis. Bias-corrected bootstrapping 95% intervals were conducted to assess the mediating effects using 5.000 bootstrap iterations. Confidence intervals that do not contain zero are considered statistically significant. We used Hayes' PROCESS macro model number 4 (Hayes, 2017) with SPSS (version 26; IBM Corporation, Armonk, NY, USA).

For the main analyses (hierarchical regression), we estimated a sample size of 77 participants for a medium effect size (f^2 =0.15, alpha = 0.05 and power = 0.80). Our initial sample was composed of 82 participants to anticipate possible missing data. Moreover, our mediation analyses are based on the bootstrap technique, which draws random samples of a fixed sample size with replacements from the dataset, increasing the statistical power. This approach considers the real sample size and controls for this factor in the analyses (Hayes, 2017).

Standardized residuals were used to detect multivariate outliers (± 3 SD). Specifically, one woman was excluded due to the relationship between depression and SMCs, anxiety and SMCs, and, for the mediation analyses, between resilience and SMCs through depression and anxiety. Tolerance and variance inflation factor values indicated that there were no collinearity issues for the variables included in the regressions.

To perform the statistical analyses, version 26.0 of SPSS was used (IBM Statistics, Chicago, IL, USA). All *p* values were two-tailed, and the level of significance was taken as $p < 0.05$. *Post hoc* planned comparisons were performed using Bonferroni adjustments for the *p*-values. For an easy interpretation of the figures, the values shown represent raw values and not log-transformed values.

Table III.2 Pearson's correlation values among all the factors used

	BMI	Level of studies	SES	BDI	BAI	CD-Risc	CAR	MFE-30
Age	.285*	.666**	−.010	−.071	−.175	−.003	−.016	−.232*
BMI		.210	−.068	.139	−.101	−.165	.167	−.055
Level of studies			−.127	.005	−.095	−.037	.100	−.280*
SES				−.026	.067	−.003	.128	−.145
BDI					.680**	−.607**	.004	.444**
BAI						−.339**	−.058	.326**
CD-Risc							−.118	−.268*
CAR								.049

Note: BMI = body mass index; SES = Subjective socioeconomic status; MFE-30 = Everyday Life Memory Failure Questionnaire; BDI = Beck Depression Inventory; BAI = Beck Anxiety Inventory; CD-Risc = Connor-Davidson Resilience; CAR = cortisol awakening response *p<.05. **p<.01. N= 77, except for CAR analyses where N=73.

Results

Preliminary results

Adherence to the salivary sampling protocol

Some authors have indicated that the delay in the first saliva sample affects the reliability of the CAR measurement and should be controlled. Previous research has suggested that a negative CAR (i.e., a decrease in cortisol levels after awakening) may be caused by a delay in collecting the first saliva sample (Thorn et al., 2006). Following these authors, and in line with previous research by our group (Almela et al., 2012; Hidalgo et al., 2016; Puig-Perez et al., 2016; Pulopulos et al., 2016a, 2016b), we identified the possible non-adherent subjects. To control this issue, we divided the sample into two groups: (i) those who had a positive CAR on both days (2 Day-CAR group) and (ii) those who had a positive CAR on only one day or none (1 or 0 Day-CAR group). Of the total sample, 69.9% of the participants showed a positive CAR on both days (25 women and 26 men), 17.8% of the participants showed a positive CAR on only one day (6 women and 7 men), and the other 12.3% of the participants did not show a positive CAR on either of the two days (3 women and 6 men). When exploring the pattern of cortisol levels, whereas the 2 Day-CAR subgroup showed a steeper rise from awakening to 30 min ($p< 0.001$), the 1 or 0 Day-CAR subgroup showed a flatter rise (Awakening: 2 Day-CAR vs. 1 or 0 Day-CAR $p = 0.001$) (see Figure III.1). No significant differences in age, SES, BMI, educational level, SMCs, affective states, resilience, or sex were found between the 2 Day-CAR and the 1 or 0 Day-CAR (all $p> 0.060$). The differences in the pattern of cortisol levels between subgroups suggest that participants with 1 or 0 Day-CAR might have had a delay when collecting the first saliva sample, although other explanations cannot be ruled out (i.e., unreported diseases). To control for a possible confounder effect of non-adherence to the protocol,

we repeated all the analyses, but excluding those participants who were suspected of being non adherent (1 or 0 Day-CAR). Results did not change when analyses were performed only with the 2 Day-CAR group. Moreover, following Stalder et al.'s (2016) criteria, in order to obtain an accurate measure of the CAR, an interval of 15 minutes is needed within a time window of ±5 between the samples. In our sample, 19% of the participants did not accurately collect their saliva samples on the first or second day of CAR. Results did not change after excluding these participants from the analyses.

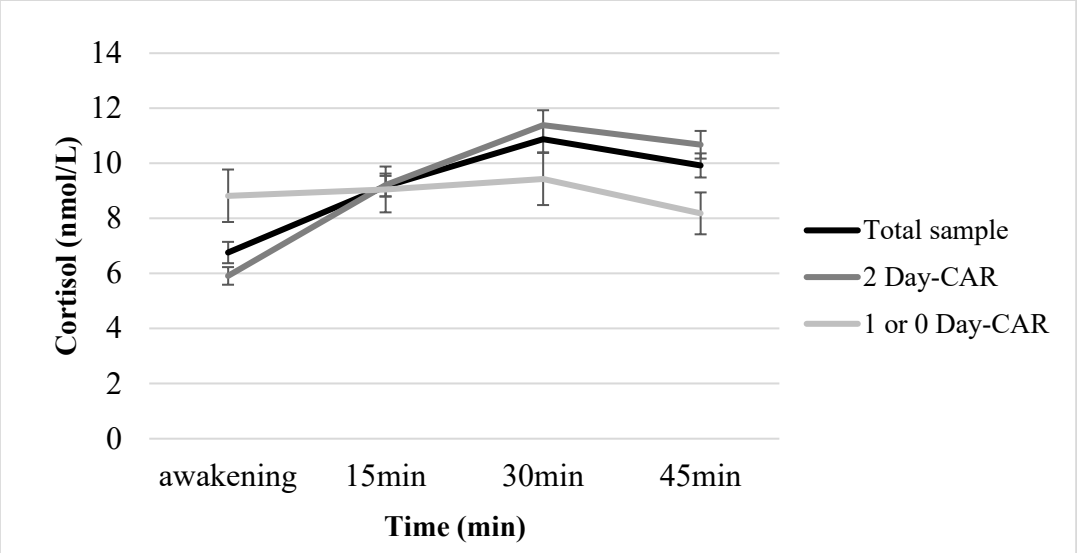
Sample characteristics

Descriptive characteristics are presented in Table III.1. No significant differences between men and women were found for age (t = 1.842, p = 0.072), SES (t = 1.088, p = 0.280), BMI (t = 1.268, p = 0.209), educational level (χ^2 =2.342, p = 0.504), perceived stress (t = −0.439, p = 0.662), SMCs (t = −1.216, p = 0.228), depression (t = 0.714, p = 0.477), anxiety (t = − 0.408, p = 0.685), resilience (t = −0.765, p = 0.447), or CAR (t = − 0.138, p = 0.891).

Cortisol pattern

ANOVAs for repeated measures showed effects of Time ($F_{(1.41, 91.77)}$ = 49.426, p < 0.001, η^2_p = 0.432) and Sex ($F_{(1, 65)}$ = 16.424, p < 0.001, η^2_p = 0.202), but not the Time x Sex interaction ($F_{(1.41, 91.77)}$ = 0.032, p = 0.992, η^2_p < 0.001). Post hoc analyses revealed that cortisol levels significantly increased from awakening to 30 min later (awakening vs. 30 min: p < 0.001). After peaking (+30 min), cortisol concentrations decreased (30 vs. 45 min: p = 0.001). In addition, women showed higher cortisol levels than men. When the same analyses were performed in the 2 Day-CAR group, the results were similar (time: $F_{(1.54, 75.39)}$ = 90.381, p < 0.001, η^2_p = .648; sex: $F_{(1,49)}$ = 5.691, p = 0.021, η^2_p = .104; time x sex interaction, p = 0.555). Figure III.1 shows the CAR pattern for the total sample and considering adherence to the salivary sampling protocol.

Figure III.1 CAR for the total sample and 1 or 0 Day-CAR and 2 Day-CAR groups



Note: Depicted values are means, and error bars represent SEM.

Relationship between SMCs and affective symptoms and HPA axis

Correlation analyses

Pearson's correlation values between all the study variables are presented in Table III.2. Results showed that SMCs were positively related to depression (p < 0.001) and anxiety (p = 0.004), and negatively related to resilience (p = 0.019). No significant correlations were found between SMCs and CAR (all p = 0.678).

Predictors of SMCs

Linear regression analyses showed that SMCs were positively associated with depression and anxiety (p < 0.001 and p = 0.002, respectively). No significant associations were observed between SMCs and CAR (p = 0.507). Hierarchical multiple regression analysis was performed, including depression and anxiety together, in Step two. Results showed that depression was the only predictor of SMCs (p = 0.001) after controlling for anxiety. No relationship was found between SMCs and anxiety (p = 0.868).

Including cortisol in the third step showed that CAR did not predict SMCs when controlling for affective states in Step two (p = 0.482) (Table III.3).

Table III.3 Linear regression and hierarchical multiple regression analyses with subjective memory complaints as dependent factor and affective symptoms or CAR as independent factors

Linear regression analyses

			Dependent variable: Subjective memory complaints	
	R^2	Adj R^2	R^2 change	Beta
BDI	.299	.270	.225	.476**
BAI	.186	.152	.112	.340**
CAR	.078	.022	.006	.083

Hierarchical multiple regression analyses

			Dependent variable: Subjective memory complaints	
	R^2	Adj R^2	R^2 change	Beta
Model	.299	.260	.225	
BDI				.461**
BAI				.023
				Step 3
	R^2	Adj R^2	R^2 change	Beta
Model	.274	.220	.005	
CAR				.075

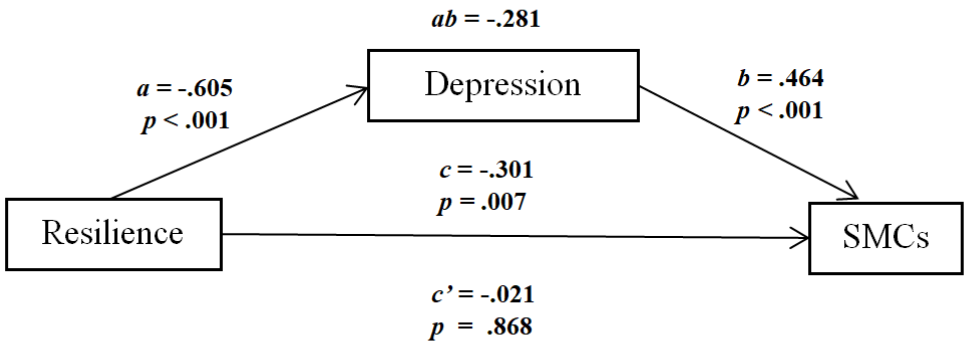
Note: BDI= Beck Depression Scale; BAI= Beck Anxiety Inventory; CAR = cortisol awakening response; *p<.05. **p<.01. N = 76, except for CAR analyses where N = 73.

Mediation analyses between resilience and SMCs

Depression

The mediation analysis showed that higher resilience was associated with less depression (path *a*: $B = -0.605$, $SE = 0.093$, $t = -6.491$, $p < 0.001$). The relationship between depression and SMCs was positive and significant (path *b*: $B = 0.464$, $SE = 0.126$, $t = 3.689$, $p < 0.001$). Analysis of the direct effect of the relationship between resilience and SMCs, controlling for depressive symptoms, showed no effect (path *c'*: $B = -0.021$, $SE = 0.125$, $t = -0.166$, $p = 0.868$). The total effect was negative and significant (Total effect c: $B = -0.301$, $SE = 0.108$, $t = -2.796$, $p = 0.007$). The indirect effect of resilience on SMCs through depression was significant (path *ab*: $B = -0.281$, 95% CI = −0.441 to −0.148) (Figure III.2).

Figure III.2 Mediation analyses

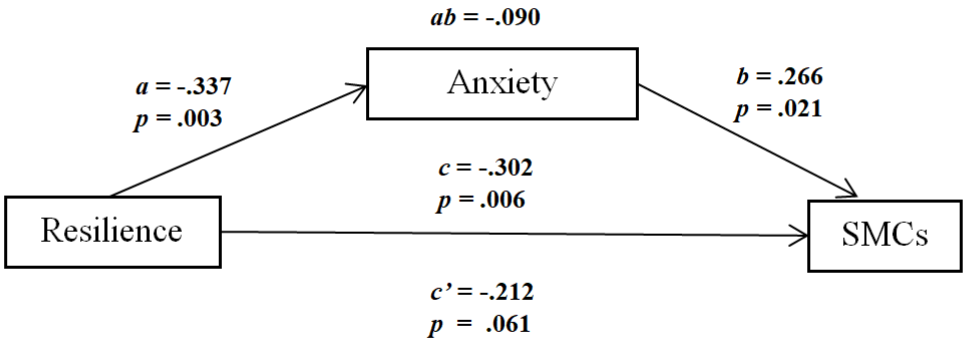


Note Mediation analysis to test the indirect effect of resilience on SMC through depression. Numbers on the lines show *B* and *p* values

Anxiety

The mediation analysis showed that higher resilience was associated with less anxiety (path *a*: *B* =−0.337, *SE* = 0.109, *t* =−3.097, *p* =0.003). The relationship between anxiety and SMCs was positive and significant (path *b*: *B* = 0.266, *SE* = 0.113, *t* = 2.356, *p* = 0.021). Analysis of the direct effect of the relationship between resilience and SMCs, controlling for anxiety, showed no effect (path *c'*: *B* =−0.212, *SE* = 0.111, *t* =−1.901, *p* = 0.061). The total effect was negative and significant (Total effect c: *B* =−0.302, *SE* = 0.108, *t* =−2.796, *p* = 0.006). The indirect effect of resilience on SMC through anxiety was significant (path *ab*: *B* =−0.090, 95% CI =−0.193 to−0.011) (Figure III.3).

Figure III.3 Mediation analyses



Note Mediation analysis to test the indirect effect of resilience on SMC through anxiety. Numbers on the lines show *B* and *p* values

Cortisol

Mediation analysis of the relationship between resilience and SMCs through CAR shows that the relationships between resilience and CAR (path *a*: $B = -0.073$, $SE = 0.059$, $t = -1.232$, $p = 0.222$) and between CAR and SMCs (path *b*: $B = 0.160$, $SE = 0.238$, $t = 0.672$, $p = 0.504$) were not significant. The conditional direct effect (path *c'*) of the relationship between resilience and SMCs, controlling for CAR, was significant ($B = -0.305$, $SE = 0.115$, $t = -2.655$, $p = 0.010$). The total effect (*c*) was negative and significant ($B = -0.317$, $SE = 0.113$, $t = -2.800$, $p = 0.007$). However, the conditional indirect effect (*ab*) of resilience on SMCs through CAR was not significant ($B = -0.012$, 95% CI $= -0.058$ to 0.027).

Discussion

This study investigated the relationships between negative affective states (i.e., depression and anxiety), physiological variables (i.e., the CAR), and SMCs in young people. In addition, it explored their potential mediating effect in the relationship between resilience and SMCs. In agreement with our hypotheses, lineal regression analyses showed that depression and anxiety positively predicted SMCs, but depression was the only significant variable related to SMCs after controlling for anxiety. However, a significant association was not found between SMCs and the CAR. Finally, we confirmed that the negative association between resilience and SMCs was due to the better psychological adjustment in stressful situations shown by resilient individuals. Thus, greater resilience was associated with less depression or anxiety and, consequently, fewer SMCs. No relationship was found between resilience and SMCs through the CAR. These results highlight the importance of resilience in the psychological adjustment to stressful situations, and they explain the negative association between resilience and SMCs.

Our results showed that both depression and anxiety states were significant predictors of SMCs, with depression explaining greater variance in SMCs. The impaired concentration and low motivation and energy associated with depression, along with the attention deficits that characterize anxiety, could influence memory and recall processes and, therefore, contribute to SMCs (Weaver Cargin et al., 2008). In this regard, Molina-Rodríguez et al. (2018) found a positive linear relationship between motivational and attentional problems and SMCs. Moreover, following the *Comprehensive Cognitive Model of Depression*, SMCs could be explained by negative cognitive biases; that is, people with depression tend to selectively pay more attention to negative aspects of experiences and have dysfunctional attitudes or beliefs about themselves (Beck, 2008). Thus, negative cognitive biases associated with depression might lead to an overestimation of their memory failures (Montejo-Carrasco et al., 2013). Therefore, low self-confidence, along with negative attribution patterns about their own memory, can lead individuals to make less effort when remembering, avoid everyday tasks that require memorizing, and experience increased anxiety during tasks that involve memory (Rowell et al., 2016). This could lead to a decline in memory performance and, in turn, further strengthen the subjects' beliefs about their declining memory capacity (Ponds et al., 1997). However, it is also possible that SMCs can act as a stressor and influence the appearance of emotional stress-related states such as depression and anxiety (Jessen et al., 2020). Our findings are in line with studies that have investigated this pattern in young populations, and they corroborate the positive association between SMCs and depression (Loprinzi, 2019; Pearman, 2009; Pellicer-Porcar et al., 2014) and anxiety (Pellicer-Porcar et al., 2014).

Although depression and anxiety seem to be intervening separately in the prediction of SMCs, after including them together in a multiple regression model, the only

significant variable was depression, suggesting that it is the most important factor associated with SMCs. This result is supported by other studies in young people that found that the main predictor of SMCs is depressive symptoms (Derouesné et al., 1999; Montenegro et al., 2013). However, two other studies found that SMCs were more associated with neuroticism (Pearman, 2009) or social anxiety (Pellicer-Porcar et al., 2014). Previous studies about the relationship between affective states and SMCs have yielded inconclusive results because the samples did not exclusively represent the young population (Derouesné et al., 1999; Montenegro et al., 2013). For example, Derouesné et al. (1999) divided the sample into people above and below 50 years of age, and Montenegro et al. (2013) used a sample between 22 and 64 years of age. In our study, we studied a sample consisting exclusively of young adults between 18 and 34 years old. Moreover, Pearman et al. (2009) used the Geriatric Depression Scale (GDS; Sheikh & Yesavage, 1986) as a depression scale, but it is important to mention that this scale was developed for older people. In our study, we used the Beck Depression Inventory (Beck et al., 1996), which has been used more with younger people. Pellicer-Porcar et al. (2014) reported that social anxiety was more strongly associated with SMCs than depression, perhaps because of the strong collinearity between these two variables, as described in Montenegro et al. (2013). In our study, although depression and anxiety were strongly associated, variance inflation factor values (VIF) indicated no collinearity (Depression: VIF = 1.885; Anxiety: VIF = 1.925). Moreover, the questionnaire used in their study divided anxiety into different subtypes (e.g. social anxiety, obsessive compulsive anxiety, etc.), and the results are not comparable with other studies evaluating overall anxiety, as in the present study. Our study tried to solve the limitations of previous literature regarding the age of the participants and the variables involved in the study. Thus, we want to emphasize that specifically studying a

younger population (18 to 34 years old), using adequate depression scales for this population, and finding no multicollinearity between the variables increase the reliability of the results. Despite this, further studies are needed to delve into psychological factors related to SMCs in the young population.

Finally, we failed to find a relationship between SMCs and the CAR. This result could suggest that this physiological stress index is not related to SMCs, or that, in early stages, people with SMCs do not have an altered cortisol pattern. In our sample, perceived stress was low, with scores below the mean of the test. Only one study on the relationship between diurnal HPA-axis activity and SMCs considered the stress perceived by the participants, and it was high, and so the results could not be replicated in less distressed samples (Fiocco et al., 2006). In our sample, the stress experienced by the participants may not have been sufficient to alter the HPA axis. In addition, our results differ from those found in older individuals, where subjects with SMCs had a lower CAR than those without SMCs and a decreased CAR was related to a greater possibility of having SMCs (Peavy et al., 2013). These differences found between young and older adults suggest that the maintenance of a stressor such as the chronic presence of memory complaints could have a significant impact on cortisol secretion (Kudielka et al., 2006). Thus, because our sample consisted of young people, the stress would have to increase, or the SMCs would have to persist over time in order to see an altered HPA axis and its neuropathological effects. Therefore, reducing memory complaints in young people who do not yet present this alteration in cortisol secretion could reduce the presence of pathologies associated with HPA axis dysregulation (Lundberg, 2005).

Regarding the contribution of resilience, we found that it is significantly associated with SMCs, in contrast to Montejo-Carrasco et al.'s results (2013) in a mixed-age

sample. However, these authors found that SMCs were correlated with one resilience factor: trust in one's own instincts. Given that SMCs in young and older individuals are attributable to different causes; different protective factors may be at work in each age group (Ginó et al., 2010). Moreover, the results found in our study could be due to the personal resources present in resilient individuals, as Montejo-Carrasco et al. (2013) showed in their study. Resilience has been related to the use of active coping strategies (i.e., problem-focused coping) and higher self-confidence, which in turn increase well-being (Feder et al 2009; Mayordomo et al., 2016). Resilience can be understood as the ability to maintain psychobiological stability and avoid adversity in situations of acute and chronic stress (Liu et al., 2018). To test this, we studied whether resilience was associated with variables such as depression, anxiety, and the CAR. We found that resilience was negatively related to depression and anxiety, which in turn predicted fewer SMCs. However, resilience was not related to SMCs through the CAR. Resilient individuals may have a greater perception of their own memory because of their psychological characteristics, which specifically involve less depressive and anxious symptomatology, or because resilience attenuates the negative consequences of depression and anxiety symptoms. As stated in the *Broaden-and-Build Theory of Positive Emotions* (Fredrickson, 1998), resilient individuals experience positive emotions even in stressful situations (e.g., trying to remember something, performing difficult tasks under pressure). These positive emotions promote adaptive coping and emotion regulation related to greater flexibility in thinking and exploration and a broadened focus of attention (Fredrickson, 2001). It has also been argued that positive emotions can correct or undo the effects of negative emotions. Therefore, resilient individuals may experience positive emotions in stressful situations, which would decrease the development of psychopathological symptoms (Tugade & Fredrickson,

2004). These findings are consistent with the results found in previous studies regarding the role of resilience as a predictor of psychiatric symptoms such as depression or anxiety (Hjemdal et al 2006; 2011). In addition, these results indicate the importance of resilience in maintaining psychological homeostasis, which in turn could explain its negative relationship with SMCs.

A limitation of the present study is that, due to its cross-sectional nature, we could not assume causality, although the regression analyses performed demonstrated the weight of depression in predicting SMCs. Further studies should observe the relationship between SMCs and psychological or physiological factors longitudinally in order to determine whether depression and anxiety are the cause or the consequence of the complaints. Moreover, only a longitudinal study would help us to find out whether SMCs in young individuals lead to a dysregulation of the HPA axis in older stages. Moreover, participants showed scores below the mean on perceived stress, and so we cannot generalize to other stress levels. Further studies should analyze the relationship between emotional (depression and anxiety) and physiological (HPA axis activity) factors and SMCs in different samples with higher levels of perceived stress that could lead to differences in the relationship between psychological and physiological variables and SMCs. Although we used the time provided in the diary to check for adherence to the sampling time, we did not use objective monitoring of awakening. To control whether the time of awakening was correctly reported, we performed the same statistical analyses for the complete sample and then for the 2 Day-CAR group. Future studies should use recording devices to measure the CAR more objectively.

To date, this is the first study to investigate the importance of depression, anxiety, and HPA axis activity in SMCs, as well as the relationship between resilience and SMCs through these variables in the young population. Overall, our results suggest that

SMCs are related to psychological factors, but not to physiological factors, such as the CAR. They also highlight the importance of resilience in facing stressful situations, thus reducing adverse effects such as SMCs. Intervention programs for young people with SMCs should take into account the negative effect of psychological factors such as depression and anxiety and the protective role of variables such as resilience in the association with SMCs.

Chapter V

Study 4: Relationship between psychological traits, the HPA axis, and Subjective Memory Complaints in young, middle-aged, and older healthy people

The main results of this study have been published in:

Zapater-Fajarí, M., Crespo-Sanmiguel, I., Pérez, V., Hidalgo, V., & Salvador, A. (2022). Subjective Memory Complaints in young and older healthy people: Importance of anxiety, positivity, and cortisol indexes. *Personality and Individual Differences, 197*, 111768. https://doi.org/10.1016/j.paid.2022.111768

Introduction

Subjective Memory Complaints (SMCs) are common in elderly people, but also in younger adults (Derouesné et al., 1999; Ginó et al., 2010; Mendes et al., 2008). For this reason, there is a need to study SMCs across the lifespan. In both young and older adults, SMCs have been related to stress perception (Lozoya-Delgado et al., 2012; Molina- Rodriguez et al., 2016; Ruiz-Sánchez de León et al., 2014) and negative affective states such as anxiety and depression (Derouesné et al., 1999; Loprinzi, 2019; Montenegro et al., 2013; Pearman, 2009; Pellicer-Porcar et al., 2014; Söğütlü & Alaca, 2019). Moreover, some stable factors have also been found to be potential predictors of SMCs (Carrigan & Barkus, 2016; Pearman & Storandt, 2004; Pearman, 2009).

Trait anxiety is a stable factor that has been related to SMCs in studies with younger (Mecacci et al., 2004), older (Balash et al., 2013; Norman et al., 2020; Pavisic et al., 2021; Sabatini et al., 2022), and mixed-aged samples (Mahoney et al., 1998). Trait anxiety can be understood as the predisposition to perceive situations as threatening, making people more vulnerable to stress conditions (Spielberger et al., 1970) and, thus, affecting attention and memory processes (Eysenck et al., 2007). Based on the association between SMCs and perceived stress, trait anxiety could be related to SMCs. To date, however, only one study has addressed the relationship between trait anxiety, along with other trait negative affect factors such as depression and neuroticism, and SMCs across the lifespan, finding a positive relationship in all the age groups studied: young (ages 18–39), early middle-aged (40–54), middle-aged (ages 55–64), older (ages 65–74), and oldest-old (ages 75–99) adults (Rowell et al., 2016). Hence, more studies are needed to investigate the exclusive relationship between trait anxiety and SMCs while considering the age factor.

In contrast to negative affective dimensions, positive traits or strategies have been studied less. Among them, positivity is a personality dimension that facilitates effective coping with stressful events, as the *Positive Orientation Theory* states (Caprara et al., 2010, 2012). Given the positive association between stress perception and SMCs, and the negative association between stress and positivity, it makes sense to study the possible negative relationship between positivity and SMCs. In fact, it has been reported that people with higher positivity due to their lower stress perception have less negative affect and more positive affect (Horiuchi et al., 2018). At the same time, positive affect has been negatively related to SMCs in older adults (Lee, 2016). Thus, positive individuals, through better adjustment to stressful situations and higher positive affect, could be attenuating the negative effects of stress on memory (Lupien et al., 2018). However, to our knowledge, no study has specifically investigated the relationship between positivity and SMCs in older or young individuals, although a few studies have focused on other related dimensions, such as positive coping, extraversion, and optimism. Specifically, positive coping was negatively related to SMCs in young adults (Molina-Rodriguez et al., 2016), and extraversion was negatively associated with SMCs in both young and older adults (Sutin et al., 2020). Recently, optimism has been related to fewer SMCs in older adults (Fastame, 2022). More research on the importance of positivity could help us to understand individual differences in SMCs across the lifespan and prevent or slow down their appearance.

The Hypothalamic- pituitary- adrenal axis (HPA axis) has been related to both personality traits and memory processes (Adam et al., 2017; Ouanes & Popp, 2019). In healthy conditions, the HPA axis follows a circadian pattern characterized by a significant decrease in cortisol, one of the main products of the HPA axis, from morning to bedtime (Adam & Kumari, 2009). Higher awakening cortisol levels and a steeper

diurnal cortisol slope (DCS; i.e., the decrease in cortisol levels secreted throughout the rest of the day) have been associated with anxiety, although further research is required (Adam et al., 2017; Vreeburg et al., 2010). Low positivity has also been related to higher awakening cortisol levels (Pasquali et al., 2020). Additionally, it is well known that hyperactivity of the HPA axis has been associated with cognitive impairment (Wolf, 2003). Moreover, as people age, the DCS (measured as the difference between wake-up to bedtime levels) changes, becoming flatter (Nater et al., 2013). In addition, in healthy older people, poor memory performance and memory decline have been associated with a flatter DCS, measured as both regression slopes (Abercrombie et al., 2004) and as the difference between 30 after-awakening to bedtime measures (Gerritsen et al., 2011).

To date, only two studies have investigated the correspondence between SMCs and the DCS. These two studies found that people with SMCs had overall higher diurnal cortisol concentrations (Peavy et al., 2013), as well as a flatter DCS calculated by regression slopes (Fiocco et al., 2006). However, no previous studies have investigated this relationship in younger adults. Although this association could be directly caused by age-related factors in older individuals, other causes may be underlying this relationship in young adults. Given the relationship between psychological traits with the HPA axis and SMCs, individual differences in these traits may determine the predisposition to or protection against experiencing problems associated with the HPA axis, such as memory problems (Wingenfeld & Wolf, 2011). Thus, psychological traits may be modulating the relationship between the HPA axis and SMCs in young adults. Moreover, this relationship could be different depending on the age, given that the pattern of cortisol secretion changes during the aging process (Nater et al., 2013). Looking for the physiological mechanisms related to SMCs and their differences

throughout aging could help to reveal the causes or consequences of this daily life forgetfulness. Thus, given that stress is related to SMCs, and that anxiety, positivity, and cortisol measures have been related to better or worse coping with stress, it is reasonable that these psychological traits and cortisol indexes would be related to SMCs.

In two independent samples of young and older individuals, the present study aimed to find out whether: (1) stable psychological traits such as trait anxiety and positivity are associated with SMCs; and (2) the relationship between the HPA axis and SMCs is direct or modulated by these psychological traits. We expected that higher trait anxiety would be related to more SMCs (Rowell et al., 2016), whereas higher positivity would be related to fewer SMCs in both age groups (Lee, 2016; Molina- Rodriguez et al., 2016; Sutin et al., 2020). Although we could not establish a hypothesis about the direct relationship between cortisol indexes and SMCS because no study has addressed this issue in young populations, we expected that higher awakening cortisol and a steeper DCS would be related to more SMCs, mainly in individuals with high anxiety and low positivity (Adam et al., 2017; Pasquali et al., 2020; Vreeburg et al., 2010). In older samples, we expected the relationship between DCS and SMCs to be direct. In line with previous studies in this age range, a flatter DCS would be related to more SMCs (Fiocco et al., 2006; Peavy et al., 2013). Regarding the awakening cortisol-SMCs link, we could not propose any hypotheses, although if this association were moderated by psychological traits as in young people, then higher awakening cortisol would be associated with more SMCs in people with high anxiety and low positivity. Moreover, given the influence of aging on HPA functioning (Nater et al., 2013) and the suggested different meanings of SMCs in older individuals before and after 65 years of age (Jessen et al., 2014), we also explored whether the association between psychological

traits and the HPA axis and SMCs is different in these two age groups (aged 55 to 64 vs 65 to 75). We wanted to study the importance of changes during aging in depth, and so we selected the cut-off of 65 years because it is a period of change from middle age to early elderly. It has been suggested that in older people below the mid-60s (around 65 years old and less), the presence of SMCs is more related to non-degenerative causes, whereas above this age, SMCs start to be associated with an increasing likelihood of preclinical Alzheimer's disease (Jessen et al., 2014, 2020).

Materials and Methods

Participants

Participants in Study 1 and Study 2 were volunteers recruited at university courses or through posters at the university campus. After the volunteers enrolled in the study, they were interviewed to assess whether they met the inclusion criteria. Inclusion criteria were: smoking <10 cigarettes a day, no alcohol or other drug abuse, no visual or hearing problems, diabetes, or the presence of an HPA axis, neurological, or psychiatric disease, not using any medication directly related to cognitive or emotional functioning or able to influence hormonal levels and sleep (e.g., glucocorticoids, psychotropic substances, or sleep medications), not having been under anesthesia in the past three months, and age between 18 and 35 years old (Study 1) or between 55 and 75 years old (Study 2). Participants were also asked a question by telephone about the presence of a stressful life event or change in their habits during the past year. To guide the participants, some examples were given (e.g., death of a relative, serious personal illness, serious personal accident or injury, serious illness in the family, breaking up a relationship, change in work/educational activities, change in residence, and so on), and those who reported any event they subjectively considered stressful to them were excluded from participating in the study. All the women who participated in Study 1

were in their follicular phase (6th to 10th day) and were not contraceptives users, whereas all the women who participated in Study 2 were postmenopausal, and none of them were receiving hormone replacement therapy. In both studies, socioeconomic status (SES) was measured with the MacArthur Scale of Subjective Social Status (Adler & Stewart, 2007). Participants rated themselves from 1 (people with the lowest education and income and the worst jobs) to 10 points (people with the best education, income, and jobs). The characteristics of the samples in both studies are shown in Table IV.1.

Table IV.1 Sample characteristics for the total sample and for men and women in the two studies

	Study 1			**Study 2**					
	Young adults			*Middle-aged adults* (55-64 years)			*Older adults* (65-75 years)		
	Total sample (n=75)	Men (n=37)	Women (n=38)	Total sample (n=35)	Men (n=16)	Women (n=19)	Total sample (n=38)	Men (n=23)	Women (n=15)
Age (years)	22.19 (3.82)	23.05 (3.99)	21.34 (3.51)	59.60 (2.79)	59.69 (2.85)	59.53 (2.82)	69.11 (3.06)	69.39 (3.16)	68.87 (2.94)
SES	5.85 (1.18)	6.03 (1.19)	5.68 (1.17)	5.83 (1.54)	5.56 (1.75)	6.05 (1.35)	6.47 (1.62)	6.61 (1.34)	6.27 (2.02)
BMI *(kg/m2)*	22.66 (3.69)	23.47 (4.08)	21.88 (3.12)	26.48 (4.42)	28.09 (3.87)	25.12 (4.49)	26.74 (3.67)	28.17 (3.52)	24.39 (2.60)
MFE-30	23.16 (15.94)	20.35 (15.50)	25.89 (16.09)	28.06 (18.12)	26.50 (16.35)	29.37 (19.83)	22.37 (13.88)	20.96 (11.98)	24.53 (16.59)

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Continuation of **Table IV.1**

	Study 1			**Study 2**					
	Young adults			*Middle-aged adults* (55-64 years)			*Older adults* (65-75 years)		
	Total sample (n=75)	Men (n=37)	Women (n=38)	Total sample (n=35)	Men (n=16)	Women (n=19)	Total sample (n=38)	Men (n=23)	Women (n=15)
Education Level (%)									
Without studies	0	0	0	2.9	0	5.3	2.7	0	6.7
Primary school	0	0	0	11.4	18.8	5.3	2.7	0	6.7
Secondary school	66.7	59.5	73.7	37.1	18.8	52.6	21.6	31.8	6.7
Upper grade	1.3	2.7	0	0	0	0	0	0	0
Graduate (3 years)	18.7	21.6	15.8	20	25	15.8	35.1	31.8	40
Graduate (5 years)	13.3	16.2	10.5	28.6	37.5	21.1	37.8	36.4	40
STAI	18.99	17.38	20.55	16.54	15.81	17.16	14.50	13.17	16.53
	(9.05)	(9.91)	(7.94)	(7.10)	(7.77)	(6.63)	(8.79)	(8.15)	(9.63)
POS	30.79	31.00	30.58	32.00	31.94	32.05	30.68	29.61	32.33
	(5.41)	(5.92)	(4.94)	(3.84)	(4.04)	(3.78)	(4.87)	(4.69)	(4.82)
Awakening Cortisol	.796	.742	.852	.831	.846	.819	.786	.812	.747
	(.21)	(.22)	(.20)	(.17)	(.18)	(.17)	(.27)	(.24)	(.32)
DCS	.781	.778	.783	.682	.683	.681	.613	.646	.563
	(.32)	(.33)	(.32)	(.32)	(.31)	(.34)	(.30)	(.27)	(.35)

Note: In Study 2 for BMI and level of studies (N=72). SES = Subjective socioeconomic status; MFE-30= Everyday Life Memory Failure Questionnaire; STAI = State Trait Anxiety; POS = Positivity Scale; DCS = diurnal cortisol slope. Data represent means (standard deviations), except for educational level (%).

Study 1

Participants in Study 1 were recruited from different bachelor's degrees at the University of Valencia. Eighty-two healthy young adults participated in the study (42 men and 40 women). In order to exclude comorbidities with depression, all the participants completed the Beck Depression Inventory-II (BDI-II; Beck et al., 1996), and three men who scored higher than 20 were excluded from the analyses. Additionally, four participants (two men and two women) were excluded from the analyses due to missing data (two from anxiety and two from awakening cortisol). Therefore, the final sample was composed of 75 participants (37 men and 38 women).

Study 2

Participants in Study 2 were mainly recruited from a study program for people over 55 years old offered by the University of Valencia. Eighty-two healthy adults participated in the study (40 men and 42 women). In order to exclude comorbidities with depression, all the participants completed the BDI-II, and three women who scored higher than 20 were excluded from the analyses. Additionally, six participants (one man and five women) were excluded from the analyses due to missing data (two from positivity, one from anxiety, and three from DCS). Hence, the final sample was composed of 73 participants (39 men and 34 women). In accordance with the Subjective Cognitive Decline Initiative working group (SCD-I), the construct of SMCs implies the absence of an objective cognitive impairment (Jessen et al., 2014). Therefore, to exclude older participants with possible cognitive impairment, we measured it with the Mini-Mental State Examination (MMSE), and the sample mean was below the clinical threshold for cognitive impairment (Folstein et al., 1975). All subjects' scores were ≥24 (M = 28.93; SD = 1.378).

Procedure

Both studies were designed and carried out in accordance with the Declaration of Helsinki, and the protocols were approved by the Ethics Committee of the University of Valencia (Code: 1034878). All participants approved and signed the written informed consent.

Participants were asked to collect salivary samples at home to measure cortisol levels immediately before going to sleep (bedtime cortisol) and immediately after awakening (awakening cortisol) on two consecutive days. Moreover, participants went to the laboratory to fill out the psychological tests. The sessions were held in the morning (10–12 h or 12–14 h) or in the afternoon (16–18 h or 18–20 h), and the time when the participants started the sessions was counterbalanced across sex.

Instruments

Subjective Memory Complaints (SMCs)

SMCs were evaluated using the Spanish adaptation (Lozoya-Delgado et al., 2012) of the Everyday Life Memory Failure (MFE-30) questionnaire (Sunderland et al., 1986). This questionnaire contains 30 items about situations and activities of daily life (e.g., My memory failures cause me problems in everyday life; I forget something that was told to me yesterday or a few days ago). Items are rated on a 5-point Likert scale ranging from 0 (never or almost never) to 4 (always), where higher scores indicate more everyday memory failures. Internal consistency of the scale in both studies was $\alpha = 0.931$.

Trait anxiety

Trait anxiety was evaluated with the Spanish version (Seisdedos, 1988) of the Trait Anxiety Inventory (STAI-T) (Spielberger et al., 1970). Participants have to rate how

they feel most of the time using a Likert scale ranging from 0 (rarely) to 3 (usually). The Cronbach's alpha in both studies was $\alpha = 0.867$.

Positivity

Positivity was evaluated using the Spanish version of the Positivity scale (POS) (Caprara et al., 2012). This is an 8-item scale designed to assess a positive view of oneself, one's life, and one's future, as well as one's confidence in others. POS is a common factor that includes self-esteem, optimism, and life satisfaction (e.g., I have faith in the future, others are generally here for me when I need them, I feel I have many things to be proud of, or I generally feel confident about myself). Items are rated on a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Results obtained range from 1 to 45, and so higher scores indicate higher positivity or a more positive outlook on life. Internal consistency of the scale in both studies was $\alpha = 0.859$.

Salivary Cortisol

Saliva samples were collected using salivettes (Sarstedt, Nümbrecht, Germany). To measure cortisol levels, all the saliva samples for each participant were analyzed in the same assay and in duplicate. The cortisol concentrations were determined by the Salimetrics commercial salivary cortisol enzyme-linked immunosorbent assay kit (Newmarket, UK). Assay sensitivity was 0.007 μg/dL, and the intra- and inter-assay coefficients of variation were all below 10 %. Cortisol levels were expressed in nmol/L. Participants were told how to provide the saliva samples, that is, keeping the cotton swab in their mouths for exactly 2 min, not chewing the cotton, and moving the swab around in a circular pattern to collect saliva from all the salivary glands. Participants were given written instructions and advised to drink only water and not eat, smoke, or brush their teeth at least 1 h prior to each saliva sample. To objectively verify participant adherence to the saliva sampling time at home, salivettes were stored in

MEMS TrackCap containers (MEMS 6 TrackCap Monitor, Aardex Ltd. Switzerland), which recorded the exact time the participants opened the container to obtain a cotton swab and provide a sample. Participants also recorded the time of awakening and each saliva collection in a diary.

Statistical analyses

Cortisol values were log transformed because they did not follow a normal distribution. In some cases, we had data from only one day, which were included in the analyses rather than the average (In Study 1, we had four cases, one man for awakening cortisol and two men and one woman for bedtime cortisol; in Study 2, we had eight cases, one man and three women for awakening cortisol and one man and three women for bedtime cortisol). The DCS was calculated by subtracting average bedtime cortisol from awakening cortisol. This method was used rather than including the Cortisol Awakening Response (CAR) in the DCS because it is influenced by different biological mechanisms from the rest of the diurnal cortisol rhythm (Adam et al., 2017; Clow et al., 2010). For the DCS, a larger value is interpreted as a steeper slope, whereas a smaller value is interpreted as a flatter slope, reflecting less cortisol decline during the day.

Student's *t*-tests for independent samples were performed to evaluate sex (in Study 1 and Study 2 separately) and age group (only in Study 2: aged 55 to 64 vs 65 to 75) differences in sociodemographic variables, SMCs, trait anxiety, positivity, awakening cortisol, and DCS. However, sex and age group differences in educational level were analyzed with Chi-square tests. Bivariate Pearson correlation analyses were used in both studies to investigate the relationships among the sociodemographic variables, SMCs, trait anxiety, positivity, awakening cortisol, and DCS (see Table IV.2). In Study 1, according to the results found in the correlation analyses, age and level of studies were included as covariates in all posterior analyses because they significantly correlated

with SMCs. In Study 2, no correlations were found between SMCs and sociodemographic variables, and so we did not add covariates. In both studies, when analyzing cortisol indexes, we included as covariates: mean awakening time for awakening cortisol or mean time from awakening to bedtime for DCS.

Table IV.2 Pearson's correlation values among all the factors used

Study 1 / Study 2	Age	BMI	Level of studies	SES	STAI	POS	Awakening cortisol	DCS	MFE-30
Age		.256*	.670**	−.051	−.057	.024	.007	.141	−.267*
BMI	−.024		.191	−.63	.098	−.192	−.071	.125	−.047
Level of studies	.183	−.004		−.141	−.030	.083	−.128	.109	−.342**
SES	.214	−.059	.213		−.099	.208	−.181	−.154	−.187
STAI	.009	−.086	−.076	−.157		−.652**	.007	−.066	.346**
POS	−.240*	.067	−.269*	.045	−.360**		−.019	.007	−.339**
Awakening cortisol	−.126	.045	.018	-.007	−.094	−.091		.425**	.062
DCS	−.048	−.152	−.158	−.085	−.074	.004	.564**		.039
MFE-30	−.100	−.056	-.165	.001	.526**	−.055	−.079	−.137	

Note: N = 75 (Study 1) and N = 73 (Study 2), except for BMI and level of studies (N = 72). The correlations for Study 2 are shown below the diagonal. BMI = body mass index; SES = Subjective socioeconomic status; MFE-30= Everyday Life Memory Failure Questionnaire; STAI = State Trait Anxiety; POS = Positivity Scale; DCS = diurnal cortisol slope. *p<.05. **p<.01

In both studies, to investigate the relationship between SMCs and psychological traits or the HPA axis, linear regression analyses were performed with anxiety, positivity, awakening cortisol, or DCS as independent variables and SMCs as dependent variable. Separate linear regression analyses were performed, including the covariates in the first step and anxiety, positivity, awakening cortisol, or DCS in the second step. In both studies, moderation analyses were performed following Preacher et al. (2007) to investigate whether the relationship between psychological traits, awakening cortisol or DCS, and SMCs was moderated by sex. Additionally, in Study 2, we divided the sample into two subgroups: middle-aged (participants below 65 years of age) and older adults (participants above 65 years of age). Moderation analyses of psychological traits, awakening cortisol or DCS, and SMCs were performed using the age factor as a moderator. We included anxiety, positivity, and awakening cortisol or DCS as independent variables, SMCs as dependent variable, and sex or age as moderators. Moreover, moderation analyses were also used to investigate the relationship between cortisol (awakening cortisol or DCS) and SMCs, moderated by anxiety or positivity. Moderated regression models and bootstrapped bias-corrected 95 % confidence intervals of the interaction effect were computed using Hayes' PROCESS macro model number 1 (Hayes, 2017) with SPSS (version 26; IBM Corporation, Armonk, NY, USA), with 5000 bootstrap iterations. Confidence intervals that do not contain zero indicate a significant interaction effect. We included cortisol indexes (awakening cortisol or DCS) as independent variables, SMCs as dependent variable, and anxiety and positivity as moderators. Standardized values were used to perform the moderation analysis.

In Study 1, we did not find any outliers. In Study 2, two univariate outliers (one woman from anxiety and one woman from awakening cortisol analyses) were eliminated for being ±3 SD from the mean. Linear regression assumptions of linearity of

the relationships, no collinearity of the predictors, as well as independence, homoscedasticity, and normality of residuals were met. Tolerance and variance inflation factor values indicated that there were no collinearity issues for the variables included in the regressions.

Statistical analyses were carried out using SPSS v.26 (IBM Statistics, Chicago, IL, USA). All *p* values were two-tailed, and the level of significance was taken as $p < .05$. Post hoc planned comparisons were performed using Bonferroni adjustments for the *p*-values.

Results

Study 1

Preliminary analyses

No significant differences between men and women were found for SES (t (73) = 1.261, p = 0.211), BMI (t (73) = 1.891, p = 0.063), educational level (χ^2 (3) = 2.393), SMCs (t (73) = − 1.519, p = 0.133), trait anxiety (t (73) = − 1.533, p = 0.130), positivity (t (73) = 0.335, p = 0.739), or DCS (t (73) = − 0.071, p = 0.944). However, women were marginally younger (t (73) = 1.977, p = 0.052) and had significantly higher awakening cortisol levels than men (t (73) =−2.307, p = 0.024).

Relationships between SMCs and psychological traits and HPA axis indexes

To test the partial associations between the variables involved in the study, linear regression analyses showed that SMCs were positively associated with anxiety (R^2 = 0.231; B = 0.334, p = 0.002) and negatively associated with positivity (R^2 = 0.218; B =− 0.316, p = 0.004). No relationships were found between SMCs and awakening cortisol (R^2 = 0.116; B = 0.033, p = 0.780), or DCS (R^2 = 0.140; B = 0.077, p = 0.500). The results did not show an interaction between sex and psychological traits or awakening cortisol (all $p > 0.176$). However, a significant sex x DCS interaction (p = 0.042) was

found, showing a marginally positive relationship between the DCS and SMCs in women ($p = 0.059$), but not in men ($p = 0.285$).

Moderation analyses between SMCs and HPA axis indexes: the role of psychological traits

The results showed a significant interaction between anxiety and awakening cortisol ($p = 0.017$). Thus, a significant positive relationship between awakening cortisol and SMCs in people with high trait anxiety was found ($p = 0.046$), but this relationship was no longer significant in people with medium and low levels of trait anxiety (all $p > 0.125$). Furthermore, a trait anxiety x DCS interaction was found ($p = 0.011$), showing a significant positive relationship between the DCS and SMCs in people with high trait anxiety ($p = 0.011$). This relationship was no longer significant in people with medium and low levels of trait anxiety (all $p > 0.163$) (Table IV.3).

Regarding positivity, no significant positivity x awakening cortisol interaction was found ($p = 0.242$). However, there was a significant interaction between positivity and the DCS ($p = 0.003$). That is, there was a significant positive relationship between the DCS and SMCs in people with low positivity ($p = 0.003$), but not in people with medium or high positivity (all $p > 0.151$) (Table IV.3).

Table IV.3 Conditional effect of awakening cortisol and DCS on SMCs at different values of trait anxiety or positivity, Study 1

Moderator variable (W): Anxiety

Independent variable (X): Awakening cortisol

Dependent variable (Y): SMCs

ΔR^2 interaction=.064 F = 6.001 p =. 017

STAI	Effect	SE	*t*	*p*	LLCI	ULCI
−1.104	−.235	.151	−1.554	.125	−.538	.067
−.220	−.016	.106	−.151	.881	−.228	.196
1.107	.313	.154	2.033	.046	.005	.621

Independent variable (X): DCS

Dependent variable (Y): SMCs

ΔR^2 interaction =.066 F = 6.922 p =. 011

STAI	Effect	SE	*t*	*p*	LLCI	ULCI
−1.104	−.216	.153	−1.412	.123	−.522	.089
−.220	.027	.101	.267	.790	−.175	.229
1.107	.392	.149	2.616	.011	.093	.691

Moderator variable (W): Positivity

Independent variable (X): Awakening cortisol
Dependent variable (Y): SMCs

ΔR^2 interaction =.016 F = 1.394 p =. 242

POS	Effect	SE	*t*	*p*	LLCI	ULCI
−1.284	.189	.173	1.099	.276	−.155	.535
.039	.025	.109	.227	.821	−.194	.243
.808	−.071	.139	−.507	.614	−.349	.208

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Continuation of **Table IV.3**

Moderator variable (W): Positivity

Independent variable (X): DCS

Dependent variable (Y): SMCs

ΔR^2 interaction =.096 $F = 9.710$ $p =. 003$

POS	Effect	SE	*t*	*p*	LLCI	ULCI
−1.284	.632	.206	3.073	.003	.221	1.042
.039	.113	.100	1.126	.264	−.087	.313
.808	−.188	.129	−1.453	.151	−.447	.071

Note: N =75; DCS = diurnal cortisol slope; values for quantitative moderators are the 16th, 50th, and 84th percentiles**.**

Study 2

Preliminary analyses

In the total sample, no significant differences between men and women were found for age (t (71) = 1.528), SES (t (71) = 0.110), educational level (χ^2 (4) = 2.997), SMCs (t (71) = − 1.280), trait anxiety (t (71) = −1.684), positivity (t (71) = −1.569; all p > 0.096), awakening cortisol levels (t (71) = 0.878) or DCS (t (71) = 0.805, all p > 0.386). Sex differences were only found in the BMI, given that men had a higher BMI than women (t (70) = 3.834, p < 0.001).

No significant differences between people under and over 65 years old (middle-aged vs older adults) were found in SES (t (71) = −1.737), BMI (t (70) = −0.273), educational level (χ^2 (4,) = 5.406), SMCs (t (71) = 1.513), trait anxiety (t (71) = 1.086), positivity (t (71) = 1.275; all p > 0.087), awakening cortisol levels (t (71) = 0.864), or DCS (t (71) = 0.939, all p > 0.351).

Relationship between SMCs and psychological traits and HPA axis indexes

To test the partial associations between the study variables, linear regression analyses showed that SMCs were positively associated with anxiety (R^2 = 0.277; B = 1.060, p < 0.001). No relationships were found between SMCs and positivity (R^2 = 0.003; B = − 0.200, p = 0.646), awakening cortisol (R^2 = 0.015; B = −5.678, p = 0.556), or DCS (R^2 = 0.019; B = −6.875, p = 0.285). The results did not show an interaction between sex and anxiety or the cortisol index (all p > 0.202). However, a sex x positivity interaction was found (p = 0.028). Thus, there was a significant negative relationship between positivity and SMCs in women (p = 0.035), but not in men (p = 0.349).

Moderation analyses of SMCs and HPA axis indexes: The role of psychological traits

In the total sample, no significant interactions between trait anxiety and awakening cortisol or the DCS were found (p = 0.738, p = 0.917, respectively). Regarding positivity, results did not show a significant positivity x awakening cortisol or DCS interaction (p = 0.970, p = 0.831, respectively) (Table IV.4).

Table IV.4 Conditional effect of awakening cortisol and DCS on SMCs at different values of trait anxiety or positivity, Study 2

Moderator variable (W): Anxiety

Independent variable (X): Awakening cortisol

Dependent variable (Y): SMCs

ΔR^2 interaction =. 001 F = .113 p =.738

STAI	Effect	SE	*t*	*p*	LLCI	ULCI
−1.090	−.080	.175	−.456	.650	−.429	.270
−.060	−.040	.113	−.349	.728	−.266	.187
1.060	.004	.161	.025	.980	−.319	.327

(Continue on next page)

Continuation of **Table IV.4**

Independent variable (X): DCS

Dependent variable (Y): SMCs

ΔR^2 interaction =.001 F = .011 p =. 917

STAI	Effect	SE	t	p	LLCI	ULCI
−1.090	−.101	.182	−.553	.582	−.465	.263
−.060	−.086	.105	−.823	.414	−.295	.123
.881	−.073	.156	−.468	.642	−.384	.239

Moderator variable (W): Positivity

Independent variable (X): Awakening cortisol
Dependent variable (Y): SMCs

ΔR^2 interaction =.000 F = .001 p =.969

POS	Effect	SE	t	p	LLCI	ULCI
-1.002	-.086	.190	-.449	.655	-.466	.295
.155	-.092	.142	-.649	.519	-.375	.191
1.059	-.097	.220	-.442	.660	-.536	.342

Independent variable (X): DCS

Dependent variable (Y): SMCs

ΔR^2 interaction =.001 F = .046 p =.83**1**

POS	Effect	SE	t	p	LLCI	ULCI
-1.002	-.089	.234	-.381	.705	-.557	.379
.155	-.129	.124	-1.038	.303	-.378	.119
1.059	-.161	.184	-.873	.386	-.528	.207

Note: N =73 except for anxiety N=72; DCS = diurnal cortisol slope; values for quantitative moderators are the 16th, 50th, and 84th percentile**s.**

Moderation analyses of SMCs and psychological traits and HPA axis indexes: The role of age

The age x anxiety (p = .072) and age x positivity (p = .629) interactions were not significant; however, anxiety was related to SMCs in both age groups, middle-aged and older adults (all p < .05) (Table IV.5). In addition, the age x awakening cortisol interaction

was significant (p = .011). Results showed that in people under 65 years old (middle-aged), awakening cortisol levels were negatively associated with SMCs (p = .012), but this relationship was not found in people over 65 (older adults) (p = .406). Moreover, the results showed that the age x DCS interaction was significant (p = .023), with a significant negative relationship between DCS and SMCs in people under 65 (middle-aged) (p = .013), but not in people over 65 (older adults) (p = .461) (Table IV.5).

Table IV.5 Conditional effect of awakening cortisol and DCS on SMCs depending on age, Study 2

Moderator variable (W): Age

Independent variable (X): Anxiety

Dependent variable (Y): SMCs

ΔR^2 interaction=.033			F = 3.336		p =. 072	
Age	Effect	SE	t	p	LLCI	ULCI
Middle-age (55-64 years	.750	.165	4.558	.000	.422	1.078
Elderly (65-75 years)	.370	.127	2.908	.005	.116	.624

Independent variable (X): Positivity

Dependent variable (Y): SMCs

ΔR^2 interaction =.003			F = .236		p =. 629	
Age	Effect	SE	t	p	LLCI	ULCI
Middle-age (55-64 years	−.006	.197	−.033	.974	−.401	.387
Elderly (65-75 years)	−.127	.149	−.848	.399	−.425	.171

(Continue on next page)

Continuation of **Table IV.5**

Independent variable (X): Awakening cortisol

Dependent variable (Y): SMCs

ΔR^2 interaction =.095			F = 6.834		p = .011	
Age	Effect	SE	t	p	LLCI	ULCI
Middle-age (55-64 years	−.588	.228	−.2.575	.012	−.1.045	−.132
Elderly (65-75 years)	.126	.151	.836	.406	−.176	.429

Independent variable (X): DCS

Dependent variable (Y): SMCs

ΔR^2 interaction =.076			F = 5.406		p =. 023	
Age	Effect	SE	t	p	LLCI	ULCI
Middle-age (55-64 years	−.434	.170	−.2.547	.013	−.775	−.093
Elderly (65-75 years)	.16	.171	.742	.461	−.214	.476

Note: N =72 for anxiety and awakening cortisol and N = 73 for positivity and DCS analyses; DCS = diurnal cortisol slope.

Discussion

The present study aimed to investigate the importance of some psychological traits (anxiety and positivity) in the appearance of SMCs in two independent samples of young (18 to 35 years) and older (55 to 75 years) healthy individuals. In agreement with our hypotheses, in young people, higher trait anxiety and lower positivity were related to more SMCs. However, in older people, whereas trait anxiety was associated with SMCs, positivity was negatively related to SMCs only in women. In addition, we aimed to investigate whether the relationship between cortisol indexes and SMCs was direct or modulated by these psychological traits (anxiety and positivity). We found that in young people with higher trait anxiety, higher awakening cortisol and a steeper DCS were related to more SMCs. However, positivity only moderated the relationship between the

DCS and SMCs, but not between awakening cortisol and SMCs. In young individuals with low positivity, a steeper DCS predicted more SMCs. In older people, we did not find any direct relationships between the cortisol indexes and SMCs, or anxiety or positivity mediations. However, we found a direct relationship between the cortisol indexes and SMCs only in the group of middle-aged adults (55 to 64 years). Lower awakening cortisol and a flatter DCS predicted more SMCs in this age group. No effect of age (middle-aged vs older adults) was found on the relationship between psychological traits and SMCs. Therefore, this study shows some similarities in the factors related to SMCs in young and older populations, but it also highlights clear differences associated with the lifespan.

Our results showed that higher trait anxiety was related to more SMCs in both young and older people, and this relationship did not differ in middle-aged adults (55 to 64 years) and older adults (65 to 75 years). Therefore, anxiety seems to be related to SMCs, independently of age. Our results are in line with other studies showing that the relationship between anxiety and SMCs was relatively stable across age (Mahoney et al., 1998; Rowell et al., 2016). Moreover, our results support previous studies that found a contribution of anxiety to SMCs in both young (Mecacci et al., 2004) and older individuals (Balash et al., 2013; Norman et al., 2020; Pavisic et al., 2021; Sabatini et al., 2022). One explanation would be that the perception of memory loss and increased concern and negative affect would be more pronounced in people with high trait anxiety (Pearman, 2021). It is also possible that people with higher trait anxiety have a poor evaluation of their memory abilities, along with higher vulnerability and worse coping strategies in stressful situations (Mecacci et al., 2004; Norman et al., 2020). Thus, following the *Attentional Control Theory* (Eysenck et al., 2007), more anxious people tend to have a reduced attentional focus on normal tasks because they are more

vulnerable to distraction and focus their attention on threat-related stimuli. Threat stimuli can be internal (e.g., worrisome thoughts) or external (e.g., threatening task-irrelevant distractors) (Eysenck et al., 2007). Trait anxiety has been related to diminish prefrontal attentional control and poor attentional mechanisms to inhibit distractors, even in non-threatening situations, which could explain everyday concentration difficulties (Bishop, 2009). In this regard, several studies have highlighted the relationship between SMCs and executive function and attention deficits (Molina-Rodriguez et al., 2018; Pedrero-Pérez & Ruiz-Sánchez de León, 2013; Ruiz-Sánchez de León et al., 2010), as well as cognitive flexibility and attention shifting (Stenfors et al., 2013). Thus, SMCs would be related to attention deficits associated with trait anxiety, which seems to be stable across age.

Regarding positive factors, in Study 1 (young people), regression analyses showed that positivity was related to SMCs. However, Study 2 (older people) showed that positivity correlated with SMCs only in women. The results of Study 1 highlight the importance of positive constructs in protecting against memory complaints in young samples, although they could also indicate the positive consequences of experiencing fewer SMCs. Positivity can be understood as a positive perception of oneself, one's life, one's future, and one's confidence in others. In this line, it could be viewed as a construct that includes optimism, self-esteem, and life-satisfaction (Caprara et al., 2010, 2012). Following the *Positive Orientation Theory*, these psychological characteristics could make them more likely to effectively cope with stressful situations (Caprara et al., 2010), thus reducing the well-known negative effects of stress on memory (Lupien et al., 2018). The *Broaden-and-Build theory of Positive Emotions* (Fredrickson, 1998) also strengthens this idea by outlining that experiencing positive emotions, even in stressful situations, broadens the focus of attention, allowing these people to access their mental

functioning and, thus, protect their cognitive capabilities (Fredrickson, 2001). However, in Study 2 (older people), we only found this association in women. This result slightly differs from Lee (2016), who found that SMCs could be reduced by positive affective states, and that this relationship did not vary with age; however, they did not look for sex differences. Future research should further examine whether there are sex differences that vary throughout the lifespan.

Regarding the cortisol indexes, regression analyses showed that neither awakening cortisol nor the DCS was significantly correlated with SMCs in young and older healthy people. However, in Study 1 (18 to 35 years), we found that anxiety mediated the relationship between both HPA axis indexes (awakening cortisol and the DCS) and SMCs, whereas positivity mediated the relationship between the DCS and SMCs. These results indicate that higher anxiety and lower positivity are associated with an HPA axis dysregulation, which in turn is related to SMCs. This could be explained by the hypervigilance, proneness to negative affective states, concern, and higher vulnerability to stress shown by anxious participants, which could be reflected in their HPA axis functioning (Chow & Mercado, 2020; Schlotz et al., 2006). HPA axis dysregulation is related to brain regions associated with emotion regulation, such as the amygdala, hippocampus, and prefrontal cortex, which are also involved in anxiety disorders (Arnsten, 2015; Bishop, 2007; Hur et al., 2019; Jung et al., 2020; McEwen, 2003). In contrast, the optimism about positive outcomes and effective coping with stressful situations that characterize positivity would also contribute to explaining the HPA regulation (Puig- Perez et al., 2018; Scheier et al., 1986). Our results are similar to those found on HPA axis dysregulation in anxious patients (Adam et al., 2017; Vreeburg et al., 2010), as well as in individuals with low positivity (Pasquali et al.,

2020). Moreover, they emphasize the importance of the HPA axis in memory processes (Ouanes & Popp, 2019).

In Study 2 (55 to 75 years), we did not confirm the moderating role of anxiety and positivity. However, when we focused more on possible changes due to age, we found that the relationship between SMCs and cortisol indexes was mediated by age, but only in middle-aged adults (55 to 64 years). In this age group, lower awakening cortisol and a flatter DCS were related to more SMCs. Our results could suggest that the presence of memory complaints acting as a stressor in middle-aged adults can be strongly related to cortisol secretion (Kudielka et al., 2006). Likewise, previous studies indicate that this stress-associated dysregulation of the HPA axis has effects on memory (for review see: Lupien et al., 2018). This could explain why adult people with SMCs have memory deficits possibly associated with their cortisol patterns (Fiocco et al., 2006; Reid & MacLullich, 2006). It is plausible that the perception of memory loss in older people contributes to persistent distress and the anticipation of further memory loss and independence (Pearman, 2021). These reactions can initiate a vicious cycle in which chronic stress leads to an HPA axis dysfunction resulting in brain changes and further cognitive problems (Peavy et al., 2013). The differences found in age could be due to workload and lost functionality; in other words, middle-aged adults (55–64 years old) can be more aware of their memory loss because they are still working, and their memory demands are higher than for those who are already retired. Given the work demands, middle-aged adults could be more stressed and have more SMCs even memory loss and, thus, have a more dysregulated HPA axis (Cedres et al., 2019).

Additionally, in our study, the association between SMCs and cortisol indexes in older adults (65 to 75 years) was not found, perhaps also due to the successful aging exhibited. These results are not consistent with studies investigating the diurnal cortisol

pattern in older people over 65 years old with SMCs (Fiocco et al., 2006; Peavy et al., 2013). These differences between studies could be due to the intrinsic differences, given that these previous studies reported higher levels of perceived stress in the samples, which could affect the HPA axis. Our oldest participants (65–75 years old) exhibited the lowest anxiety and SMCs levels, which agrees with the results found in Mahoney et al. (1998) and Mecacci & Righi (2006) with samples from 18 to 85 years old, showing that as age increased, anxiety and SMCs were lower. Thus, in our study, healthy older participants with no cognitive impairment, no economic problems, and decreased anxiety had the lowest SMCs. Therefore, SMCs from 65 years old and up would be more related to other causes, such as preclinical stages of Mild Cognitive Impairment and dementia, and to a lesser extent to stress (HPA axis) (Jessen et al., 2014; Jonker et al., 2000). However, these results should be better tested, adding other biomarkers of preclinical Alzheimer's disease.

Some limitations warrant a cautious interpretation of the results of this study. First, the overall mean for SMCs was low in both age groups. Other samples with more SMCs could yield different results. Second, our participants were carefully screened for good health and habits, and so generalizability to clinical samples would be limited. Moreover, results for SMCs must consider the questionnaire employed. Other studies have focused on specific complaints such as memory complaints or concentration complaints separately or, on the contrary, on more general cognitive complaints. Results for DCS should be interpreted with caution when comparing them to other studies because there are many different types of cortisol slopes, and the slope methodology chosen may reflect different diurnal patterns. Finally, although we did not perform an objective monitoring of the awakening time, we used the awakening time provided in

the participants' diaries. We encourage future studies to use recording devices to measure the awakening time more objectively.

Conclusions

To our knowledge, this is the first study to investigate the interaction between trait anxiety, positivity, and cortisol indexes in SMCs in different age groups. Our results suggest that the relationship between psychological traits and SMCs did not vary significantly across the lifespan. Moreover, one underlying mechanism of the relationship between SMCs and cortisol in young adults would involve traits such as anxiety and positivity. In older samples, our results confirm that HPA axis dysregulation is directly related to SMCs in middle-aged adults, but not in elderly adults who experience successful aging. These results shed light on the complexity of SMCs in young and older samples, and they offer a possible explanation about why some people are more prone to subjective memory forgetfulness than others.

Chapter VI

Study 5: Importance of depressive symptomatology and neurodegenerative biomarkers in Subjective Cognitive Decline in older people

The main results of this study are under review in:

Zapater-Fajarí, M., Diaz-Galvan,P. , Cedres,N., Rydberg Sterner, T., Rydén ,L., Sacuiu,S., Waern ,M., Zettergren,A., Zetterberg,H., Blennow,K., Kern,S., Hidalgo, V., Salvador,A., Westman,E., Skoog,I., and Ferreira,D. Biomarkers of Alzheimer's disease and cerebrovascular disease in relation to depressive symptomatology in individuals with subjective cognitive decline. *Alzheimer's and Research Therapy.*

Introduction

Subjective cognitive decline (SCD) has been suggested as one of the first signs of Alzheimer Disease (AD) (Jessen et al., 2014). Previous studies showed a significant association between SCD and biomarkers of AD pathology, including amyloid-beta (Aβ) and tau biomarkers (Amariglio et al., 2012; Buckley et al., 2017). In addition, SCD may also be associated with other brain pathologies such as cerebrovascular disease (CVD) (Cedres et al., 2019; 2021; Diaz-Galvan et al., 2021a; Diniz et al., 2013; Minett et al., 2005). Hence, SCD is gaining interest as an early marker of various brain pathologies, which can be diagnosed before the emergence of objective cognitive impairment.

A current topic of discussion is that SCD may reflect not only brain pathologies but also other non-pathological conditions such as depressive symptomatology (Diaz-Galvan et al., 2021a; Hill et al., 2016). As a consequence, the leading international SCD initiative (SCD-I) working group has recently made a call for a better understanding of the role of depressive symptomatology in SCD (Jessen et al., 2020). Several studies have shown the strong association between SCD and depressive symptomatology (Cedres et al., 2019; Hill et al., 2016). The main difficulty lies in understanding whether depressive symptomatology found in SCD individuals is really connected to brain pathology (Diniz et al., 2013; Pomara et al., 2016; Taylor et al., 2013). For instance, depression correlates with AD biomarkers in the absence of cognitive impairment (Pomara et al., 2016; Sun et al., 2008), and AD pathology predicts increase in depressive symptomatology over time (Babulal et al., 2016). Similarly, CVD is known to affect neural connections leading to depressive symptomatology (Taylor et al., 2013). However, the association between SCD, depressive symptomatology, and AD and CVD

biomarkers is not completely understood (Jessen et al., 2020). Another difficulty is that clinical depression is an exclusion criterion for the diagnosis of SCD (Jessen et al., 2014), but how to approach subclinical depressive symptomatology in SCD is still debated (Jessen et al., 2020).

AD pathology (amyloidosis and tau neurofibrillary tangles) can be assessed *in vivo* through cerebrospinal fluid (CSF) biomarkers such as the amyloid-beta 42/40 (Aβ42/40) ratio and phosphorylated tau (p-tau) (Blennow & Zetterberg, 2018). Amyloidosis is thought to be the initiating event of the Alzheimer pathological cascade, which would be followed by tau pathology (Jack et al., 2010; 2013). SCD has been related to the preclinical stage of AD (Jessen et al., 2020), demonstrating early positivity in AD biomarkers in the absence of overt cognitive impairment (Visser et al., 2009). CVD can be measured with magnetic resonance imaging (MRI) (Wardlaw et al., 2013), for example in the form of white matter signal abnormalities (WMSA) on T1-weighted images (white matter hypointensities) and on T2-weighted or fluid-attenuated inversion recovery (FLAIR) images (white matter hyperintensities).

In a previous study, we demonstrated that SCD is independently associated with both subclinical depressive symptomatology and CVD (Diaz-Galvan et al., 2021a), but depressive symptomatology was not associated with CVD and we could not test for associations with Aβ and tau biomarkers. Hence, the question of whether depressive symptomatology in SCD is a symptom of brain pathologies remains partially unanswered. The primary aim of the current study was to investigate the role of depressive symptomatology and biomarkers of brain pathology (CVD, Aβ42/40 and p-tau) in SCD. We hypothesized that SCD would be related to both depressive symptomatology and biomarkers of AD and CVD (Diaz-Galvan et al., 2021a; Minett et

al., 2005; Sun et al., 2008).We also wanted to test whether depressive symptomatology found in SCD is associated with brain pathology. We hypothesized that depressive symptomatology would be related to biomarkers of AD and CVD (Pomara et al., 2016; Sun et al., 2008; Taylor et al., 2013). Additionally, a recent publication showed that different complaints are associated with different MRI-based biomarker profiles and depressive symptomatology (Diaz-Galvan et al., 2021b). However, there is a need to confirm this finding in independent cohorts that also include CSF biomarkers of AD. Some studies have already made the distinction between memory and concentration complaints (Grambaite et al., 2013, Topiwala et al., 2021). Therefore, our secondary aim was to investigate memory and concentration complaints separately in relation to depressive symptomatology and biomarkers of AD and CVD. Because memory impairment is a core symptom of AD and difficulties in concentration are common in individuals with high CVD burden, we hypothesized that subjective memory complaints would be more strongly associated with AD biomarkers, and concentration complaints would be more strongly associated with CVD biomarkers (Diaz-Galvan et al., 2021b; Topiwala et al., 2021). The association of depressive symptomatology with SCD is well known, so we hypothesized that both memory and concentration complaints would be associated with depressive symptomatology.

Materials and Methods

Participants

Data was derived from the 2014-2016 examinations of the Gothenburg H70 Birth Cohort 1944 Studies. A total of 1203 individuals participated in the study. All examinations and procedures have been described previously (Rydberg Sterner et al., 2019). The initial sample was composed of 297 participants, who received an MRI scan

and a CSF lumbar puncture (LP). Inclusion criteria were in concordance with the international SCD-I working group (Jessen et al., 2014):

1) Normal cognition, in this study established in two steps: Firstly, dementia was excluded based on a clinical diagnosis of dementia according to the DSM-III-R criteria, a Mini-Mental State examination (MMSE) score <24, or a Clinical Dementia Rating (CDR) score >0.5. Secondly, mild cognitive impairment (MCI) was excluded based on comprehensive neuropsychological assessment and age-, sex-, and education-adjusted normative data. Following recommendations by Jak et al. (2009) and Molinuevo et al. (2017), individuals were classified as MCI if at least one of the following two criteria were met: (Criterion 1) performance below the 16 percentile in two measures within at least one of the following cognitive domains: Memory, represented by Thurstone's Picture Memory, 10 word list, and remembering 12 objects; Verbal fluency represented by a semantic fluency task (animals); Speed / executive function, represented by Digit Span Forward and Backward test, and the Figure Logic (SRB2) of the Figure Logic of the Synonyms, Reasoning and Block Design Test; and Visuospatial ability represented by Block Design (Koh's Block Test); (Criterion 2) performance below the 16th percentile in three independent tests of three out four cognitive domains studied. When criterion 1 could not be met because the domain was evaluated with one test, criterion 2 was spent. The 16th percentile criterion was favored instead of the >1SD criterion in Jak et al. (2009) and Molinuevo et al. (2017) due to the asymmetrical distribution of several neuropsychological tests in our cohort;

2) No large infarcts or tumors on brain MRI and no history of stroke or transient ischemic attack (TIA), according to a neuroradiologist;

3) No medical history of psychiatric (e.g., major depression) or neurological disorders, systemic diseases or head trauma, nor intake of antidepressants; and

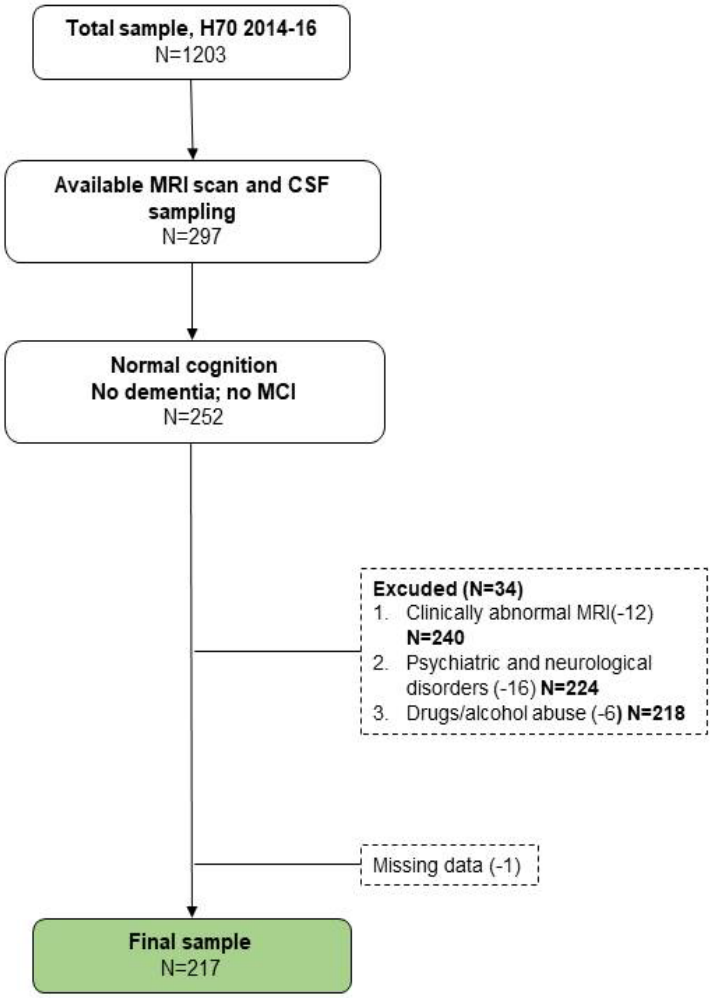
4) No history of substance abuse based on clinical interview and no alcohol use disorder identification test (AUDIT) score >20 (Bergman et al., 1994).

Among 297 participants in the initial sample, 80 were excluded after applying our inclusion criteria. One further participant was excluded due to missing data in the semi-structured Comprehensive Psychopathological Rating Scale (CPRS) (Åsberg et al., 1978). The final sample was composed of 217 participants. The process of participant selection and reason for exclusions are displayed in Figure V.1.

Standard protocol approvals, registrations, and patient consents

The H70 study was approved by the Regional Ethical Review Board in Gothenburg (Approval Numbers: 869-13, T076-14, T166-14, 976-13, 127-14, T936-15, 006-14, T703-14, 006-14, T201-17, T915-14, 959-15, T139-15), and by the Radiation Protection Committee (Approval Number: 13-64), in concordance with the 1964 Helsinki declaration and its later amendment. Informed consent was obtained from all participants or their relatives if the participant was unable to provide informed consent.

Figure V.1 Study selection flow



Note. Study selection flow diagram with the number of participants excluded from the study at each step, showing the final study sample. Abbreviations: MRI= magnetic resonance imaging; CSF= cerebrospinal fluid; MCI= Mild cognitive impairment.

Subjective Cognitive Decline (SCD)

SCD was assessed using two different questions from the semi-structured CPRS (Åsberg et al., 1978) that refer to subjective memory and concentration complaints experienced during the last month. These questions are rated in a 7-point Likert scale according to intensity, frequency, and degree of inability produced (Table V.1). The

range is from 0 (no difficulties) to 6 (severe difficulties), allowing for intermediate scores. Scores 0 and 1 represent no difficulties or difficulties within the normal range, while scores ≥2 reflect an increasing degree of complaint. This type of response in a Likert scale has been suggested as the better choice to measure change over time by the SCD-I and is commonly used in studies of the world-leading initiative (80% of the studies) (Rabin et al., 2015). To note, the concentration item further extends the study of SCD so far primarily focused on memory complaints, strengthening the notion about different phenotypes associated to different complaints (Diaz-Galvan et al., 2021b). Based on clinical experience and clinimetric considerations about the CPRS, the presence of a complaint was defined by the cutoff point of ≥2 on the concentration and memory complaints items. Indeed, the CPRS itself proposes that scores <2 represent no difficulties or difficulties within the normal range, while scores 2 or more reflect some degree of complaint. Based on this criterion, individuals were classified into people with subjective cognitive decline in memory (SCD-memory group) or concentration (SCD-concentration group) if they scored ≥2 in these items, respectively. Individuals who scored <2 on both memory and concentration complaints items were classified as controls. We favored this dichotomous classification (SCD *vs.* controls) instead of the continuous form of the items due to the study aims and the nature of our statistical approach (see below).

Table V.1 Questions for the operationalization of memory (SCD-memory) and concentration (SCD-concentration) SCD groups

SCD-memory: Failing memory. Representing subjective disturbances of recall compared with previous ability. Distinguish from concentration difficulties

Control group	0-1: Memory as usual
SCD-memory group	2-3: Occasional increased lapses of memory 4-5: Reports of socially inconvenient or disturbing loss of memory 6: Complaints of complete inability to remember

SCD-concentration: Concentration difficulties. Representing difficulties in collecting one's thoughts mounting to incapacitating lack of concentration. Distinguish from failing memory.

Control group	0-1: No difficulties in concentrating
SCD-concentration group	2-3: Occasional difficulties in collecting one's thoughts 4-5: Difficulties in concentrating and sustaining thought which interfere with reading or conversation 6: Incapacitating lack of concentration

Note: Abbreviations: SCD-memory= Subjective Cognitive Decline in Memory; SCD-concentration= Subjective Cognitive Decline in Concentration.

Depressive Symptomatology

The Montgomery-Åsberg Depression Rating Scale (MADRS) (Montgomery & Åsberg, 1979) was derived from the CPRS and used to assess the overall burden of depressive symptoms. The MADRS is a 10-items scale scored in a 7-point Likert scale from 0 (no symptoms) to 6 (severe symptoms), giving a total score from 0–60 (MADRS-10). We used the total MADRS-10 score to characterize the cohort (clinical

cut points are available for the MADRS-10 score). However, our main statistical analyses were performed on the MADRS score with the concentration item excluded (MADRS-9, based on 9 items), to avoid circularity, because the concentration item of the CPRS defines the SCD-concentration group. In addition, as explained in the 'Participants' section, none of the participants have a clinical diagnosis of major depression nor were they under treatment for depression, in agreement with the current diagnostic criteria of SCD (Jessen et al.,2014).

MRI acquisition, image processing and biomarkers of CVD

Participants were scanned using a 3.0T Philips Achieva system (Philips Medical Systems). It used a three-dimensional T1-weighted Turbo Field Echo (TFE) sequence (repetition time=7.2 ms., echo time=3.2 ms., flip angle=9°, number of slices=160, matrix size=250x250 mm, field of view = 256x256 mm, and slice thickness=1.0 mm) and a 3D FLAIR sequence (repetition time= 4800 ms., echo time= 280 ms., inversion time= 1650 ms., flip angle=90°, number of slices=140, matrix size=250x237 mm, field of view = 250x250 mm, and slice thickness=2.0 mm) for estimations of hypointense and hyperintense WMSA, respectively. Data management and image processing was done with our database system theHiveDB (Muehlboeck et al., 2014).

Both, hypointense and hyperintense WMSA have been suggested as biomarkers of CVD. Although there is a strong correlation between hypointense and hyperintense WMSA (Cedres et al., 2020; Riphagen et al., 2018), they may reflect different underlying pathologies. Hypointense WMSA has been suggested to reflect poorer white matter integrity and more chronic white matter damage than hyperintense WMSA (Riphagen et al., 2018). In contrast, hyperintense WMSA may reflect a mix of white matter damage, peri-inflammatory processes, and other pathologies related to increased blood-brain barrier permeability (Young et al., 2008). Given that age-related /

neurodegenerative CVD is usually insidious and chronic rather than acute, in this study we selected hypointense WMSA as a better proxy of CVD in our population, but reported hyperintense WMSA in supporting information for completeness of information..

Hypointense WMSA were automatically segmented with FreeSurfer 6.0.0 and hyperintense WMSA were automatically segmented with LST 2.0.15. Briefly, the T1-weighted images were processed with the FreeSurfer 6.0.0 image analyses suite (http://surfer.nmr.mgh.harvard.edu/). FreeSurfer detects white matter hypointensities and automatically labels them using a probabilistic procedure (Fischl et al., 2002). This procedure is sensitive in measuring white matter damage both in healthy individuals and in patients with AD (Leritz et al., 2014; Salat et al., 2010). LST is an open-source segmentation toolbox in the SPM software (https://www.fil.ion.ucl.ac.uk/spm/), which uses a lesion prediction algorithm (LPA) based on FLAIR hyperintensities that builds a lesion probability map for each individual. WMSA volumes in millimeters (ml) were adjusted by the total intracranial volume (TIV) obtained from FreeSurfer. This adjustment was performed by dividing the WMSA volume by the TIV of each participant (Voevodskaya et al., 2014), and TIV-adjusted WMSA measures were used for statistical analyses. Following Cedres et al., (2020) we classified WMSA into low and high WMSA burden with a cutoff value of 0.00321 for hypointense WMSA, which resembles low and high WMSA burden in the Fazekas visual rating scale (Fazekas et al., 1987). Hypointense WMSA were treated continuously in the analyses but were categorized as high and low to describe the degree of pathology for the characterization of the sample.

CSF biomarkers and APOE-ε4 genotype

CSF samples were collected in the morning through LP in L3/L4 or L4/L5 interspaces. A total of 10ml of CSF were collected in a polypropylene tube and immediately transported to the laboratory and centrifuged at 1800g at 20°C. The supernatant was gently mixed to avoid possible gradient effects, aliquoted in polypropylene tubes and stored at -70°C. CSF phosphorylated tau at threonine 181 (p-tau) concentrations were determined using sandwich enzyme-linked immunosorbent assay (ELISA) (INNOTEST® htau Ag and PHOSPHO_TAU (181P) (Fujirebio, Ghent Belgium) (Vanmechelen et al., 2000). We used the CSF Aβ42/40 ratio as a biomarker of amyloid-beta pathology. The CSF Aβ42/40 ratio was obtained using the V-PLEX Aβ peptide panel 1 (6E10) kit (Meso Scale Discovery, Rockville, MD) (Andreasson et al., 2018). This variable was treated continuously in the main analyses. To characterize the sample, following Samuelsson et al. (2021) we classified CSF p-tau and Aβ42/40 values into positive and negative using the following cutoff values: ≥80 pg/mL for p-tau and ≤.082 for Aβ42/40. The *APOE*-ε4 allele was determined using the KASPar PCR SNP genotyping system (LGC Genomics, Hoddesdon, Herts, UK) as described in Skoog et al. (2021). To characterize the sample, participants were classified as *APOE*-ε4 carriers if they had at least one ε4 allele.

Statistical analyses

Box-Cox transformations were performed when continuous variables did not follow the normal distribution (Osborne, 2010). Firstly, we conducted univariate analyses consisting of Student t-tests and Pearson's Chi-square tests. We tested for differences between individuals with subjective complaints in memory and controls (SCD-memory vs. controls) across sociodemographic variables, MMSE, *APOE* ε4, depressive

symptom scores, CSF p-tau, CSF Aβ42/40, and WMSA. The same analyses were also performed for the comparison between individuals with subjective complaints in concentration and controls (SCD-concentration vs. controls). Effect sizes were calculated using Cohen's d for continuous variables ion t tests, and using phi (φ) for nominal variables on chi square (χ^2) tests. For Cohen's d 0.20, 0.50 and 0.80 and for φ 0.1, 0.3 and 0.5 represent small, medium, and large effect sizes, respectively. Secondly, we conducted multivariable analysis consisting of multiple binary logistic regression models. These models were used to further investigate partial associations of depressive symptom scores, CSF p-tau, CSF Aβ42/40, and WMSA (predictors) with SCD-memory vs. controls or SCD-concentration vs. controls (outcome variables), in two separate models. We report models for hypointense WMSA in the main text and models for hyperintense WMSA in supporting information. Finally, we approximated the question of whether depressive symptomatology is a symptom of brain pathology through correlations of depressive symptom scores with WMSA, CSF p-tau, and CSF Aβ42/40. All statistical analyses were carried out using SPSS v.26 (IBM Statistics, Chicago, IL, USA). All *p* values were two-tailed and the level of significance was set at $p<.05$.

Results

Cohort description

Cohort characteristics are shown in Table V.2. Regarding biomarkers, 32% of the participants were CSF Aβ42/40-positive, 6% were p-tau-positive, and 15% had a high hypointense WMSA burden. Total MADRS-10 scores ranged from 0 to 20 (*mean* = 2.97, *SD* = 3.66). Hence, consistent with the SCD-I criteria, virtually all the participants had depressive symptom scores within the normal range when using clinical cut points for the MADRS (Svanborg & Ekselius, 2003), while 6 participants had mild depressive symptomatology, and 1 participant had moderate depressive symptomatology. Total MADRS-9 scores used in the analyses ranged from 0 to 18 (*mean* = 2.67, *SD* = 3.43). The distributions of total MADRS-10 and MADRS-9 scores are shown in Figure V.2.

Table V. 2 Demographic and clinical characteristics of the total sample and by SCD-memory and SCD-concentration groups

						SCD-Memory vs Control group			***SCD-Concentration vs Control group***		
	Total sample (n=217)	**Min-Max.**	**SCD-memory group (n=119)**	**SCD-concentration group (n=23)**	**Control group (n=89)**	***t*/χ^2**	***p-value***	***d*/φ**	***t*/χ^2**	***p-value***	***d*/φ**
Age (years)	70.54(.26)	69.71-71.92	70.55(.24)	70.56(.18)	70.54(.30)	−.22	.827	−.03	−.283	.777	−.07
Sex (% women)	52.5		47.9	56.5	57.3	1.804	.179	.10	.005	.946	.01
Years of education	13.30(4.09)	7-35	12.99(3.67)	13.26(4.05)	13.65(4.70)	1.13	.261	.16	.361	.719	.09
***APOE* ε4 (%)**	32		29	55	33	.136	.712	−.03	3.652	.056	.18
MMSE (total score)	29.19(1.07)	25-30	29.16(1.16)	28.96(1.29)	29.17(.97)	.058	.954	.01	.870	.386	.20
Depressive symptomatology (MADRS-9)	2.67(3.43)	0-18	3.04(3.85)	4.39(4.95)	2.034(2.61)	−2.25	.026	−.30	−2.205	.037	−.73
P-tau levels (pg/L)	49.38(17.19)	18-128	51.41(18.12)	50.26(17.06)	47.27(16.36)	−1.70	.091	−.24	−.775	.440	−.20
P-tau (% positive)	6	-	7	9	6	.106	.745	.02	.295	.587	.05
Aβ 42/40 ratio	.087(.022)	.026-.121	.086(.023)	.070(.024)	.089(.019)	.968	.334	.06	3.641	.001	.86

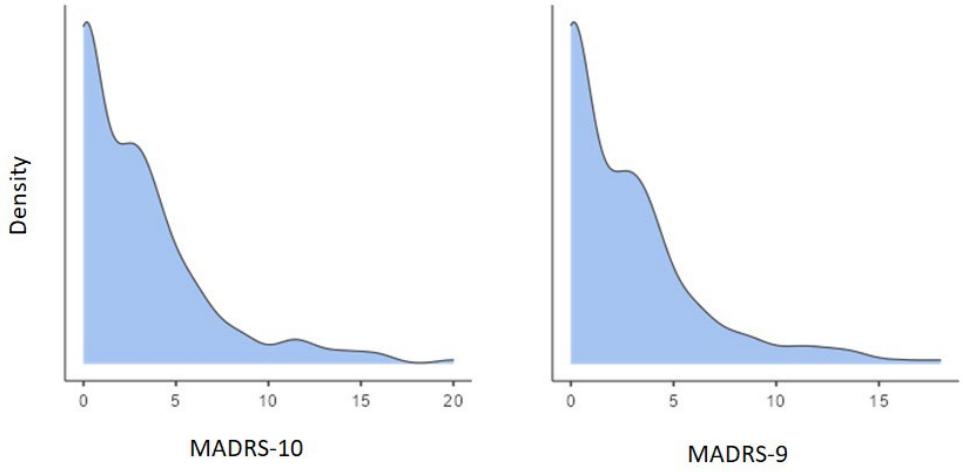
(Continue on next page)

Continuation of **Table V. 2**

						SCD-Memory vs Control group			*SCD-Concentration vs Control group*		
	Total sample (n=217)	**Min-Max.**	**SCD-memory group (n=119)**	**SCD-concentration group (n=23)**	**Control group (n=89)**	***t*/χ^2**	***p-value***	***d*/φ**	***t*/χ^2**	***p-value***	***d*/φ**
Aβ 42/40 (% positive)	32	-	33	61	26	1.062	.303	.07	9.899	.002	.30
Hypointense WMSA (ICV-corrected volumes)	.0023(.0026)	.0004-.022	.0023(.0024)	.0025(.0033)	.0022(.0029)	−.136	.892	−.13	−.387	.699	−.13
Hypointense WMSA (high burden) (%)	15	-	16	13	12	.537	.464	.05	.008	.930	.01

Note: Data represent means (standard deviation) except for sex, *APOE*-ε4, and the dichotomized version of biomarkers, where percentage is shown. *p-values* are shown for the differences between SCD-memory and control group, and between SCD-concentration and control group. Analyses involving years of education, Aβ 42/40, and *APOE*-ε4 were performed on n=216, n=216, and n=213 individuals, respectively. SCD-memory= Subjective Cognitive Decline in Memory; SCD-concentration= Subjective Cognitive Decline in Concentration; *APOE*-ε4 =participants with at least one *APOE* ε4 allele; MMSE= Mini-Mental State examination; MADRS-9= The Montgomery-Åsberg Depression Rating Scale with the concentration item excluded; p-tau= phosphorylated tau; Aβ 42/40 = Amyloid-beta 42/40 ratio; WMSA= White matter signal abnormalities; χ^2= Chi-square; d = Cohen's d; *φ* =phi.

Figure V.2 Depressive symptomatology - Distribution of MADRS scores.



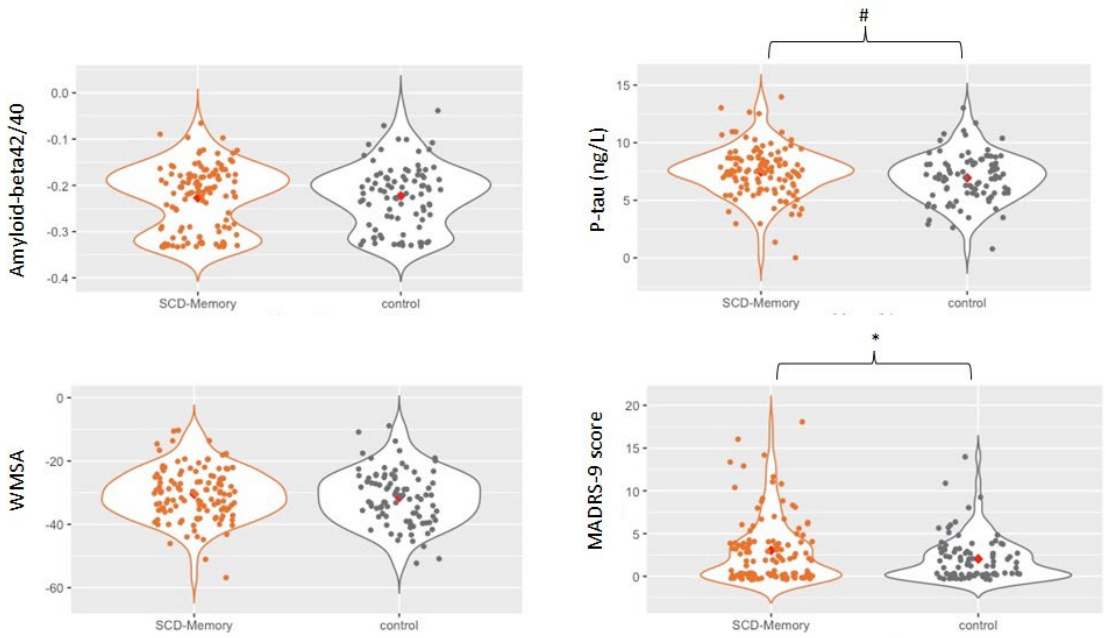
Note. Distribution of total MADRS-10 and MADRS-9 scores in the study. MADRS scores on the x-axis and density on the y-axis. MADRS-10= The Montgomery-Åsberg Depression Rating Scale 10-items; MADRS-9= The Montgomery-Åsberg Depression Rating Scale with the concentration item excluded.

Regarding subjective cognitive complaints, 119 (54.8%) participants endorsed subjective complaints in memory and were thus classified into the SCD-memory group. A total of 23 participants (10.6%) reported subjective complaints in concentration and were thus classified into the SCD-concentration group. A total of 89 participants (41%) did not have any subjective complaint in memory or concentration and were thus classified into the control group. There was no association between SCD-memory and SCD-concentration groups ($\chi^2 = .378$; $p = .539$).

We found no significant differences in age, sex, years of education and MMSE between the SCD-memory group and controls, nor between the SCD-concentration group and controls (all $p>.05$). Participants in the SCD-memory group had significantly higher MADRS-9 scores ($p = .026$) and showed a tendency to have higher CSF p-tau

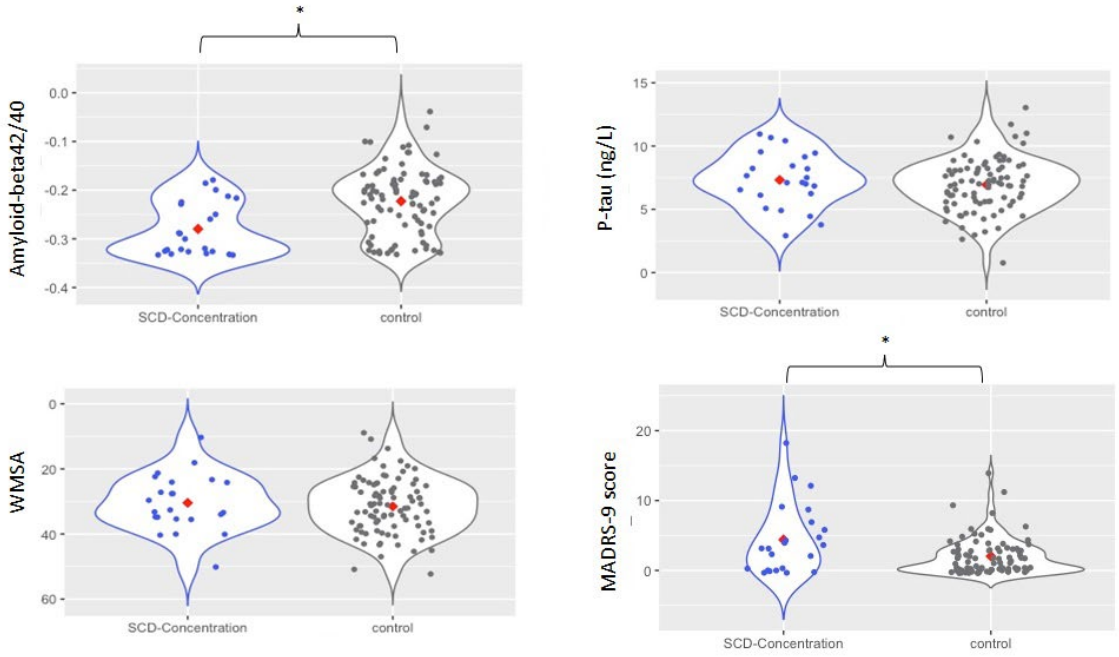
levels (p = .091), as compared with the control group. The SCD-concentration group had a significantly higher frequency of CSF Aβ42/40-positive individuals (p = .002), had lower levels of CSF Aβ42/40 (p = .001), had higher MADRS-9 scores (p = .037), and showed a tendency to have a higher frequency of *APOE*-ε4 carriers (p = .056) than the control group (Table V.2). Figure V.3 and Figure V.4 depict the differences in CSF Aβ42/40, p-tau, WMSA, and MADRS-9 scores between SCD-memory and SCD-concentration groups and the control group.

Figure V.3 Aβ 42/40, p-tau, hypointense WMSA, and MADRS-9 scores in SCD-memory and control groups.



Violin plots where observations and data distribution are represented. Red dots represent median values for SCD-memory and control groups. p value <.05*(p = .026 for MADRS-9), # = trend towards significant differences (p = .091 for p-tau). SCD-memory= Subjective Cognitive Decline in Memory; p-tau= phosphorylated tau; Aβ 42/40 = Amyloid-beta 42/40 ratio; MADRS-9 = The Montgomery-Åsberg Depression Rating Scale with the concentration item excluded; WMSA = White Matter Signal Abnormalities.

Figure V.4 Aβ 42/40, p-tau, hypointense WMSA, and MADRS-9 scores in SCD-concentration and control groups



Violin plots where observations and data distribution are represented. Red dots represent median values for SCD-concentration and control group. *p* value <.05*(*p* = .001 for Aβ 42/40 and *p* = .037 for MADRS-9). SCD-concentration= Subjective Cognitive decline in concentration; p-tau= phosphorylated tau; Aβ 42/40 = Amyloid-beta 42/40 ratio; MADRS-9= The Montgomery-Åsberg Depression Rating Scale with the concentration item excluded; WMSA = White Matter Signal Abnormalities.

Logistic regression analyses

Partial association of CSF biomarkers, WMSA, and depressive symptomatology with SCD-memory

Binary logistic regression was conducted including SCD-memory group as the criterion variable (SCD-memory *vs.* controls), and MADRS-9, CSF Aβ42/40, CSF p-tau, and hypointense WMSA as the predictors. The model was significant (*p* =.014) showing that higher MADRS-9 scores (*p* = .025, OR = 1.114) and p-tau levels (*p* =

.053, OR = 1.142) predicted SCD-memory group. In contrast, CSF Aβ42/40 (p = .969, OR = .922) and hypointense WMSA (p = .265, OR = 1.019) were not associated with SCD-memory group (Table V.3). We repeated the same model with hyperintense WMSA instead of hypointense WMSA and the results were similar (please see supplementary material).

Partial association of CSF biomarkers, WMSA, and depressive symptomatology with SCD-concentration

We performed similar models for the SCD-concentration group. SCD-concentration group was included as the criterion variable (SCD-concentration *vs.* controls), and MADRS-9, CSF Aβ42/40, and CSF p-tau and hypointense WMSA were included as the predictors. The model was significant (p <.001). CSF Aβ42/40 (p = .001, OR = .000) was the main predictor of SCD-concentration group, followed by MADRS-9 (p = .008, OR = 1.223). The SCD-concentration group had a lower CSF Aβ42/40 ration and higher MADRS-9 scores than the control group. In contrast, p-tau (p =.941, OR = 1.010) and hypointense WMSA (p =.291, OR = 1.032) were not associated with SCD-concentration group (Table V.3). When performing the same model with hyperintense WMSA instead of hypointense WMSA, results were similar (please see supplementary material).

Table V.3 Logistic regression models

Dependent variable: SCD-memory group (SCD-memory coded as 1, controls as 0)

$\chi^2(2) = 8.550$	$R^2 = .054$ (Nagelkerke)			$p =. 014$	
	Beta	Wald	*SE*	*p*	OR
MADRS-9	.108	5.042	.048	.025	1.114
p-tau	.133	3.744	.069	.053	1.142
Aβ 42/40	−.082	.002	2.100	.969	.922
Hypointense WMSA	.019	1.242	.017	.265	1.019

Dependent variable: SCD-concentration group (SCD-concentration coded as 1, controls as 0)

$\chi^2(2) = 21.422$	$R^2 = .274$ (Nagelkerke)			$p < .001$	
	Beta	Wald	*SE*	*p*	OR
Aβ 42/40	−15.302	10.813	4.653	.001	.000
MADRS-9	.201	7.045	.076	.008	1.223
p-tau	.010	.006	.131	.941	1.010
Hypointense WMSA	.032	1.114	.030	.291	1.032

Note: SCD-memory= Subjective Cognitive Decline in Memory; SCD-concentration= Subjective Cognitive decline in Concentration; p-tau= phosphorylated tau; Aβ 42/40 = Amyloid-beta 42/40 ratio; WMSA= White matter signal abnormalities; MADRS-9= The Montgomery-Åsberg Depression Rating Scale with the concentration item excluded; χ^2= Chi-square; OR: odds ratio.

Correlations of depressive symptomatology with biomarkers of brain pathology

We did not find any significant correlation between MADRS-9 scores and p-tau, Aβ42/40, or WMSA biomarkers (all $p > .05$) (Table V.4).

Table V.4 Correlations between depressive symptomatology and biomarkers

	Aβ 42/40	Hypointense WMSA	Hyperintense WMSA	MADRS-9
p-tau	−.119	−.082	−.055	−.087
Aβ 42/40		.029	.011	−.070
Hypointense WMSA			.863*	−.015
Hyperintense WMSA				−.051

Note: Analyses for Aβ 42/40 based on n=216. *p* value <.001*. p-tau= phosphorylated tau; Aβ 42/40 = Amyloid-beta 42/40 ratio; WMSA= White matter signal abnormalities; MADRS-9 = The Montgomery- Åsberg Depression Rating Scale with the concentration item excluded.

Discussion

The primary aim of this study was to investigate the association between SCD, depressive symptomatology, and biomarkers of brain pathology (Aβ42/40, p-tau, and WMSA). Additionally, we investigated whether depressive symptomatology is associated with biomarkers of brain pathologies or is rather independent of the underlying pathological process. We extended the research in previous studies by investigating associations between SCD, depressive symptomatology, and biomarkers of AD and CVD pathologies in the same sample of cognitively unimpaired older adults, and by reporting the findings for two common subjective complaints separately, i.e. memory and concentration complaints.

There has been an intense discussion about the potential confounding effect of depressive symptomatology in SCD (Hill et al., 2016; Diaz-Galvan et al., 2021a). Major depression is an exclusion criterion for SCD (Jessen et al., 2014), but subclinical depressive symptomatology is recognized and further research on its role in SCD has recently been promoted by the SCD-I work group (Jessen et al., 2020). However, very

little is known about what should be the exact threshold to exclude depression in SCD studies and how this type of symptomatology should be assessed, while the common approach is to exclude clinical depression. Instead, subclinical depressive symptomatology can be included in SCD studies and the current need is to clarify its role in SCD (Jessen et al., 2020; Molinuevo et al., 2017). Our main finding confirmed the well-known association between subclinical depressive symptomatology and SCD. In addition, we demonstrated that the association between depressive symptomatology and SCD was independent of AD and CVD biomarkers. Our logistic regression analyses showed that depressive symptom scores do not seem to influence the association of amyloid-beta, tau, and WMSA biomarkers with SCD. While this association has not been extensively investigated, some previous studies also showed that depressive symptomatology can co-exist with brain pathologies (Buckley et al., 2017; Diaz-Galvan et al., 2021a; Minett et al., 2005).

Depressive symptom scores did not correlate with AD and CVD biomarkers in our study. Similarly, Diaz-Galvan et al. (2021a) did not find any significant association between depressive symptomatology and a CVD biomarker. However, other studies did find significant associations between depression and AD biomarkers (Harrington et al., 2015) and CVD (Minett et al., 2005). These contradictory results may be due to the fact that in the articles reviewed in Harrington et al. (2015), participants had higher levels of depression than in our sample, mostly representing major depression or dysthymia; and in Minett et al. (2005), depression was operationalized as a past history of major depression. Hence, in these previous studies, the measures of depression reflected clinical depression. In contrast, our cohort primarily reflects variability in the subthreshold spectrum of depressive symptomatology (subclinical depressive symptoms). Only 7 participants had scores above the clinical cut point for depression: 6

were within the mild range and 1 was within the moderate range of depressive symptomatology. As per exclusion criteria, our participants did not have a clinical diagnosis of major depression nor were they under treatment for depression, in agreement with the current diagnostic criteria of SCD (Jessen et al., 2014). Altogether, our results suggest that subclinical depressive symptomatology in our SCD individuals did not reflect AD or CVD pathologies.

We found different associations depending on the type of subjective complaint. Concentration complaints were mainly associated with the amyloid-beta biomarker, followed by depressive symptom scores. In contrast, memory complaints were mainly associated with depressive symptoms scores, followed by the tau biomarker. To the best of our knowledge, only two studies have addressed the distinction between subjective complaints of memory and concentration (Grambaite et al., 2013, Topiwala et al., 2021). Both studies partially agree with our results by showing that more depressive symptomatology was associated with memory and concentration complaints, although with a stronger association with concentration complaints (Grambaite et al., 2013, Topiwala et al., 2021). However, these previous studies did not exclude concentration items from the depression scales. In contrast, our current study did exclude the concentration item from the depression score to avoid circularity. This could influence the strength of the associations because difficulties in concentration are frequent in people with depressive symptomatology.

Regarding AD biomarkers, we found that concentration complaints were associated with the amyloid-beta biomarker, while memory complaints were associated with the tau biomarker. Despite not specifically assessing concentration complaints, Amariglio et al. (2012) found an association of non-memory subjective complaints with their amyloid-beta biomarker. In contrast, Grambaite et al. (2013) reported that memory and

concentration complaints were not associated with amyloid-beta, p-tau, or total tau CSF biomarkers in individuals with memory complaints (Grambaite et al., 2013). The lack of a significant association in Grambaite et al. (2013) could be related to the small sample size (N = 23) and/or inclusion of a younger cohort (mean age, 58.8 years), since AD biomarker positivity increases with age (Spires-Jones et al., 2017). Additionally, several studies that did not differentiate the type of complaint but investigated an SCD group, found significant associations with AD biomarkers (Amariglio et al., 2012; Buckley et al., 2017; Visser et al., 2009).

The AD continuum of the NIA-AA classification system (Sperling et al., 2011) places SCD at the latest stage of the preclinical phase, also known as the transitional stage (Stage 2). In that stage, amyloid-beta and tau biomarkers are positive, but there is no formal evidence of objective cognitive impairment (Jessen et al., 2020). Our differential associations of concentration and memory complaints with amyloid-beta and tau biomarkers could be interpreted using the *Hypothetical Model of Dynamic Biomarkers* proposed by Jack et al. (2010; 2013). In that model, amyloid-beta positivity precedes positivity in tau biomarkers. Following this hypothesis, our results could be interpreted as concentration complaints being an early sign of amyloid-beta pathology, hence reflecting an AD pathological change (Jack et al., 2018). In contrast, memory complaints would be a sign of tau pathology, hence reflecting AD pathology and signifying a more developed disease status closer to the clinical transition to MCI (Jack et al., 2018). This is also an interesting finding when it comes to interpreting the role of depressive symptomatology in SCD. In our cohort, depressive symptomatology seems to be an important factor at the end of the SCD continuum, showing a significant association with memory complaints, which in turn reflect tau pathology in our cohort. However, in the context of amyloid-beta pathology, depressive symptomatology

rendered a weaker although significant association with subjective complaints, in this case, concentration complaints. It should be clarified that we modelled amyloid and tau CSF biomarkers as continuous variables in our analyses and the proportion of biomarker positive amyloid and tau SCD individuals was low. Therefore, subjective complaints are differentially associated with these AD biomarkers but these biomarker levels may be normal and might remain normal over time. One thus needs to be aware that this finding could reflect normal age-related changes and not pathological changes. However, if this differential association of amyloid-beta and tau biomarkers with concentration and memory complaints and depressive symptomatology can be replicated in other cohorts, our findings may have clinical implications. One could suggest that people may be able to detect different stages of the biological process of AD through concentration and memory complaints before cognitive decline can be detected with objective neuropsychological tests. This is a first step to disentangle the underpinnings of these different complaints, as well as the different implications of depressive symptomatology in the AD continuum. In addition, such a finding could support the use of certain complaints to enrich research cohorts and clinical trials with certain biomarker profiles (Diaz-Galvan et al., 2021b).

We did not find any significant association between SCD and CVD biomarkers in our cohort, in line with other studies that included similar populations (Bartley et al., 2012; Topiwala et al., 2021; van Norden et al., 2008). However, there are studies showing a significant association between SCD and CVD (de Groot et al., 2001; Diaz-Galvan et al., 2021a; Minett et al., 2005). These diverging results from de Groot et al. (2001) could be explained by SCD mostly being related to periventricular WMSA, while our WMSA biomarker is a measure of global burden. The discrepancy with Diaz-Galvan et al. (2021a) could be related to their wide age range, while our participants

were all 70 years old by design. Furthermore, the average age of the cohort in Diaz-Galvan et al. (2021a) (mean 54.6 years) was younger than in our cohort, including an age strata where CVD can already be present, but it is more difficult to find amyloid-beta or tau positivity. In Minett et al. (2005) WMSA were related to the severity of SCD, rather than the presence of SCD, and complaints were assessed with a memory questionnaire. Further, individuals sought medical help and complaints were elicited by the physician, a different setup than in our study. In addition, we cannot exclude that AD and CVD are two additive pathologies, and together they increase the risk for cognitive impairment (Vemuri et al., 2015). In our cohort, the presence of both AD pathology and CVD is likely associated with cognitive impairment, which was an exclusion criterion in our current study. This could also explain the diverging results with the findings of de Groot et al. (2001), Diaz-Galvan et al. (2021a), and Minett et al. (2005).

The present study has some limitations. To completely capture the interaction between biomarkers, complaints, and depressive symptomatology throughout the different preclinical phases of AD, our current findings should be complemented with longitudinal studies. To the best of our knowledge, findings from our cross-sectional analyses are the first on the association between these three factors, and may be useful for the design of future longitudinal studies. Another consideration is that Jessen et al. (2014) suggested that the complaints should have a duration of 6 months, while in the current study our questions referred to complaints within the last 1 month. This criterion was included in Jessen et al. (2014) mostly to increase the ability of SCD to reflect a neurodegenerative disease. It is reassuring that despite referring to the last 1 month in our study, we still captured associations of the complaints with both amyloid-beta and tau biomarkers of AD. We used two items from the CPRS to assess SCD. Most of

previous studies are based on only one item of memory, so the addition of the concentration items is an advantage. However, in the future, it would be important to apply more comprehensive questionnaires of memory and concentration complaints, perhaps including other non-memory domains as well. Finally, we comment on some non-significant statistical trends due to their potential clinical interest. To substantiate this approach, we provided with effect sizes (or odds ratios) in the respective analyses. Effect sizes are also informative in the context of greater statistical power for the SCD-memory group than for the SCD-concentration group, since we anticipated that it would take larger differences or stronger associations to become statistically significant in the smaller SCD-concentration group. We confirmed that none of the large effect sizes (or odds ratios) in the smaller SCD-concentration group were missed to become significant (i.e., we ruled out any potential false negative results).

We conclude that in our cohort, depressive symptomatology in SCD can be interpreted as an independent phenomenon of AD and CVD biomarkers. The role of depressive symptomatology may be different depending on the actual stage within the spectrum of preclinical AD (as determined by amyloid-beta and tau positivity). Our findings help to advance the current knowledge on the role of subclinical depressive symptomatology in SCD, a topic that has recently been urged by the international SCD-I (Jessen et al., 2020; Molinuevo et al., 2017). Moreover, we suggest that subjective complaints of memory and concentration may reflect different stages of AD pathology. This study adds to the still scant literature on the potential association of different subjective cognitive complaints with distinct syndromic and biomarker profiles.

Supplementary Material

Logistic Regression Analyses

Partial Association of CSF Biomarkers, Hyperintense WMSA, and Depressive Symptomatology with SCD-memory

Binary logistic regression was conducted including SCD-memory as the criterion variable (SCD-memory *vs.* controls), and MADRS-9, Aβ42/40, p-tau, and hyperintense WMSA as the predictors. The model was significant ($\chi^2(2) = 8.550$, $p = .014$, $R^2 = .054$ (Nagelkerke), showing that higher MADRS-9 scores ($B = .108$, Wald = 5.042, SE = .048, $p = .025$, OR = 1.114) and higher p-tau ($B = .133$, Wald = 3.744, SE =.069, $p = .053$, OR = 1.142) significantly predicted SCD-memory. The SCD-memory group had higher p-tau levels and MADRS-9 scores. However, Aβ42/40($B = -.032$, Wald = .000, SE = 2.094, $p = .988$, OR = .968) and hyperintense WMSA ($B = 2.745$, Wald =.186, SE = 6.365, $p = .666$, OR = 15.559) were not significantly associated with SCD-memory.

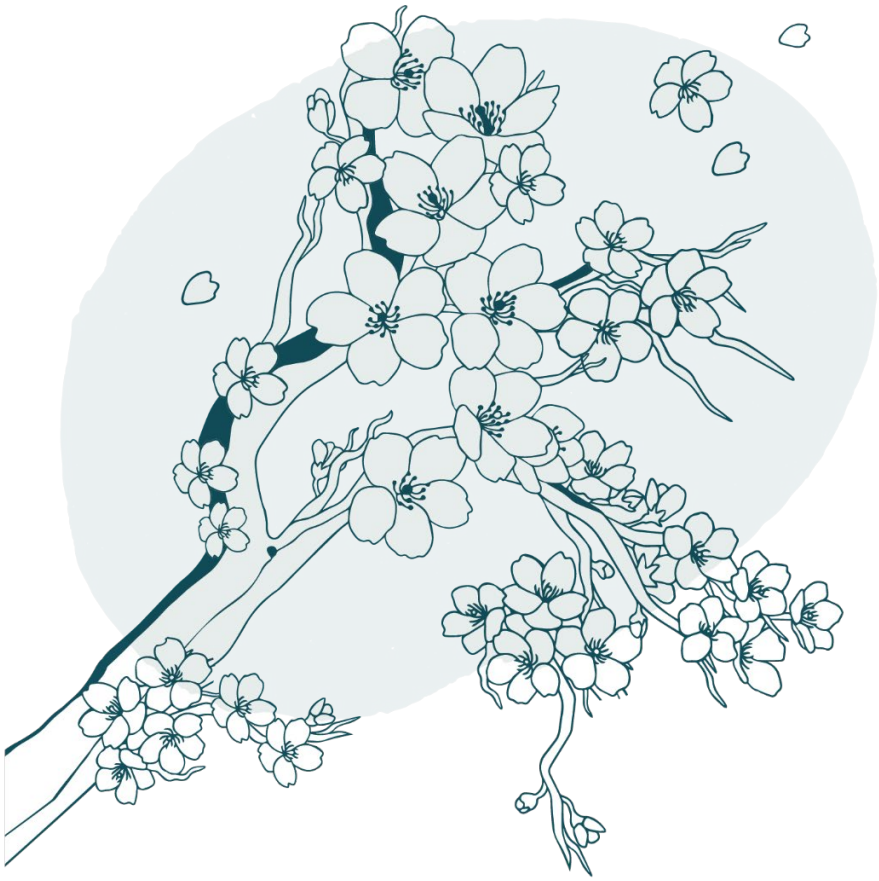
Partial Association of CSF Biomarkers, Hyperintense WMSA, and Depressive Symptomatology with SCD-concentration

We performed similar models for SCD-concentration. SCD-concentration was included as the criterion variable (SCD-concentration *vs.* controls), and MADRS-9, Aβ42/40, and p-tau as well as hyperintense WMSA as the predictors. The model was significant ($\chi^2(2) = 21.422$, $p < .001$, $R^2 = .274$ (Nagelkerke). Aβ42/40 ($B = -15.302$, Wald = 11.813, SE = 4.653, $p = .001$, OR = .000) was the main predictor of SCD-concentration, followed by MADRS-9 ($B = .201$, Wald = 7.045, SE = .076, $p = .008$, OR = 1.223). The SCD-concentration group had lower levels of Aβ42/40 and MADRS-9 scores. In contrast, p-tau ($B = -.001$, Wald =.000, SE =.132, $p = .991$, OR =.999), and

hyperintense WMSA (B = 9.013, Wald =.596, SE = 11.675, p =.440, OR = 8206.437) were not significantly associated with SCD-concentration.

Chapter VII

General Discussion



General Discussion

This doctoral thesis aimed to investigate the role of vulnerability and resilience factors to stress and early dementia detection or cognitive health in young, middle-aged, and older people. More specifically, the first and second studies investigated the relationship between some psychological traits considered positive or harmful for health, such as resilience, optimism, or pessimism, and the acute or chronic stress response measured via the HPA axis functioning in healthy older people. The third, fourth, and fifth studies examined the relationship between positive and negative psychological factors, the HPA axis, and neurodegenerative factors in different age populations. Specifically, the third and fourth study examined the relationship between negative affective states (depression and anxiety), negative (trait anxiety) and positive (resilience and positivity) psychological traits, the HPA axis and SMCs in healthy young, middle-aged, and older participants. The fifth study examined the relationship between depressive symptomatology, biomarkers of AD, and CVD and SCD in healthy older participants.

The following is a general discussion of the main results obtained, a global reflection on their implications, general limitations, and future directions based on the main findings of this thesis.

Summary of main findings

Study 1

Previous literature has shown that some people experience growth and adaptation rather than developing health problems in stressful situations. The aim of this study was to test the relationship between resilience and the psychobiological response to an acute stressor in 66 healthy older people between 56 and 75 years old. We also

wanted to test the possible mediation effect of active and avoidance coping strategies in the relationship between resilience and the physiological (cortisol reactivity) and psychological (anxiety reactivity) response to an acute laboratory stressor. Acute stress was elicited by the TSST, and the stress response was measured at endocrine (cortisol) and psychological (anxiety) levels. Moderation analyses showed that resilience was related to cortisol reactivity through the use of active coping strategies. Thus, highly resilient individuals engaged in more active coping strategies and, consequently, experienced lower cortisol reactivity. However, active coping did not mediate in the relationship between resilience and anxiety reactivity. Moreover, avoidance coping did not mediate in the relationship between resilience and the psychobiological response to a stressor. Our results highlight the relevance of active coping strategies as an important component of resilience when facing a stressor.

The main results of our study are consistent with other studies that found an association between resilience and active coping strategies (Mayordomo et al., 2016; Thompson et al., 2018; Tomás et al., 2012), and between active coping strategies and better stress regulation (Villada et al., 2017). The results also agree with a study that showed no association between resilience and avoidance coping (Smith et al., 2016). Following Lazarus and Folkman's model (1984), resilient individuals may have greater resources to cope with stress, including the use of adaptive coping strategies. Moreover, an absence of passive (avoidant) coping strategies (i.e., mental disengagement and denial) may have an influence on stress appraisal, enhancing resilience and predisposing them to engage in more active coping strategies (Gloria & Steinhardt, 2016; Thompson et al., 2018). Thus, our hypothesis about resilience influencing less avoidant coping strategies could be viewed as the opposite, with the lack of avoidance (maladaptive) coping being an influential factor in resilience. This new perspective should be further

tested. According to the *Broaden and Build Theory of Positive Emotions* (Fredrickson, 1998), resilient individuals may experience positive emotions rather than negative ones in stressful situations, which makes them react more effectively and develop more adaptive strategies to deal with stress (Feder et al., 2009; Fredrickson, 2001; Gloria & Steinhardt, 2016; Tugade & Fredrickson, 2004).

Finally, we failed to find an association between resilience and anxiety reactivity through active coping strategies. These results could be viewed from the *Emotion Regulation Perspective*. Emotion regulation increases with age and can help to manage psychological stress responses more easily (Charles & Piazza, 2009; Nielsen et al., 2008). Overall, the different results related to the physiological and psychological stress response shed light on the existing literature about the independence between psychological and physiological responses to laboratory stressors (Campbell & Elhert, 2012; Villada et al., 2014b).

In sum, our results add evidence about the importance of resilience and adaptive (active) coping strategies in HPA axis regulation, suggesting their possible protective role in stress-related pathologies associated with aging.

Study 2

Chronic stress is an important factor related to health, especially in older populations that are at a greater risk of developing stress-related disorders. Thus, current perspectives focus on which psychological traits can explain individual differences in chronic stress. This study aimed to investigate the association between dispositional optimism, and its optimism and pessimism subscales, and chronic stress biomarkers measured in hair. We also wanted to explore sex differences in chronic stress biomarkers as well as in their association with psychological traits. To this end, we

measured hair cortisol (HC), hair dehydroepiandrosterone (HDHEA), and their ratio HC:$HDHEA_{ratio}$ in 121 healthy older people (46 men and 75 women) between 56 and 81 years old. Linear regression analyses indicated that higher dispositional optimism was related to lower HC and a lower HC:$HDHEA_{ratio}$. Specifically, lower scores on the pessimism subscale were related to lower HC and a lower HC:$HDHEA_{ratio}$, whereas higher scores on the optimism subscale were only related to a lower HC:$HDHEA_{ratio}$. No sex differences were found. These results suggest that positive and negative expectations about the future (i.e., optimism and pessimism) may play an important role in elderly people's health due to their relationship with the HPA axis. The present findings are in line with other investigations that have studied the relationship between optimism or other resilience factors and HC in young adults (García-León et al., 2019; Milam et al., 2014), and the HC:$HDHEA_{ratio}$ in mixed-aged samples (Bürgin et al., 2020). Our results could be explained by the *Self-Regulatory Behaviour Theory* (Scheier & Carver, 2000), which states that positive expectancies about the future (i.e., optimism) lead to better sources to cope with stress (Carver et al., 2010). In this line, optimistic people show lower perceived stress (Endrighi et al., 2011; Milam et al., 2014) and higher engagement in active coping strategies (Nes & Segerstorm, 2006). In contrast, anticipating that negative things are going to happen and having lower expectancies about achieving goals (i.e., pessimism) lead to less effort when dealing with stressors and greater use of avoidant (maladaptive) coping strategies. This is translated into higher vulnerability to stress-related diseases (Carver et al., 2010).

Moreover, the stronger relationship found between dispositional optimism and its pessimism subscale, compared to optimism, and chronic stress biomarkers highlights the idea that the optimism and pessimism subscales should be studied separately in older people (Herzberg et al., 2006; Puig-Perez et al., 2015; Rasmussen et al., 2009).

Our results suggest that the effect of dispositional optimism on HPA axis regulation is mainly due to the pessimism subscale, given the greater weight of pessimism over optimism in predicting chronic stress biomarkers. Thus, aspects of pessimism, such as focusing more on negative aspects of life, may have a greater influence on health and HPA axis functioning than the protective effects of optimism (Puig-Perez et al., 2018; Robinson-Whelen et al., 1997; Scheier & Carver, 2018). Overall, the results for the HC:$HDHEA_{ratio}$ could mainly be due to HC levels, possibly because, on the subscales, pessimism is significantly related to HC, whereas optimism is not, which could explain why the relationship with the HC:$HDHEA_{ratio}$ is stronger for the pessimism subscale than for the optimism subscale. Moreover, the absence of relationships with HDHEA could be further investigated in different age periods because, before our study, the relationship between HDHEA and positive factors had been only investigated in younger samples. Finally, our results show no sex differences in any of the stress biomarkers measured in hair, suggesting that the chronic stress response may be similar in healthy older men and women, as previously found (Dettenborn et al., 2012; Gao et al., 2010; Manenschijn et al., 2011; Raul et al. 2004; Thomson et al., 2010). More research is needed on sex differences in chronic stress biomarkers, given the inconclusive results in the literature.

Study 3

SMCs have been proposed as an indicator of cognitive health. Psychophysiological states produced by stress exposure have been suggested as associated factors in SMCs, although little is known about the young population. This study investigated the relationship between depression, anxiety states, and HPA axis functioning and SMCs. We also studied whether resilience was associated with SMCs through depression, anxiety, and HPA axis activity. To do this, we evaluated 77 healthy

young people between 18 and 34 years old. Basal HPA axis functioning was addressed on two days through four saliva samples each day, and the CAR index was obtained. Although linear regression analyses showed that depression and anxiety were significantly related to SMCs, hierarchical multiple regression showed that depression was the only predictor of SMCs after controlling for anxiety. The CAR was not related to SMCs. Mediation analyses showed that higher resilience was related to fewer SMCs through less depression and anxiety, but not the CAR. Together, our results point out that negative affective states have greater importance in SMCs than the HPA axis in young populations, and they highlight the role of resilience as a protective factor in SMCs through better psychological stress regulation (lower depressive and anxiety states).

We corroborated previous literature on depression and anxiety as factors associated with SMCs in young populations (Loprinzi, 2019; Pearman, 2009; Pellicer-Porcar et al., 2014). We also added new evidence showing that depression is the most influential factor in the SMCs of young individuals. Our results could be explained in light of the *Comprehensive Cognitive Model of Depression*, which states that people with depression have more negative cognitive biases, paying more attention to negative aspects of life and themselves (Beck, 2008). Thus, this negative perception may lead to an overestimation of memory failures (Montejo-Carrasco et al., 2013). Moreover, depression tends to be associated with impaired concentration and low motivation, which may influence people's low effort and focus when they have to remember or memorize something (Rowell et al., 2016). These behaviors could lead to a real subtle decline in cognitive performance, starting a vicious cycle and increasing people's subjective perception about memory decline. The results obtained could also be viewed in the opposite direction, with SMCs acting as a stressor and, thus, preceding depressive

and anxiety states (Jessen et al., 2020). The main finding of this study was the protective role of resilience against SMCs through adaptive emotional regulation. This result agrees with studies that found a relationship between resilience and lower depression and anxiety (Hjemdal et al., 2006; 2011). It should be noted that the *Broaden and Build Theory of Positive Emotions* points out that resilient individuals, by experiencing positive emotions rather than negative emotions in stressful situations, broaden their focus of attention and enhance flexibility in thinking (Fredrickson, 2001). Thus, our findings suggest that resilient people may have fewer SMCs due to their psychological characteristics, or that resilience attenuates the negative consequences of depression and anxiety for memory perception. Further interventions should continue to investigate resilience as a key factor in affective processes and health.

We failed to find associations between SMCs and the CAR, contrary to the results reported in older participants (Peavy et al., 2013). These discrepancies suggest that, in early stages, SMCs are not related to HPA dysregulation, and that, in older samples, the maintenance of a stressor such as the subjective perception of memory failures over time may influence cortisol secretion. Other modulating factors strongly related to the HPA axis, such as personality characteristics or psychological traits, could shed light on the association between the HPA axis and SMCs.

Study 4

After not finding an association between SMCs and the HPA axis in young people in the third study, the fourth study in this dissertation investigated (i) whether SMCs were related to psychological traits such as positivity and trait anxiety, and (ii) whether these psychological traits moderated the association between the HPA axis and SMCs. Here, we studied 75 healthy young adults (from 18 to 35 years old; Study 1) and

73 older adults (from 55 to 75 years old; Study 2). In addition to trait anxiety and positivity measures, cortisol was collected after awakening and before going to bed on two consecutive days. Thus, two cortisol indexes were obtained: (i) cortisol awakening levels and (ii) the DCS. In both age groups, higher anxiety was related to more SMCs, but higher positivity was related to fewer SMCs only in young people and older women. Moderation analyses showed that psychological traits moderated the relationship between the HPA axis and SMCs in young individuals. This means that higher awakening cortisol and a steeper DCS were related to more SMCs in those with higher anxiety and lower positivity. In older samples, age was the moderator in this relationship (when we compared those aged 55 to 64 vs 65 to 75). Lower awakening and blunted DCS were directly related to more SMCs only in participants from 55 to 64 years old, but not in those older than 64. These results emphasize the different nature of SMCs across the lifespan and clarify the association between the HPA axis and SMCs in different age periods.

The present findings add evidence to the growing literature about the relationship between personality traits and SMCs in young and elderly people (Carrigan & Barkus, 2016; Pearman & Storandt, 2004; Pearman et al., 2009). Thus, SMCs were related to trait anxiety independently of age. One explanation is that, as mentioned in the *Attentional Control Theory*, trait anxiety is associated with lower attentional focus during tasks because highly anxious people put their focus of attention on threat stimuli such as irrelevant distractors or worrisome thoughts (Eysenck, 2007). This lowered attention could explain why anxious people report more SMCs given that they cannot retrieve things they paid little attention to (Bishop, 2009). It has also been suggested that anxious people tend to experience higher negative affect, and so it is more likely that these individuals also focus on negative aspects of life, increasing their perception

of memory loss (Pearman et al., 2021). Moreover, anxious people, in addition to perceiving more situations as threatening, use worse coping strategies and are more vulnerable to stress and its effects on memory (Lupien et al., 2018; Norman et al., 2020). The relationship between cortisol indexes (awakening cortisol and DCS) and SMCs was found in young individuals with higher anxiety, corroborating the hypothesis about higher stress vulnerability and a more dysregulated HPA axis in anxious people (Adam et al., 2017; Chow & Mercado, 2020; Vreeburg et al., 2010).

Our results regarding positive traits and SMCs in healthy young and older women point out the protective role of positivity in subjective indicators of memory. Following the *Positive Orientation Theory* (Caprara et al., 2010), some characteristics of positive individuals, such as optimism, self-esteem, and life satisfaction, make them more able to cope with stressful situations by broadening their focus of attention and reducing the negative effects of stress on memory consolidation and retrieval (Lupien et al., 2018). This was also corroborated by our moderation analyses, which showed the moderating effect of positivity in the relationship between cortisol indexes and SMCs in young individuals.

We also emphasize the role of age in the relationship between HPA axis indicators and SMCs. In our group of healthy older people, we found that there was a direct relationship between cortisol indexes and SMCs in middle-aged adults (55-64 years old), although this relationship was not found in older people (65-75 years old). This finding agrees with our hypothesis about memory complaints acting as a stressor, thus affecting cortisol secretion in middle-aged people (Kudielka et al., 2006). The perception of memory loss led to higher stress and, therefore, HPA axis dysregulation, which in turn predisposes them to brain changes and subtle cognitive problems, initiating a vicious cycle (Peavy et al., 2013). Differences between middle-aged and

older participants could be due to the fact that middle-aged individuals are more aware of their memory deficits because they continue to work and have higher demands in terms of memorizing things for work, which increases their SMCs. These greater work demands could also lead to higher stress and, thus, dysregulated HPA axis activity (Cedres et al., 2019). Moreover, although our results for older adults are different from those of other studies investigating the role of cortisol in SMCs in this age range (Fiocco et al., 2006; Peavy et al., 2013), these differences could be attributable to the sample characteristics. Our older adults were healthy and had no economic problems, lower anxiety and perceived stress, and not many SMCs. Overall, the lack of relationship between the HPA axis and SMCs over 65 years old suggests that, when SMCs appear at older ages, causes other than stress indicators might be more associated with SMCs, such as preclinical stages of MCI and dementia (Jessen et al., 2014; Jonker et al., 2000).

Study 5

A collaboration at the Karolinska Institutet in Sweden through a research stay allowed us to test the possible association between SMCs and neurodegenerative factors and the role of depressive symptomatology in people over 65 years old. In the last study in this thesis, we analyzed a sample of 217 older people, all of whom were 70 years old, from the Gothenburg H70 Birth Cohort 1944 Studies. We measured AD and CVD through cerebrospinal fluid levels of the Aβ42/40 ratio and phosphorylated tau (p-tau) and white matter signal abnormalities on an MRI. SCD was assessed through memory and concentration complaints. Additionally, we measured depressive symptomatology. In this study, we wanted to test the role of depressive symptomatology and biomarkers of brain pathology (CVD, Aβ42/40 and p-tau) in SCD. We also aimed to investigate whether depressive symptomatology was related to brain pathology. Logistic regression

analyses showed that depressive symptomatology was strongly related to SCD. Correlation analyses showed no relationship between depression and AD and CVD biomarkers. Interestingly, we found different associations depending on the type of complaint. Thus, memory complaints were mainly related to depression, followed by the p-tau biomarker, whereas concentration complaints were mainly associated with the amyloid-beta biomarker, followed by depression. These results help to clarify the current, intense discussion about the role of depressive symptomatology in SCD, as well as adding new evidence about the different meanings of memory and concentration complaints.

First, we corroborated the well-known association between depressive symptomatology and SCD (Cedres et al., 2019; Hill et al., 2016). Furthermore, results of the logistic regression showed that this relationship was independent of AD and CVD biomarkers, as reported in other studies, showing that depressive symptoms can co-exist with brain pathology (Buckley et al., 2017; Diaz-Galván et al., 2021a; Minett et al., 2005). We did not find any association between depression and AD and CVD biomarkers, which is in line with a previous study that found no association between depressive symptomatology and CVD, although AD pathology was not included (Diaz-Galván et al., 2021a). One explanation could be that higher levels of depressive symptomatology are more likely to be related to AD and CVD pathology (Harrington et al., 2015; Minett et al., 2005). In contrast, in our cohort we addressed subclinical depressive symptomatology following the SCD-I (Jessen et al., 2014) criteria, and we excluded participants who had major depression or were undergoing treatment for depression. Thus, the logistic regression and correlation analyses suggest that depressive symptomatology did not reflect AD or CVD pathologies in SCD individuals.

The differential association between memory and concentration complaints and different biomarker profiles within the spectrum of preclinical AD could be viewed from the perspective of the *Hypothetical Model of the Dynamic Biomarkers* (Jack et al., 2010; 2013). In this model, amyloid-beta positivity is the initiating event in the AD pathological cascade, followed by tau positivity. Thus, our results could be interpreted as indicating that concentration complaints are the first sign of the pathological cascade through their association with amyloid-beta. The association between tau pathology and memory complaints suggests a later stage of AD pathology more closely related to MCI (Jack et al., 2018). These results are consistent with studies that have associated SCD with AD biomarkers (Amariglio et al., 2012; Buckley et al., 2017; Minett et al., 2005) and others that specifically found a relationship between non-subjective memory complaints and amyloid beta positivity (Amariglio et al., 2012). Our results also suggest that the role of depressive symptomatology may be different depending on the stage on the AD continuum. Depressive symptomatology has greater weight at the end of the SCD continuum because it is more strongly associated with memory complaints, which in turn reflect tau pathology. To our knowledge, this is the first study to investigate the relationship between depressive symptomatology and different types of SCD in the context of brain pathology.

Finally, we did not find any association between SCD and CVD biomarkers. With advancing age, the presence of AD positivity has been found to increase (Harrington et al., 2015). It should be noted that AD and CVD are two additive pathologies that together increase the risk of MCI, and that the presence of cognitive impairment was one of the exclusion criteria. It is possible that, in our 70-year-old participants, the presence of both pathologies together is more closely related to MCI, and that these individuals were excluded from our final sample. Overall, our findings

clarify the role of depressive symptomatology in SCD, an issue that has been emphasized by the SCD-I (Jessen et al., 2020; Molinuevo et al., 2017). We also highlight the potential power of using different complaints to detect different stages of the AD pathological continuum.

In sum, this doctoral thesis adds evidence about the role of resilience as a protective factor in the stress response of the older population. We have seen that some resilience/protective factors play a key role in stress reactivity and basal HPA axis activity, enhancing the use of active coping strategies to deal with stressors and reducing the negative consequences of chronic stress on health. We also clarified the meaning of SMCs, understood as subjective cognitive health indicators, in young, middle-aged, and older people. Our studies shed light on their relationships with non-degenerative causes (psychological factors and stress) in different periods in the aging process. We corroborated the importance of negative psychological factors, depression and anxiety, and provided new evidence about the protective role of resilience factors in all age ranges. Stress biomarkers also seem to be related to SMCs through different moderators across the lifespan. Overall, in young people, SMCs are related to psychological causes and stress factors. With age, it appears that SMCs continue to be related to these non-degenerative factors, although in people over 65, they start to be related to AD biomarkers. In sum, this doctoral thesis helps to clarify the role of some psychological and stress indicators, in order to enhance and encourage future research and interventions focused on improving health and healthy aging.

Limitations and strengths

Each study in this dissertation has addressed its specific limitations. Therefore, in this section, we will focus on the general aspects that need to be considered. First,

due to the cross-sectional nature of all our studies, we could not ascertain causality. Another important limitation is that participants in all the studies were healthy people, given that we had strict control over factors related to health and medication (e.g., no neurological diseases, substance abuse, medication or psychiatric diseases, among others). Although this restrictive exclusion criterion was followed to avoid any confounding factor that could affect our aims, the results should be generalized with caution. Moreover, three out of four of the studies involving older people had a sample formed by participants from a university program. Thus, the inclusion of these participants could introduce some bias, given that a larger number of these people had active aging, lower levels of perceived stress, a higher socioeconomic status, and preserved cognitive capabilities. With all this in mind, generalization of the results to clinical samples or other samples involving the general population should be done with caution. Although several psychological factors and biological markers have been taken into account in this thesis, in psychology many variables are interconnected with each other, and so other factors could also be intervening, yielding alternative explanations.

Despite the limitations, the present doctoral thesis has some strengths that have to be highlighted. First, we studied several psychological factors related to stress and aging. More importantly, we focused on protective factors such as resilience or optimism and positivity, which have hardly been studied in the literature. This has allowed us to more deeply understand the role of positive psychological factors in stress and health, as well as encouraging future interventions to consider these variables in order to enhance general well-being and reduce age-related diseases associated with stress exposure. We also shed light on the meaning of SMCs in different age periods, in addition to clarifying the role of depressive symptomatology. Methodologically, we used different stress biomarkers, such as hair cortisol and DHEA and cortisol in saliva.

The control of saliva sampling was also rigorous, considering the factors that could affect its collection (Adam et al., 2017; Stalder et al., 2016). Moreover, precise statistical analyses, such as multivariate and logistic regression analyses as well as moderation and mediation using the bootstrap technique, allowed us to strengthen the power and validity of the results.

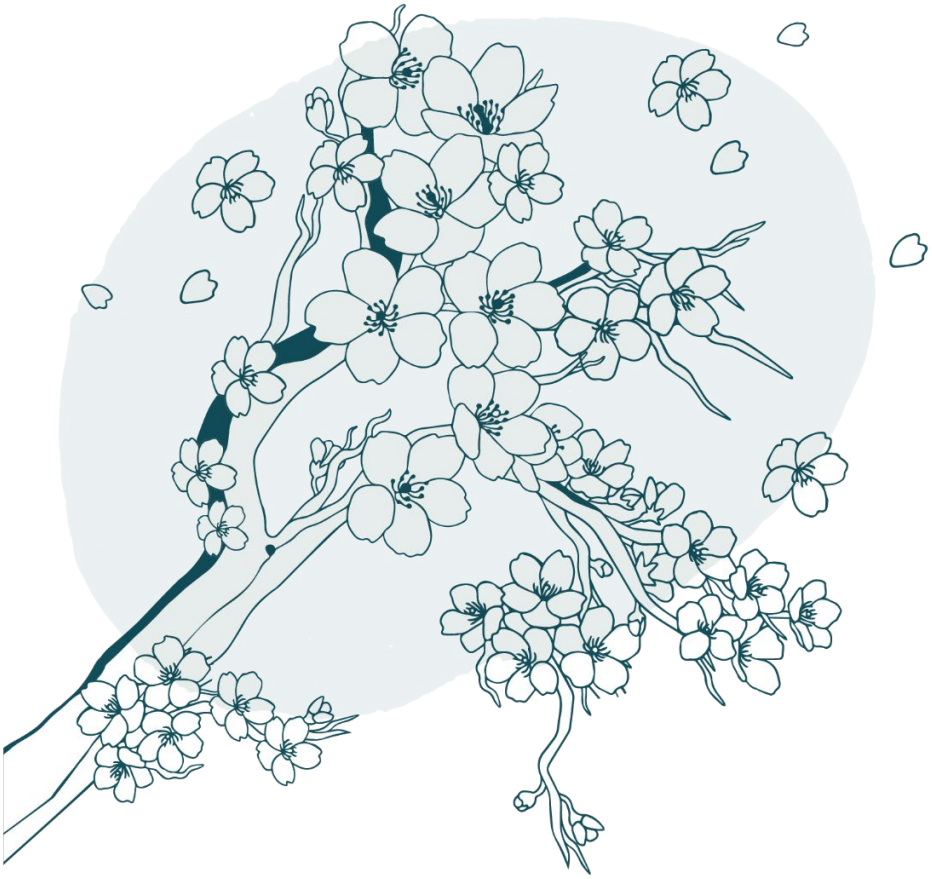
Future directions

The findings of this dissertation open up new, interesting research questions that should be answered in future studies.

The first interesting question comes from a result found in the first study in this dissertation, which showed a positive direct association between resilience and cortisol reactivity in older people when controlling for active coping strategies. In this regard, this competitive mediation suggests the existence of secondary mediators that should be tested in the future. We also encourage further studies to include different resilience biomarkers other than cortisol, such as heart rate variability. In this regard, heart rate variability has been suggested as a potential biomarker of resilience, due to its association with cognitive flexibility and stress adaptation. Another important issue is the fact that the results from our studies should be replicated in larger samples and samples with higher stress levels and more SMCs, in order to support the relationships found. Moreover, longitudinal studies will help to clarify causality between the variables involved, especially the variables related to SMCs. Finally, the last study in this doctoral thesis opens the door to studying subjective complaints different from memory, specifically through comprehensive validated questionnaires.

Chapter VIII

Conclusions



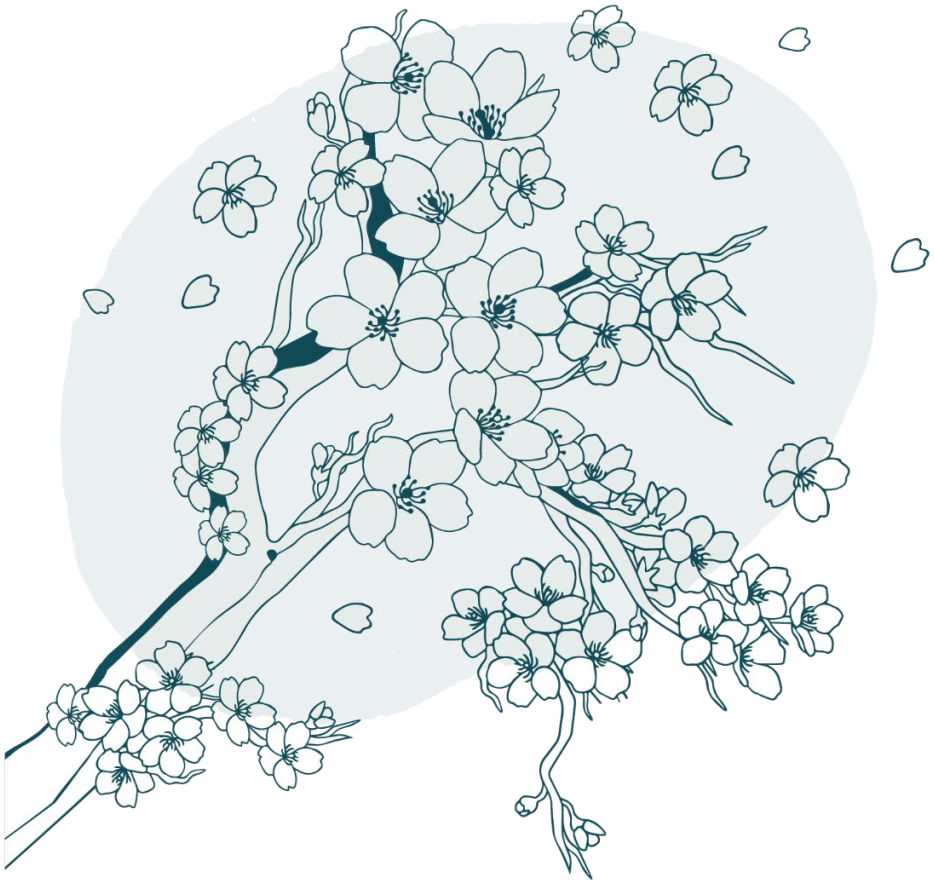
Main Conclusions

1) Resilience is associated with active coping strategies, which, in turn, explain a more adaptive cortisol reactivity to a laboratory stressor in healthy middle-aged and older people.
2) Positive and negative expectations about the future (i.e., optimism and pessimism) are related to chronic stress biomarkers measured in hair (HC and HC:HDHEA$_{ratio}$) in healthy middle-aged and older people.
3) Depressive and anxious symptomatology, but not basal HPA axis functioning (i.e., CAR), are related to more SMCs in healthy young people.
4) Depression has greater weight than anxiety in explaining SMCs in healthy young people.
5) Resilience is negatively related to SMCs through the mediation of negative affective states (i.e., depression and anxiety), but not basal HPA axis functioning (i.e., CAR), in healthy young people.
6) Trait anxiety is related to more SMCs in healthy young, middle-aged, and older people.
7) Higher positivity is associated with fewer SMCs in healthy young people and in middle-aged and older women.
8) Anxiety and positivity traits moderate the relationship between basal HPA axis functioning (i.e., awakening and DCS) and SMCs in healthy young people, but not in middle-aged and older individuals.
9) There is a direct association between basal HPA axis functioning (i.e., awakening and DCS) and SMCs in healthy middle-aged people, but not in older individuals.

10) SCD is independently related to depressive symptomatology and brain pathology in healthy seventy-year-old people.
11) Different memory and concentration complaints are related to different AD biomarkers in healthy seventy-year-old people.
12) Depressive symptomatology found in SCD is not related to AD and CVD biomarkers in healthy seventy-year-old people.

Chapter IX

General summary in Spanish



Introducción general

Depresión, ansiedad y estrés

Los trastornos mentales son una de las principales causas de enfermedad mundial, con aproximadamente 500 millones de personas viviendo con alguna de estas condiciones (World Health Organization [WHO], 2022b; Global Health Data Exchange [GHDx], 2021). Por ello se está dando mayor importancia al estudio de la salud mental y sus repercusiones en el bienestar de la población.

En este sentido, la depresión y la ansiedad se han asociado con diversos problemas de salud como la enfermedad cardiovascular o la diabetes mellitus, además de tener un impacto sobre el bienestar físico y psicológico (Allgulander, 2016; Clarke, 2009; Engum, 2007). Uno de los factores que más se ha relacionado con el desarrollo y mantenimiento de estas enfermedades es el estrés (Daviu et al., 2019; Hammen, 2005; McEwen, 2018).

El estrés es considerado un problema principal de salud de las sociedades actuales debido a su asociación con numerosas enfermedades mentales y físicas (O'Connor et al., 2021) y trastornos relacionados con el envejecimiento (Zsoldos et al., 2014). La respuesta de estrés incluye la activación de numerosos sistemas cognitivos, comportamentales, emocionales y fisiológicos (Campbell & Elhert, 2012). El primer nivel comprende la valoración y percepción que la persona tiene de la situación amenazante. Esta percepción de la situación predispone a la persona a responder de una determinada manera, ejecutando tareas específicas. A nivel emocional, las personas experimentan un incremento en ansiedad y afecto negativo (Campbell & Elhert, 2012; Villada, 2014a; 2017). Desde la perspectiva fisiológica, el organismo responde a los cambios en el ambiente a través de la activación de dos sistemas (eje Hipotalámico-

Pituitario-Adrenal [eje HPA] y Sistema Nervioso Autónomo [SNA]) para adaptarse y responder a las actividades demandantes.

El eje HPA se puede estudiar en su respuesta aguda y en su actividad basal. Situaciones de estrés agudo dan lugar a la respuesta de estrés, sobreponiéndose a esta actividad basal. Sin embargo, dicha actividad basal del eje HPA puede ser indicador de una exposición continuada o crónica al estrés midiéndose a través de la respuesta matutina de cortisol (Cortisol Awakening Response [CAR]) o la pendiente diurna de cortisol (Diurnal Cortisol Slope [DCS]) en saliva o mediante otros indicadores de estrés crónico como son los niveles de cortisol (Hair cortisol [HC]) o de dehidroepiandrosterona en pelo (Hair dehydroepiandrosterone [HDHEA]) (Adam & Kumri, 2009; Greff et al., 2019; Stalder et al., 2016; Ullman et al., 2016).

La respuesta fisiológica de estrés es adaptativa permitiendo al individuo lidiar con la situación estresante satisfactoriamente y, por lo tanto, manteniendo su homeostasis interna (Sterling & Eyer, 1988). Sin embargo, como el *Modelo de Carga Alostática* señala, cuando los recursos que la persona necesita para afrontar la situación exceden la energía que la persona tiene para afrontarla, aparece la patología (McEwen, 1998). Por lo tanto, una activación repetida o exacerbada de estos sistemas, comúnmente llamado estrés crónico o agudo, lleva a una sobrecarga alostática y, por lo tanto, a un aumento del riesgo de padecer problemas físicos y mentales como enfermedad cardiovascular, alteraciones cognitivas, depresión o ansiedad (McEwen, 2018). Según el *Modelo de Umbral de Reactividad de Cortisol*, una respuesta adaptativa de estrés se caracterizaría por una respuesta moderada del eje HPA, y niveles también moderados de emociones negativas (Herman et al., 2016; Vrshek-Schallhorn et al., 2018). En relación con la actividad basal del eje HPA, su disrupción a nivel crónico (bajo CAR, un DCS aplanado o un aumento general de los niveles de cortisol durante el

día) se ha asociado con diversas enfermedades. Asimismo, niveles altos de depresión y ansiedad se han relacionado con una desregulación basal del eje HPA (Adam et al., 2017; Chida & Steptoe, 2009). Es por ello que estos indicadores de cortisol han sido considerados indicadores de salud objetivos (Adam et al., 2017; Dedovic & Ngiam, 2015; Lupien et al., 2009; Stalder et al., 2016).

Por lo tanto, una respuesta de estrés exagerada a largo plazo o de manera crónica o una disrupción del eje HPA basal se ha asociado con consecuencias negativas a largo plazo en la memoria (Allen et al., 2014; Lupien et al., 2018), el bienestar y la salud (McEwen et al., 2008). En este sentido, aunque los resultados son poco concluyentes acerca de la diferencia en la respuesta de estrés entre jóvenes y mayores, existen repercusiones a largo plazo sugiriéndose la senectud como un periodo de mayor vulnerabilidad a los efectos negativos del estrés. Así, una respuesta exagerada del eje HPA se presenta como un predictor de deterioro cognitivo futuro en personas mayores (de Souza-Talarico et al., 2020). Respecto al funcionamiento basal del eje, existe evidencia de que las personas mayores muestran niveles más altos de cortisol en general, menor CAR y DCS más aplanado (Heaney et al., 2012; Kudielka & Kirschbaum, 2003; Nater et al., 2013). Estas diferencias entre jóvenes y mayores, así como la mayor vulnerabilidad a los efectos del estrés en etapas posteriores de la vida destacan la importancia de estudiar la respuesta de estrés y el funcionamiento basal del eje HPA en personas mayores, además de sus efectos sobre la salud.

Resiliencia al estrés: eje Hipotalámico-Pituitario-Adrenal (HPA)

Existen diferencias individuales en cómo las personas afrontan las situaciones estresantes pudiendo estas incrementar o reducir las condiciones crónicas asociadas al estrés (Chida & Steptoe, 2009). La teoría de Lazarus y Folkman (1984), plantea que la percepción que una persona tiene de la situación así como los recursos con los que

cuenta para hacerle frente explican las diferencias individuales en la respuesta de estrés. Desde esta perspectiva, gran número de estudios se han centrado en el concepto de resiliencia.

La resiliencia se puede entender como un rasgo psicológico por el cual las personas entienden las situaciones estresantes como un reto, experimentando crecimiento y adaptación más que únicamente recuperación (Connor & Davidson, 2003). Las personas resilientes se recuperan más rápido y eficientemente de las experiencias estresantes debido al uso de estrategias de afrontamiento activas (Thompson et al., 2018), así como a sus altos niveles de positivismo, optimismo y emociones positivas (Souri & Hasanirad, 2011; Tugade & Fredrickson, 2004). La resiliencia se ha relacionado con un mayor bienestar (MacLeod et a., 2016), y con menores niveles de depresión y ansiedad (Bonanno, 2004; Hjemdal et al., 2006; Smith et al., 2016), protegiendo de la adversidad y, por lo tanto, promoviendo un envejecimiento saludable (Hildon et al., 2010; MacLeod et al., 2016).

Puesto que uno de los factores mediadores entre el estrés y la salud es el eje HPA, varios estudios han analizado qué factores podrían modular esta relación, entre los que se encuentra la resiliencia. Sin embargo, la investigación es escasa y con diversas poblaciones y grupos de edad habiendo únicamente tres estudios que investiguen la relación resiliencia-eje HPA basal y dos con la respuesta de estrés. Respecto a la respuesta basal del eje HPA, se ha descrito que la alta resiliencia se relaciona con un mayor CAR y un DCS más pronunciado en jóvenes (Lai et al., 2020). También, cuidadores resilientes de personas con autismo mostraron niveles bajos al despertar y durante los 60 min después de despertar (Ruiz-Robledillo et al., 2014). En niños con padres con Virus de la Inmunodeficiencia Humana (VIH), niveles altos de cortisol al despertar y una pendiente DCS más pronunciada se relacionaron con altos niveles de

resiliencia (Chi et al., 2015). Respecto a la respuesta de estrés, se encontró que hombres jóvenes con alta resiliencia tenían menores concentraciones de cortisol en anticipación a un estresor (Mikolajczak et al., 2008). Asimismo, en hombres y mujeres cuidadores de personas autistas con alta resiliencia, se ha descrito una menor secreción de cortisol durante el estresor (Ruiz-Robledillo et al., 2017). Sin embargo, ningún estudio ha investigado específicamente la relación entre la resiliencia y la respuesta psicobiológica a un estresor en personas mayores, ni en qué medida las estrategias de afrontamiento podrían estar mediando esta relación. Por ello, en el primer estudio de esta tesis doctoral se propuso contestar a esta pregunta.

Positivismo, Optimismo y eje Hipotalámico-Pituitario-Adrenal (HPA): biomarcadores de estrés crónico

El positivismo y el optimismo se han considerado dos componentes importantes de la resiliencia (Milioni et al., 2016; Souri & Hasanirad, 2011; Yu & Zhang, 2007). El positivismo o percepción positiva sobre uno mismo, la vida, el futuro y la confianza en los demás también engloba los pensamientos optimistas, ya que el optimismo se define como un pensamiento positivo sobre el futuro (Caprara et al., 2010). Se ha argumentado que el optimismo disposicional en personas mayores en lugar de ser un constructo unidimensional, englobaría dos dimensiones separadas (optimismo versus pesimismo) (Carver et al., 2010; Ferrando et al., 2002; Mroczek et al., 1993; Robinson -Whelen et al., 1997). El positivismo, el optimismo y el pesimismo se han definido como rasgos psicológicos dada su relativa estabilidad y se han relacionado con el eje HPA (Endrighi et al., 2011; Lai et al., 2005; Pasquali et al., 2020). La *Teoría de la Orientación Positiva* afirma que las características de los individuos positivos los hacen más propensos a afrontar con éxito situaciones estresantes (Caprara et al., 2010). Concretamente, parece

que las expectativas positivas sobre el futuro (es decir, un alto optimismo disposicional) juegan un papel clave en la respuesta al estrés (Puig-Perez et al., 2021).

No obstante, los estudios sobre la asociación entre optimismo y pesimismo con medidas estables de cortisol en saliva muestran resultados no concluyentes (Ebrecht et al., 2014; Endrighi et al., 2011; Lai et al., 2005; Pasquali et al., 2020; Puig-Perez et al., 2015; 2018). Por tanto, es conveniente emplear otro tipo de biomarcadores de estrés crónico, y analizar en qué medida se relacionan con rasgos psicológicos más positivos, para alcanzar resiliencia al estrés y, por tanto, mejor salud (Rasmussen et al., 2009). En este sentido, el análisis en muestras de cabello parece un buen método para capturar la actividad del eje HPA de manera crónica durante al menos los últimos 6 meses (Kirschbaum et al., 2009).

Dos estudios recientes han investigado la relación entre HC, HDHEA o la ratio HC: HDHEA (HC:$HDHEA_{ratio}$) con la resiliencia y factores relacionados con ella (Bürgin et al., 2020; García-León et al., 2019). Por un lado, estos últimos autores encontraron que la resiliencia estaba relacionada con un menor HC en jóvenes (García-León et al., 2019). Por otro lado, algunas medidas estrechamente relacionadas con la resiliencia, como son el sentido de coherencia y el autocuidado, se asociaron positivamente con los niveles de HDHEA y negativamente con la HC:$HDHEA_{ratio}$ en personas jóvenes y mayores (Bürgin et al., 2020). Sin embargo, hasta donde sabemos, solo un estudio ha investigado la relación entre el optimismo y la HC y mostró que un alto optimismo estaba asociado con menores niveles de HC (Milam et al., 2014).

Es importante conocer los factores asociados a un menor HC y mayor HDHEA, especialmente en la población mayor, debido a los peores efectos del estrés crónico sobre su salud y envejecimiento (Feller et al., 2014; Maggio et al., 2015). De hecho,

debido a los efectos positivos de la DHEA y al efecto negativo del cortisol sobre la salud y el bienestar, los niveles más altos de HDHEA y los niveles más bajos de HC en estas edades podrían estar asociados con un envejecimiento saludable (Kamin & Kertes, 2017; Maggio et al., 2015). A pesar de ello, no hay estudios relacionando el optimismo y el pesimismo con biomarcadores de estrés crónico medidos en muestras de cabello en personas mayores sanas. Este tema se abordó en el segundo estudio de esta tesis doctoral.

Eje Hipotalámico-Pituitario-Adrenal (HPA), factores psicológicos y Quejas Subjetivas de Memoria (QSM)

El estrés repetido puede afectar la función cerebral, especialmente los procesos de memoria, por la hiperactivación del eje HPA y el efecto posterior del cortisol en el hipocampo, entre otras regiones del cerebro. La demencia es actualmente la séptima causa de muerte entre todas las enfermedades y una de las principales causas de discapacidad en la sociedad actual, con una estimación de 55 millones de personas viviendo con esta condición (WHO, 2022a). Por tanto, es prioritario identificar estrategias para prevenir o retrasar el deterioro cognitivo o la demencia, dado su impacto en la salud, en la sociedad y la economía (Anstey et al., 2013).

Las percepciones de olvidos repetidos sobre aspectos cotidianos, conocidas como Quejas Subjetivas de Memoria (QSM), podrían ser un indicador importante para la detección temprana de la demencia y la salud cognitiva (Sunderland et al., 1986). La percepción de QSM a lo largo del tiempo, o una percepción de declive cognitivo que no se acompaña de un deterioro objetivo real, se define actualmente como Deterioro Cognitivo Subjetivo (Subjective Cognitive Decline [SCD]) (Jessen et al., 2014). Ambos conceptos se han utilizado amplia e indistintamente en muestras de personas mayores. La iniciativa internacional SCD (SCD-I) ha desarrollado un marco conceptual en el que

abordan varias cuestiones relacionadas con la complejidad del concepto. Sugieren que a edades más avanzadas (alrededor de los 60 años o más), el SCD está más asociado con la enfermedad de Alzheimer (EA) y los procesos neurodegenerativos, mientras que, en personas jóvenes, otros factores como condiciones médicas, condiciones psiquiátricas, rasgos de personalidad y exposición al estrés podrían ser más importantes (Jessen et al., 2014; 2020). En este sentido, estados psicológicos negativos relacionados con el estrés como son la depresión y la ansiedad han sido ampliamente estudiados relacionándose con un mayor número de QSM (Derouesné et al., 1999; Montenegro et al., 2013; Pellicer-Porcar et al., 2014; Söğütlü & Alaca, 2019). Las QSM también aparecen en jóvenes (Derouensé et al., 1999; Ginó et al., 2010; Mendes et al., 2008), pero pocos estudios se han realizado para clarificar la relación entre los estados afectivos y las QSM en esta población (Loprinzi, 2019; Pearman, 2009). Conocer qué factores están relacionados con la presencia de QSM en poblaciones más jóvenes podría ayudar a esclarecer la aparición de estos problemas cognitivos subjetivos a estas edades e incluso ayudar a gestionar intervenciones para frenar su aparición. En el tercer estudio de esta tesis doctoral se investigó la relación entre los estados afectivos negativos (i.e., depresión y estado de ansiedad) y su peso en la predicción de QSM en una muestra de individuos jóvenes.

Por otro lado, las QSM pueden verse como un círculo vicioso en el que la percepción de la pérdida de memoria puede causar una angustia persistente que conduce a una disfunción del eje HPA que provoca cambios cerebrales y problemas de memoria (Peavy et al., 2013). Solo unos pocos estudios han abordado la correspondencia entre las QSM y la desregulación del eje HPA (Fiocco et al., 2006; Peavy et al., 2013; Wolf et al., 2005). Estos descubrieron que las QSM se asociaron con una DCS más plana (Fiocco et al., 2006), concentraciones de cortisol diurno más altas y un CAR más bajo

(Peavy et al., 2013) y concentraciones de cortisol urinario de 12 h más altas (Wolf et al., 2005), entre las personas mayores sanas. Dado el cambio en el funcionamiento del eje HPA con la edad (Nater et al., 2013), también es importante estudiar cómo los indicadores del eje HPA podrían estar relacionados con las QSM en diferentes edades. Hasta la fecha, ningún estudio ha investigado si el eje HPA está relacionado con las QSM en jóvenes. De ahí que se propusiera el tercer estudio de esta tesis doctoral para dar respuesta a esta pregunta. El cuarto estudio de esta tesis doctoral también abordó este tema en diferentes grupos de edad. Además, dado el posible significado diferente de las QSM en personas de mediana edad y mayores antes y después de los 65 años, el cuarto estudio de esta tesis investigó esta relación en mayores con una edad menor y mayor a 65 años.

Existe cierta evidencia de que los factores psicológicos estables o de personalidad son predictores importantes de las QSM (Carrigan & Barkus, 2016; Pearman & Storandt, 2004; Pearman et al., 2009). La ansiedad rasgo se considera un factor potencialmente estable relacionado con más QSM en todas las edades (Balash et al., 2013; Mahoney et al., 1988; Mecacci et al., 2004; Norman et al., 2020; Pavisic et al., 2021; Sabatini et al., 2022), aunque solo un estudio ha relacionado estas dos variables en diferentes grupos de edad en un mismo estudio (Rowell et al., 2016). El cuarto estudio de esta tesis doctoral aportó más evidencia de la relación entre la ansiedad rasgo y las QSM considerando el factor edad. Además, se está intentando dilucidar en qué medida las variables psicológicas podrían interactuar con los biomarcadores del estrés, con el fin de frenar sus efectos sobre la salud. Dada la relación entre la ansiedad y el eje HPA (Adam et al., 2017), y entre el eje HPA y las QSM (Fiocco et al., 2006; Peavy et al., 2013; Wolf et al., 2005), el cuarto estudio incluido en

esta tesis doctoral también indagó el efecto moderador del rasgo de ansiedad en la relación entre eje HPA y las QSM.

Por otro lado, el estudio de los rasgos psicológicos positivos en relación con las QSM y cómo podrían actuar como protectores frente a su aparición o desarrollo es aún incipiente. Teniendo en cuenta la relación antes mencionada entre el estrés y las QSM (Fiocco et al., 2006; Peavy et al., 2013; Wolf et al., 2005), la resiliencia emerge como un factor importante relacionado con el afrontamiento del estrés y la adversidad, que podría estar relacionado negativamente con las QSM (VanMeter & Cicchetti, 2020). El positivismo también aparece como un factor relacionado con la resiliencia que facilita el afrontamiento al estrés (Caprara et al., 2010; 2012). Solo un estudio ha abordado la asociación entre la resiliencia y las QSM encontrando que un factor de resiliencia concreto (la confianza en los propios instintos) se asociaba negativamente con las QSM (Montejo-Carrasco et al., 2013). También, otros factores como afrontamiento positivo, afecto positivo, extraversión y optimismo se han relacionado con menos QSM (Fastame, 2022; Lee et al., 2016; Molina-Rodriguez et al., 2016; Sutin et al., 2020). Más interesante aún, ningún estudio ha investigado si estos rasgos psicológicos positivos podrían estar relacionados con las QSM a través de la mediación o moderación de factores relacionados con el estrés, como los estados afectivos negativos o el eje HPA. Considerando la escasa literatura sobre la asociación entre rasgos positivos y QSM, y la necesidad de corroborar si estos rasgos psicológicos pueden interactuar con otros factores asociados con las QSM (es decir, estados afectivos y eje HPA), el tercer y cuarto estudio de esta tesis doctoral cubrieron este vacío en la literatura. Concretamente, el tercer estudio investigó la relación entre la resiliencia y las QSM a través del estado depresivo, de ansiedad y el eje HPA en población joven. Y el cuarto analizó la relación entre la positividad y las QSM, así como la moderación de la positividad en la relación

entre el eje HPA y las QSM en dos muestras diferentes de individuos jóvenes y mayores.

Depresión, Declive Cognitivo Subjetivo y patología cerebral en el envejecimiento

La aparición de QSM después de los 60 años es un factor de riesgo importante a tener en cuenta para la relación entre el SCD y el deterioro cognitivo, dado que a estas edades el SCD está muy asociado a patología cerebral y causas neurodegenerativas (Jessen et al., 2020). El sistema de clasificación del National Institute on Aging-Alzheimer's Association (NIA-AA) posiciona al SCD en la segunda etapa de la fase preclínica de la EA. En esta etapa hay positividad en biomarcadores de EA pero el deterioro cognitivo aún no está presente (Jessen et al., 2020; Sperling et al., 2011). El SCD en etapas posteriores de la vida se ha relacionado con la patología de la EA, como los biomarcadores beta amiloide (Aβ) y tau (Amariglio et al., 2012; Buckley et al., 2017). Sin embargo, el SCD también puede estar asociado con otras causas neurodegenerativas como la Enfermedad Cerebrovascular (ECV) (Cedres et al., 2019; 2021; Diaz-Galvan et al., 2021a; Diniz et al., 2013; Minett et al., 2005). Como se mencionó anteriormente, la sintomatología depresiva se ha relacionado fuertemente con el SCD (Hill et al., 2016). Actualmente, la SCD-I ha planteado la preocupación de estudiar el papel de la sintomatología depresiva encontrada en pacientes con SCD (Jessen et al., 2020). Algunos estudios han encontrado una relación entre la depresión y los factores neurodegenerativos (Diniz et al., 2013; Pomara et al., 2016; Taylor et al., 2013). Por lo tanto, en la actualidad se busca aclarar si la sintomatología depresiva que se encuentra en los individuos con SCD se debe o no a factores neurodegenerativos. Previamente, un estudio investigó la relación entre la sintomatología depresiva y la ECV en SCD y encontró que el SCD estaba relacionado de forma independiente con la sintomatología depresiva y la ECV. La sintomatología depresiva no se asoció con ECV,

pero no se incluyeron biomarcadores de patología de EA (Diaz-Galvan et al., 2021a). El quinto y último estudio de esta tesis doctoral amplió este trabajo investigando la relación entre el SCD, la sintomatología depresiva y los biomarcadores patológicos de la ECV y la EA. También comprobamos si la sintomatología depresiva encontrada en la SCD estaba asociada a patología cerebral (ECV, Aβ y tau). Además, algunos estudios han diferenciado tipos de SCD: (i) SCD en la memoria (SCD-memory) usando la presencia de QSM y (ii) SCD en concentración (SCD-concentration), usando Quejas Subjetivas de Concentración (Grambaite et al., 2013; Topiwala et al., 2021). Un estudio reciente mostró que diferentes quejas se asociaron con diferentes perfiles de resonancia magnética (RM) y sintomatología depresiva, pero no incluyeron biomarcadores cerebroespinales de EA (Aβ y tau) (Diaz-Galvan et al., 2021b). El quinto estudio que comprende esta tesis probó las asociaciones antes mencionadas para las quejas de memoria y concentración.

Objetivos e hipótesis

En general, la literatura descrita anteriormente destaca las limitaciones actuales en el estudio de la relación entre varios factores psicológicos y el eje HPA y cómo podrían estar asociados con las QSM. El objetivo central de esta tesis doctoral fue clarificar el papel de diferentes factores psicológicos de vulnerabilidad y resiliencia en el estrés y la detección temprana de demencias o salud cognitiva, dos problemáticas importantes en la sociedad actual.

Los objetivos generales de esta tesis doctoral han sido:

1. Estudiar la relación entre rasgos psicológicos positivos (resiliencia y optimismo) en la respuesta al estrés tanto agudo como crónico del eje HPA en población mayor sana. Este objetivo fue abordado en el primer y segundo estudio de esta tesis.

2. Investigar el papel de los rasgos psicológicos positivos (resiliencia y optimismo) y negativos (ansiedad y sintomatología depresiva), el eje HPA y los biomarcadores neurodegenerativos en las QSM en diferentes poblaciones de edad. Los estudios tercero, cuarto y quinto de esta tesis fueron diseñados para alcanzar este objetivo.

Estudio 1

El objetivo de este estudio fue examinar la relación entre la resiliencia y la respuesta psicobiológica al estrés (cortisol y reactividad de ansiedad) en personas mayores sanas. Además, queríamos investigar si las estrategias de afrontamiento (activas y pasivas) mediaban en esta asociación.

Esperábamos una relación negativa entre la resiliencia y la reactividad de cortisol y de ansiedad. Además, hipotetizamos que la relación negativa entre la resiliencia y la respuesta psicobiológica al estrés estaría mediada por estrategias de afrontamiento más activas y menos pasivas. Es decir, una mayor resiliencia estaría relacionada con estrategias de afrontamiento más activas y menos pasivas, y esto se traduciría en una respuesta al estrés más adaptativa (cortisol moderado y menor reactividad de ansiedad).

Estudio 2

El objetivo principal fue analizar la relación entre el optimismo disposicional, y sus subescalas optimismo y pesimismo, con biomarcadores de estrés crónico medidos en cabello (HC, HDHEA y HC:$HDHEA_{ratio}$) en personas mayores sanas. Finalmente, queríamos explorar las diferencias de sexo en las concentraciones de HC, HDHEA y HC:$HDHEA_{ratio}$, así como en su relación con los rasgos psicológicos (optimismo y pesimismo).

Esperábamos que las personas con mayor optimismo y menor pesimismo mostraran menor HC y $HC:HDHEA_{ratio}$, y mayor HDHEA. Además, planteamos que los hombres tendrían mayores niveles de HC y $HC:HDHEA_{ratio}$, y menores niveles de HDHEA en comparación con las mujeres. También esperábamos una asociación positiva entre el optimismo y HDHEA solo en mujeres.

Estudio 3

Este estudio se centró en el papel de la resiliencia, los estados afectivos y el eje HPA en las QSM de jóvenes sanos. En primer lugar, queríamos confirmar la relación de algunos estados afectivos (depresión y ansiedad) con las QSM, así como aclarar qué estado afectivo tenía mayor peso en la predicción de las QSM. Además, exploramos la relación entre el funcionamiento del eje HPA basal (CAR) y las QSM. Finalmente, el objetivo principal fue averiguar si la resiliencia se relacionaba negativamente con las QSM a través de la mediación de la depresión, la ansiedad y el CAR.

De acuerdo con la evidencia disponible, esperábamos que la depresión y la ansiedad se relacionaran positivamente con las QSM, siendo la depresión el factor más relacionado con las mismas. Como ningún estudio previo había evaluado la relación entre el eje HPA y las QSM en jóvenes, y dadas las diferencias de edad en el funcionamiento basal del eje HPA, no hicimos ninguna hipótesis. Finalmente, hipotetizamos que la asociación negativa entre resiliencia y QSM se debía a una menor depresión y ansiedad, y una mayor CAR experimentada por individuos resilientes.

Estudio 4

En este estudio probamos, en dos muestras independientes de personas jóvenes y mayores, la relación entre los rasgos psicológicos estables como el rasgo de ansiedad y positividad y las QSM. También estudiamos si la relación entre el funcionamiento del

eje HPA basal (cortisol al despertar y DCS) y las QSM era directa o moderada por estos rasgos psicológicos.

Esperábamos encontrar una mayor ansiedad y una menor positividad relacionadas con más QSM tanto en personas jóvenes como mayores. En cuanto a la relación entre el eje HPA y las QSM, en jóvenes no pudimos establecer ninguna hipótesis sobre la asociación directa dada la ausencia de estudios. Esperábamos que en aquellos individuos con mayor ansiedad y menor positividad, un nivel más alto de cortisol al despertar y una DCS más pronunciada estuvieran relacionados con más QSM. En personas mayores esperábamos una relación negativa directa entre DCS y QSM. Además, como ningún estudio previo había investigado la relación entre el cortisol al despertar y las QSM en los mayores, esperábamos que, como sucedía en los jóvenes, un nivel más alto de cortisol al despertar se asociaría con más QSM en personas con alta ansiedad y baja positividad. Además, dado el impacto del envejecimiento sobre el funcionamiento del HPA, así como las diferencias sugeridas en las QSM en menores y mayores de 65 años, exploramos la relación entre los rasgos psicológicos, los biomarcadores del eje HPA y las QSM en estos dos grupos de edad (de 55 a 64 frente a 65 a 75 años).

Estudio 5

El objetivo principal fue probar el papel de la sintomatología depresiva y los biomarcadores de patología cerebral (ECV, Aβ42/40 y p-tau) en individuos con SCD. Queríamos abordar si la sintomatología depresiva en SCD se debía a una patología cerebral. Además, hallazgos recientes sugieren que diferentes quejas están asociadas con diferentes perfiles de biomarcadores basados en RM y sintomatología depresiva. Por lo tanto, queríamos confirmar estas diferencias incluyendo biomarcadores de Líquido Céfaloraquídeo (LCR) de patología de EA en dos tipos de quejas, quejas de

memoria y de concentración. Por lo tanto, nuestro segundo objetivo fue investigar las quejas de memoria y concentración por separado en relación con la patología cerebral (ECV, Aβ42/40 y p-tau) y la sintomatología depresiva.

Siguiendo los resultados de estudios previos, planteamos que el SCD estaría relacionado tanto con la sintomatología depresiva como con biomarcadores de EA y ECV. La sintomatología depresiva también estaría relacionada con biomarcadores de EA y ECV siendo estas relaciones diferentes según el tipo de queja (Memoria vs Concentración). Siguiendo la literatura previa, las quejas de memoria estarían más fuertemente asociadas con los biomarcadores de EA y las quejas de concentración estarían más fuertemente asociadas con los biomarcadores de ECV. Finalmente, debido a la fuerte relación entre la sintomatología depresiva y las quejas, esperábamos la sintomatología depresiva estuviera relacionada tanto con las quejas de memoria como de concentración.

Metodología

A continuación se presenta una breve descripción de los participantes, del procedimiento e instrumentos empleados en cada estudio, para ofrecer una idea global de la metodología empleada.

Participantes

En la presente tesis doctoral, cuatro de los cinco estudios que la componen fueron desarrollados en su totalidad en el Laboratorio de Neurociencia Social de la Universitat de València. El quinto estudio fue fruto de una colaboración con el Instituto Karolinska gracias a una estancia predoctoral realizada por la doctoranda.

Respecto a la población mayor, el primer, segundo y cuarto estudio contaron con sujetos pertenecientes a un programa de la Universitat de València para mayores de 55

años llamado La Nau Gran. Estos participantes fueron reclutados en diferentes años y forman parte de tres muestras independientes de personas mayores. La muestra del primer estudio estuvo compuesta por 66 adultos sanos (31 hombres y 35 mujeres) entre 55 y 75 años. Los participantes del segundo estudio fueron 121 adultos sanos (46 hombres y 75 mujeres), con edades comprendidas entre los 56 y los 81 años. El cuarto estudio contó con 73 adultos mayores sanos de entre 55 y 75 años (39 hombres y 34 mujeres).

Los sujetos jóvenes del tercer y cuarto estudio fueron también reclutados de diferentes grados y masters de la Universitat de València. Para ambos estudios obtuvimos una muestra de 82 adultos jóvenes (42 hombres y 40 mujeres) de entre 18 y 35 años. El número de la muestra varió dependiendo de los objetivos y variables analizadas en cada estudio.

Los cuatro estudios contaron con los siguientes criterios de exclusión: fumar más de 10 cigarrillos al día, abuso del alcohol o drogas, haber estado bajo anestesia general en los últimos tres meses, haber experimentado un evento estresante en el último mes (por ejemplo, muerte de un familiar, divorcio, separación, haber sido despedido, haber pasado por alguna enfermedad grave, algún accidente entre otros), presentar problemas graves de vista, audición, enfermedades neurológicas, psiquiátricas, endocrinas o del eje HPA, deterioro cognitivo, usar medicación que afecte directamente al funcionamiento cognitivo, emocional o a los niveles hormonales (por ejemplo, glucocorticoides, antidiabéticos, antidepresivos, anticoagulantes, beta-bloqueantes, benzodiacepinas o hipnóticos). Adicionalmente, las mujeres mayores no tomaban terapia hormonal sustitutiva, y su último periodo ocurrió al menos 2 años antes del estudio. Las mujeres jóvenes se encontraban todas en la fase folicular (del 6º al 10º día) y no tomaban anticonceptivos orales.

Finalmente, el quinto estudio contó con 114 mujeres y 103 hombres pertenecientes a un estudio epidemiológico realizado en Gotemburgo (Suecia) sobre una cohorte de nacimiento en 1944, por tanto, de 70 años. Los criterios de inclusión siguieron la iniciativa internacional de SCD (SCD-I) y fueron: I) Cognición preservada establecida mediante dos criterios. En primer lugar, se excluyó la demencia a través de diagnósticos clínicos siguiendo los criterios de DSM-III-R, una puntuación en el test de cribado cognitivo Mini-Mental State Examination (MMSE) <24, o una calificación clínica de demencia mediante el Clinical Dementia Rate (CDR) >0.5. En segundo lugar, el deterioro cognitivo se excluyó basándose en los criterios de Jack et al. (2009) y Molinuevo et al. (2017) a partir de una evaluación neuropsicológica; II) Ausencia de infartos o tumores en RM, así como ninguna historia de ictus o ataque isquémico según el criterio de un/a neuroradiologo/a; III) Ausencia de antecedentes médicos de trastornos psiquiátricos (por ejemplo, depresión mayor) o neurológicos, enfermedades sistémicas o traumatismos craneales; IV) Sin ingesta de antidepresivos; V) Sin antecedentes de abuso de substancias o alcohol.

Procedimiento

Respecto al procedimiento, el primer estudio fue diseñado para estudiar el papel de la resiliencia y las estrategias de afrontamiento en la respuesta psicobiológica a un estresor de laboratorio. Para ello, los 66 participantes acudieron una sesión experimental de aproximadamente 2h de duración. Estos sujetos fueron aleatoriamente asignados a una situación estrés (14 hombres y 16 mujeres) o control (17 hombres y 19 mujeres). La condición de estrés consistió en el Trier Social Stress Test (TSST), un estresor psicosocial de laboratorio, mientras que la tarea control fue una tarea de la misma carga mental y física pero sin el componente de estrés. Con la finalidad de controlar el ritmo circadiano de secreción de cortisol y las diferencias sexuales en la respuesta de estrés,

las sesiones fueron por la tarde (16h-18h o 18h-20h), y la hora y el sexo fueron contrabalanceados. Asimismo, los sujetos completaron cuestionarios de ansiedad estado, estrés percibido, estrategias de afrontamiento y resiliencia y proporcionaron 7 muestras de saliva para analizar la respuesta de cortisol

El segundo estudio tuvo como objetivo estudiar el papel del optimismo disposicional y sus subescalas optimismo y pesimismo en biomarcadores crónicos de estrés en pelo. Para ello, los participantes acudieron al laboratorio donde contestaron a los test psicológicos de optimismo y estrés percibido. Asimismo, se les medía el peso, la altura, la cintura y la cadera para obtener el Índice de Masa Corporal (IMC), y la ratio cintura-cadera. Al final de la sesión se cortaron 3 mechones de pelo de 3 cm cada uno.

El tercer y cuarto estudio, que tuvieron como objetivo estudiar la relación entre las QSM, factores psicológicos y el eje HPA, siguieron el mismo procedimiento. Los participantes acudieron al laboratorio en sesiones por la mañana (10 o 12h) o por la tarde (16h o 18h). La hora a la que acudieron a las sesiones fue contrabalanceada por sexo. En las sesiones completaron los cuestionarios de QSM, estrés percibido, depresión, ansiedad, resiliencia y positivismo. Además, los participantes tuvieron que recoger 10 muestras de saliva en casa durante dos días consecutivos (inmediatamente tras despertar, a los 15, 30 y 45 minutos después de despertar e inmediatamente antes de dormir).

Por último, el procedimiento del quinto estudio se encuentra detallado en Rydberg Sterner et al. (2019). Los participantes fueron sometidos a un examen general de su funcionamiento y salud física, emocional y cognitiva, y se obtuvieron sus datos sociodemográficos. Dado que el estudio tenía como objetivo estudiar el rol de la depresión en el SCD y los factores neurodegenerativos, específicamente seleccionamos

datos sobre las quejas subjetivas de memoria y concentración, sintomatología depresiva, y biomarcadores de la EA (beta-amiloide y tau) y de ECV (anomalías en la sustancia blanca).

Variables

Variables psicológicas

En los cinco estudios que conforman esta tesis, se ha evaluado las siguientes variables psicológicas:

La ***resiliencia***, se evaluó en el primer y tercer estudio, mediante la versión de 10 ítems (Campbell-Sills & Stein, 2007) del cuestionario CD-Risc (Connor-Davidson Resilience Scale; Connor & Davidson, 2003). Mide la habilidad de evaluar y afrontar la adversidad y el estrés durante el pasado mes (por ejemplo, *soy capaz de adaptarme cuando ocurren cambios, afrontar adversidades, no me desanimo ante el fracaso, puedo lograr mis objetivos aunque haya obstáculos,* etc.).

Las ***estrategias de afrontamiento****,* empleadas en el primer estudio se evaluaron mediante el Cuestionario de estrategias de afrontamiento (COPE, Coping Orientations to Problems Experienced Inventory; Crespo & Cruzado, 1997). Es un cuestionario de 60 ítems en el que el/la participante indica qué siente usualmente y hace en una situación estresante. El cuestionario se agrupa en 15 subescalas que, a su vez, se pueden agrupar en cuatro factores de segundo orden: estrategias de afrontamiento activo, cognitivo y emocional (es decir, estrategias activas de afrontamiento) y evitación (es decir, estrategias pasivas de afrontamiento).

El ***optimismo***, se midió en el segundo estudio con la escala de optimismo (LOT-R, Life Orientation Test Revised; Scheier et al., 1994). Se trata de un cuestionario de 10 ítems en el que se puede obtener las subescalas de optimismo y pesimismo, así como

una puntuación total de optimismo disposicional. Tres ítems miden optimismo (por ejemplo, *En tiempos de incertidumbre, suelo esperar lo mejor*), tres miden pesimismo (por ejemplo, *Si algo puede ir mal para mí, irá*), y los cuatro ítems restantes son distractores

El ***positivismo,*** se midió en el cuarto estudio, usando el Cuestionario de Positivismo (Caprara et al., 2012). En este cuestionario de 8 ítems se valora la visión positiva sobre uno mismo, su vida, su futuro y la confianza en los demás. El positivismo incluye factores como la autoestima, el optimismo y la satisfacción con la vida (por ejemplo, *tengo esperanza en el futuro, los demás están ahí cuando los necesito, generalmente tengo confianza en mí*).

El ***estrés percibido***, en los estudios 1, 2, 3, y 4, fue evaluado mediante la escala de estrés percibido (PSS, Stress Perceived Scale; Cohen et al., 1983). En este cuestionario se mide el estrés percibido en el último mes mediante 14 ítems. Usamos esta escala para controlar el estrés auto percibido de nuestros participantes en todos aquellos estudios que implicaban cualquier variable relacionada con el estrés.

La ***sintomatología depresiva,*** se midió en el tercer y en el quinto estudio. En el tercer estudio se utilizó el Cuestionario de Depresión de Beck (BDI-II; Beck et al., 1996), que consta de 21 ítems y mide los síntomas cognitivos, emocionales y comportamentales de depresión durante las dos últimas semanas. En el quinto estudio se empleó la escala de depresión de Montgomery-Åsberg de 10 ítems (MADRS; Montgomery & Åsberg, 1979).

La ***ansiedad,*** se evaluó en los estudios 1, 3 y 4. En el primer estudio, la ansiedad estado se midió con el cuestionario de ansiedad estado (STAI-S; Spielberger et al., 1970), que evalúa, con 20 ítems, cómo se sienten los participantes en el momento en que contestan el cuestionario.

En el tercer estudio se evaluó mediante el Cuestionario de Ansiedad de Beck de 21 ítems (BAI; Beck et al., 1988). Este cuestionario mide los síntomas de ansiedad durante la última semana. Por último, la ansiedad rasgo evaluada en el cuarto estudio fue medida con el Cuestionario de Ansiedad rasgo (STAI-T; Spielberger et al., 1970), donde los participantes indicaban cómo se sentían la mayor parte del tiempo.

Variables biológicas

Variables hormonales

El funcionamiento del eje HPA se estudió a través de las concentraciones salivares y en pelo de cortisol, y de DHEA en pelo. Concretamente, las muestras salivares se obtuvieron en los estudios 1, 3 y 4 mediante Salivettes (Sarstedt, Rommelsdorf, Alemania). Estos Salivettes contienen un algodón que los participantes debían mantener en la boca durante 2 minutos impregnándolos de saliva. En los tres estudios, las concentraciones de cortisol se determinaron mediante ELISA (Salimetrics, Newmarket, Reino Unido). En el segundo estudio las concentraciones de cortisol y DHEA en pelo se analizaron siguiendo el protocolo descrito en Kirschbaum et al. (2009) usando la técnica de inmunoensayo en los 3 centímetros proximales de pelo que, basándose en el crecimiento de 1cm/mes, representan las concentraciones de cortisol o DHEA de los últimos 3 meses (Russel et al., 2012).

Biomarcadores de la EA

La patología de la EA (amiloidosis y ovillos neurofibrilares de proteína tau) se puede estudiar *in vivo* a través de biomarcadores en LCR como la ratio de beta-amiloide 42/40 y la proteína tau fosforilada (p-tau). Estos biomarcadores pueden ser positivos en ausencia de un deterioro cognitivo objetivo, ayudándonos a detectar etapas preclínicas de la EA (Visser et al., 2009). El quinto estudio contó con biomarcadores en LCR como beta-amiloide 42/40 medido con el kit V-PLEX Aβ peptide panel 1 (6E10) (Meso Scale

Discovery, Rockville, MD) y p-tau en LCR medido mediante el kit ELISA (INNOTEST® htau Ag and PHOSPHO_TAU (181P); Fujirebio, Gante, Bélgica).

Biomarcadores de enfermedad cerebrovascular

En el quinto estudio se usaron las hipointensidades en sustancia blanca (WMSA por sus siglas en inglés) como biomarcador de ECV. Se ha sugerido que las hipointensidades WMSA reflejan una integridad de la sustancia blanca más pobre y parecen estar relacionadas con un daño crónico de la sustancia blanca (Riphagen et al., 2018). Las hipointensidades WMSA fueron estudiadas mediante imágenes de RM usando una secuencia tridimensional T1. Estas hipointensidades se segmentaron automáticamente con FreeSurfer 6.0.0 y las imágenes fueron procesadas con la suite de análisis de imágenes FreeSurfer 6.0.0.

Variables genéticas

El alelo *APOE*-ε4 se determinó usando el sistema de genotipado KASPar PCR SNP (LGC Genomics, Hoddesdon, Herts, Reino Unido). Para la caracterización de la muestra en el quinto estudio los participantes fueron clasificados como *APOE*-ε4 si poseían al menos un alelo ε4.

Variables cognitivas

La percepción subjetiva de memoria se estudió en los estudios 3, 4, y 5, mediante la presencia de QSM o quejas subjetivas de concentración. En los estudios 3 y 4, las QSM se midieron con el cuestionario de Fallos de memoria en la vida cotidiana (MFE-30; Sunderland et al., 1986) que incluye de 30 ítems con situaciones y actividades de la vida diaria (por ejemplo, *Olvido algo que me dijeron ayer o hace pocos días; Mis fallos de mi memoria me causan problemas en la vida cotidiana*). En el quinto estudio se emplearon dos preguntas distintas de la entrevista semiestructurada

CPRS (Åsberg et al., 1978), refiriéndose a quejas subjetivas de memoria y concentración en el último mes. Asimismo, en el estudio 4 se utilizó el cuestionario Mini-Mental (MMSE) para excluir a los participantes mayores con posible deterioro cognitivo. En el quinto estudio, se contó con una evaluación neurocognitiva para excluir a todos aquellos participantes que presentaran cualquier afectación cognitiva que fuera indicadora de deterioro cognitivo o demencia.

Resultados principales y conclusiones

Esta tesis doctoral tuvo como objetivo principal investigar el papel de diferentes factores psicológicos de vulnerabilidad y resiliencia en dos contextos importantes de la sociedad actual como son el estrés y la detección temprana de demencia o salud cognitiva, en personas jóvenes, de mediana edad y mayores. Concretamente, el primer y segundo estudio investigaron la relación entre algunos rasgos psicológicos considerados positivos o perjudiciales para la salud, como la resiliencia, el optimismo o el pesimismo, y la respuesta al estrés agudo o crónico medida a través del funcionamiento del eje HPA en personas mayores sanas. Los estudios tercero, cuarto y quinto examinaron la relación entre varios factores psicológicos positivos y negativos, el eje HPA y algunos factores neurodegenerativos en diferentes grupos de edad. Específicamente, el tercer y cuarto estudio examinaron la relación entre las QSM con los estados afectivos negativos (depresión y ansiedad), los rasgos psicológicos negativos (rasgo de ansiedad) y los rasgos psicológicos positivos (resiliencia y positividad) y el eje HPA en participantes sanos jóvenes, de mediana edad y mayores. El quinto estudio examinó la relación entre la sintomatología depresiva, los biomarcadores de EA y ECV y el SCD en participantes mayores sanos.

En el *Estudio 1*, los análisis de moderación mostraron que la resiliencia estaba relacionada con la reactividad del cortisol mediante el uso de estrategias de

afrontamiento activas. Por lo tanto, las personas con alta resiliencia eligen estrategias de afrontamiento más activas y experimentan una menor reactividad al cortisol. Sin embargo, el afrontamiento activo no medió la relación entre la resiliencia y la reactividad a la ansiedad. El afrontamiento de evitación o pasivo tampoco medió la relación entre la resiliencia y la respuesta psicobiológica a un estresor. Nuestros resultados destacan la importancia de las estrategias de afrontamiento activas como un componente importante de la resiliencia cuando nos enfrentamos a una situación estresante.

En el *Estudio 2*, los análisis de regresión lineal indicaron que un mayor optimismo disposicional estaba relacionado con niveles de HC y HC:$HDHEA_{ratio}$ más bajos. Particularmente, menores puntuaciones en la subescala de pesimismo se relacionaron con niveles de HC y HC:$HDHEA_{ratio}$ más bajos, mientras que mayores puntuaciones en la subescala de optimismo solo se relacionaron con niveles de HC:$HDHEA_{ratio}$ más bajos. No se encontraron diferencias por sexo. Estos resultados sugieren que las expectativas positivas y negativas sobre el futuro (optimismo y pesimismo) pueden jugar un papel importante en la salud de las personas mayores debido a su relación con el eje HPA.

En el *Estudio 3*, los análisis de regresión lineal mostraron que la depresión y la ansiedad estaban significativamente relacionadas con las QSM. La regresión múltiple jerárquica mostró que la depresión era el único predictor de las QSM después de controlar la ansiedad. El CAR no apareció relacionado con las QSM. Los análisis de mediación mostraron que a mayor resiliencia menos QSM a través de menor depresión y ansiedad, pero no a través del CAR. En conjunto, nuestros resultados sugieren que en jóvenes los estados afectivos negativos tienen mayor importancia en las QSM que el eje

HPA y resaltan el papel de la resiliencia como factor protector a través de una mejor regulación del estrés psicológico (menores estados depresivos y de ansiedad).

En el *Estudio 4*, los análisis de regresión mostraron que, en jóvenes y mayores sanos, una mayor ansiedad rasgo se relacionó con más QSM, pero una mayor positividad se relacionó con menos QSM solo en jóvenes y mujeres mayores. Los análisis de moderación mostraron que los rasgos psicológicos moderaron la relación entre el eje HPA y las QSM en individuos jóvenes. Esto significó que mayor cortisol al despertar y una DCS más pronunciada se relacionaron con más QSM en personas con alta ansiedad y baja positividad. En los mayores, el moderador de esta relación fue la edad (al comparar mayores de 55-64 años frente a los de 65-75 años). Menos cortisol al despertar y una DCS más aplanada se relacionaron directamente con más QSM solo en las personas de 55 a 64 años, pero no en los mayores de 64 años. Estos resultados enfatizan la naturaleza diferente de las QSM a lo largo de la vida y aclaran la asociación entre el eje HPA y las QSM en diferentes periodos de edad.

Finalmente, en el *Estudio 5*, los análisis de regresión logística mostraron que la sintomatología depresiva estaba fuertemente relacionada con el SCD en una muestra de personas de 70 años. Los análisis de correlación no mostraron relación entre la depresión y los biomarcadores de EA y ECV. Curiosamente, encontramos diferentes asociaciones según el tipo de queja (Memoria vs. Concentración). Es decir, las quejas de memoria se relacionaron principalmente con la depresión, seguida del biomarcador p-tau, mientras que las quejas de concentración se asociaron principalmente con el biomarcador beta-amiloide, seguido de la depresión. Estos resultados ayudan a clarificar la discusión actual sobre el papel de la sintomatología depresiva en el SCD, y añaden nueva información sobre los diferentes significados de las quejas de memoria y concentración.

En resumen, esta tesis doctoral aporta evidencia sobre el papel de la resiliencia como factor protector en la respuesta al estrés de la población mayor. Hemos visto que algunos factores protectores juegan un papel clave en la reactividad al estrés y la actividad del eje HPA basal, mejorando el uso de estrategias de afrontamiento activas para lidiar con los factores estresantes y reduciendo las consecuencias negativas del estrés crónico en la salud. También contribuimos a clarificar el significado de las QSM, entendidas como indicadores subjetivos de salud cognitiva, en personas jóvenes, de mediana edad y mayores. Nuestros estudios arrojan luz sobre sus relaciones con causas no degenerativas (factores psicológicos y estrés) en diferentes etapas del ciclo vital. Se ha corroborado la importancia de los factores psicológicos negativos, depresión y ansiedad, y se ha avanzado en conocer el papel protector de los factores psicológicos positivos en diferentes rangos de edad. Los biomarcadores de estrés también parecen estar relacionados con las QSM a través de diferentes moderadores a lo largo de la vida. En general, en los jóvenes, las QSM están relacionadas con causas psicológicas y con el eje HPA. A medida que avanza la edad parece que las QSM siguen asociadas con estos factores no degenerativos, aunque en mayores de 65 años empiezan a estar relacionadas con biomarcadores de EA. En definitiva, esta tesis doctoral ayuda a clarificar el papel de algunos indicadores psicológicos y de estrés, con el fin de potenciar y fomentar futuras investigaciones e intervenciones enfocadas a la mejora de la salud y el envejecimiento saludable.

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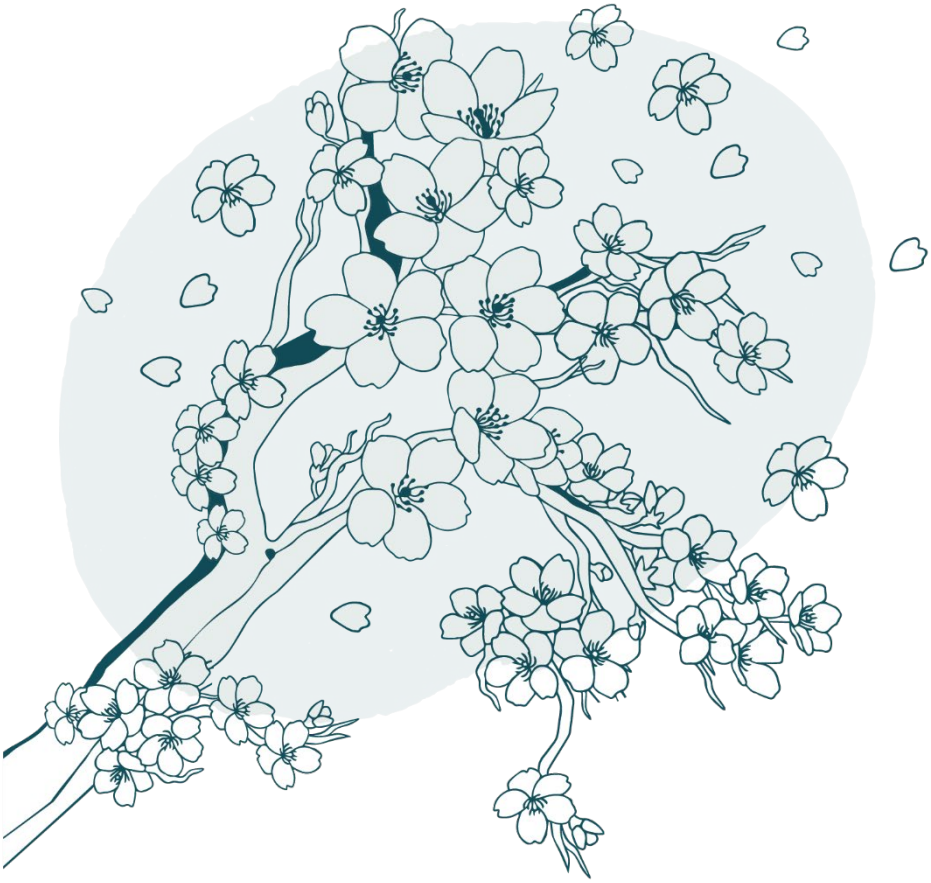

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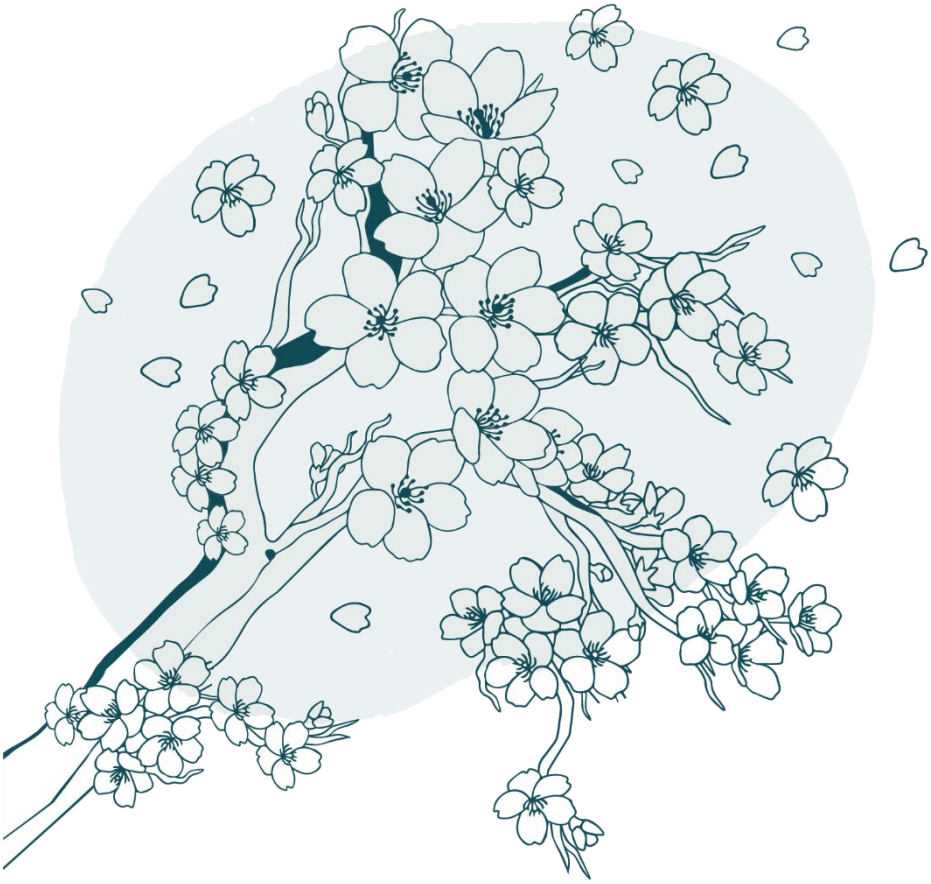

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