VNIVERSITAT DE VALÈNCIA

Departamento de Psicobiología.
Doctorado en Neurociencias (30001)
RD: 1393/2007

Facultad de Psicología

The importance of the way we are:

The role of personality in the functioning of the Autonomic Nervous System and the Hypothalamic-Pituitary-Adrenal axis in older people.

Presentada por
Presented by **Sara Puig Pérez**

Dirigida por
Directed by **Dra. Alicia Salvador Fernández-Montejo**

Valencia, 2016

“Nothing in the world can take
the place of persistence.

Talent will not; nothing in the
world is more common than
unsuccessful men with talent.

Genius will not; unrewarded
genius is a proverb.

Education will not; the world is
full of educated derelicts.

Persistence and determination
alone are omnipotent.”

Calvin Coolidge.

TABLE OF CONTENTS

Agradecimientos / Acknowledgments / Agraïments

La realización de esta tesis doctoral ha sido, en cierto modo, una carrera de fondo. Sin duda alguna, habría sido sumamente difícil llevarla a cabo con éxito sin la ayuda de las personas que me han brindado su apoyo y cariño a lo largo de todo el proceso.

En primer lugar, me gustaría agradecerle a Alicia, mi directora de tesis, abrirme la puerta al mundo de la ciencia. Sus consejos, su apoyo y su cariño a lo largo de estos años han sido fundamentales para mí. Siempre le agradeceré que me abriese los brazos cuando llamé a su puerta sin saber quién era, ofreciéndome conocer lo que hoy en día considero mi forma de vida. Para mí, eres un ejemplo a seguir y me siento muy orgullosa de haber compartido estos años contigo. Muchísimas gracias, de todo corazón.

Gracias a Alicia conocí un nuevo mundo y a gente maravillosa, que ya desde los primeros años han sido más que compañeros de trabajo. Me gustaría agradecer a Mercedes y Leander esas comidas en la "pecera", las risas y el enseñarme a disfrutar la estadística. Con María y Clara compartí momentos divertidos en el laboratorio de hormonas, en el antiguo animalario y repasando datos, momentos que nunca olvidaré. Carol ha sido y sigue siendo una de las personas más importantes que he conocido en esta aventura, ¡te echo de menos! En algunos casos sólo tuve la oportunidad de disfrutar de su presencia por un breve periodo de tiempo, pero dejasteis vuestra huella: David, Verena, Ali... ¡Echo de menos veros todos los días! Pero siempre estaréis cerca para mí.

Tengo que agradecer a Teresa su buena predisposición a todo: además de ser una trabajadora estupenda, tiene un corazón que no le cabe en el pecho. Vanesa ha sido y es una de las personas que más respeto y admiro. Sincera, rigurosa y con ese carácter *salao*, contigo he aprendido y crecido como persona lo que nunca imaginé. Y finalmente a

mi Matías, mi compañero y amigo de cueva, que con ese entusiasmo por la investigación y esos "¡*venga, carajo!*" haces que trabajar sea un juego de niños. Has sido un gran compañero y amigo durante estos años, ¡muchas gracias!

No quisiera olvidarme de todas esas mentes maravillosas que me han acompañado en las horas de las comidas: Agus, Lucas, Iva, Sabina, Robi, Casti, Víctor, Esther, Eva, Gosia… ¡sois muy grandes, chicos! Y por supuesto, también quiero dar las gracias a aquellos que en este último período se han convertido en personas muy especiales para mí: Adrián, Diana, Irene y Ruth. Tenéis un gran futuro por delante, y yo espero verlo de cerca.

Tampoco me gustaría olvidar dar las gracias a la gente con la que he compartido grandes momentos en mis estancias: al Dr. Hartmut Schächinger por su guía en Trier; al Dr. Andrew Steptoe por acogerme como una más en su equipo; y a mi querida Bianca por su generosidad y alegría cada día en la UCL, me hiciste sentir como en casa.

Hi ha una personeta especial que m'ha acompanyat des del primer dia de la carrera, en el màster i en el doctorat: la meua Marta. Hem viscut moments únics durant els últims 10 anys amb les nostres psicòlogues, amb les que tenim un vincle molt especial pel que donar gràcies: Alba, Belén, Laura, Majo, María, Roseta, Tania i Miri. Tampoc no puc oblidar-me de les meues amigues de la infància, Ana i Laia, que d'una manera o altra sempre han estat al meu costat... ¡Us estime xiques!

He d'agrair haver gaudit de l'amor incondicional de la meua família, eixa energia que em feia seguir endavant a pesar dels obstacles: ma mare, mon pare, el meu Jordi i la meua uela guerrera preferida. Heu sigut l'impuls que m'ha fet volar, no seria res sense vosaltres. Espere, ser mereixedora del recolzament incondicional que he rebut per la vostra part. Gràcies per fer-me creure que podia, i per fer tot el possible perquè ho aconseguís.

I finalment, he de donar gràcies a la persona que m'ha escollit com a companya en aquesta aventura que és la vida, tant en els bons i no tan bons moments. Andrés, gràcies per estimar-me i per fer-me sentir que ho puc tot. Fas que tot l'esforç valga la pena, i em sent afortunada de tenir-te al meu costat cada dia que passa.

Moltes gràcies a tots per fer d'aquesta experiència un viatge inoblidable. ¡Açò només acaba de començar!

Sara Puig Pérez.

València, Juliol de 2016.

Dissertation Outline

One of the most important social changes in the world in the past century was the increase in life expectancy (European Commission, 2014). This change involves an aging process in the population that produces novel needs, especially regarding health problems related to aging. In fact, The European Commission and the European Council (2015) recognized the need to address the aging process by incorporating new strategies into the European Social Models. For this reason, new research programs have appeared, such as Horizon 2020 in Europe, in order to find out how to maintain good health ratings in the older population as long as possible. Due to the rise in fertility rates, life expectancy, and migration, the age structure in the EU will change dramatically in the coming decades (European Commission, 2015). In fact, Eurostat (EUROPOP2013) announced that the overall population size is expected to be slightly higher but older than it is now. Along these lines, people over 65 years old are projected to increase from 27.8% to 50.1% in 2060, which could be explained by the increase in life expectancy of 7.1 years in men (84.8 in 2060) and 6.0 years in women (89.1 in 2060). Additionally, migration flows to the EU are expected to increase to 1.364.000 by 2040, but decline later to 1.037.000 by 2060. Taking the aforementioned into account, it makes sense for the research to focus on investigating what promotes a pathological or satisfactory aging process. Clearer and more in-depth knowledge of those variables that protect from or lead to disease could help to develop more effective prevention and intervention strategies.

In line with these ideas, this thesis presents the results of a number of studies focused on investigating the relationship between specific personality traits and the functioning of basic physiological mechanisms in older people: the autonomic nervous system (ANS) and the hypothalamus-pituitary-adrenal axis (HPA axis).

The first chapter contains a brief description of the main characteristics and functioning of the ANS and the HPA axis. Additionally, it describes how they can be activated when facing a stimulus or situation perceived as stressful. Finally, the last part of this chapter shows an overview of the main factors present in the previous literature that have been related to the maladaptive or adaptive functioning of these physiological systems, as well as their relationship with an increased or decreased risk of health problems. The second chapter includes the main objectives and hypotheses of this thesis, which are then developed in the following chapters. Each study described in the chapters of the present thesis contains a brief introduction, methods, results, and discussion of the main findings. Most of the results presented in this dissertation have been published in international journals or are currently under review.

The third and fourth chapters describe two different approaches to test the relationship between the neuroticism trait and the ANS and HPA axis functioning. Considering that neuroticism has been extensively related to a high risk of physical and mental disease, the aim was to investigate the underlying mechanisms through which this trait may contribute to worse health in older people. Specifically, the third chapter investigated the relationships among neuroticism, depressive mood, and the functioning of the ANS and HPA axis in stressful conditions. Additionally, we tested whether neuroticism and depressive mood are related to a dissociated response between the ANS and HPA axis functioning. The fourth chapter focused on the relationship between neuroticism and extraversion traits and morning cortisol release (awakening concentrations, awakening cortisol response, and overall cortisol release during the first 45 min after awakening).

The last part of the dissertation describes the relationship between optimism and the ANS and HPA axis functioning. Considering that optimism has been extensively related to a better prognosis for physical and mental health, the aim was to investigate the underlying mechanisms through which this trait may contribute to better health in older people. The fifth chapter describes a study designed to test the relationship between

optimism and the psychophysiological stress response in healthy older people. In the sixth chapter, we wanted to know, in healthy older people, whether optimism was related to adequate circadian HPA axis functioning and past-life perception, as a reflection of well-being. Finally, we considered the fact that different stress-related pathologies, such as type II diabetes, have been related to a malfunctioning of the ANS and HPA axis. Thus, in the seventh chapter, we investigated whether optimism helps older people diagnosed with type II diabetes to show similar circadian HPA axis and ANS and HPA axis functioning to healthy people in stressful situations.

The last chapter of the dissertation contains a general discussion, main conclusions, limitations, and future considerations. Additionally, a brief summary of the thesis has been included in Spanish.

ABBREVIATIONS

ACTH = Adrenocorticotropic Hormone

ANS = Autonomic Nervous System

AOCg = sAA Variations Controlling for Cortisol Variations

AUC_G = Area Under the Curve with Respect to the Ground

AUC_I = Area Under the Curve with Respect to the Increase

AVP = Arginine-Vasopressin

BMI = Body Mass Index

BPS = Biopsychosocial Model

CAR = Cortisol Awakening Response

COAg = Cortisol Variations Controlling for sAA Variations

CRH = Corticotrophin-Releasing Hormone

CV = Cardiovascular

CVD = Cardiovascular Disease

ENS = Enteric Nervous System

GRs = Glucocorticoid receptors

HF = High Frequency

HPA axis = Hypothalamic-Pituitary-Adrenal axis

HR = Heart Rate

HRV = Heart Rate Variability

LF = Low Frequency

LOT = Life Orientation Test

MPFC = Medial Prefrontal Cortex

MRs = Mineralcorticoid receptors

NA = Negative Affect

NCE = Negative Cognitions-Emotions

NE = Negative Events

PA = Positive Affect

PANAS = Positive and Negative Affect Schedule

PCE = Positive Cognitions-Emotions

PE = Positive Events

PNS = Parasympathetic Nervous System

PNV = Paraventricular Nucleus

SCN = Suprachiasmatic Nucleus

S.D. = Standard Deviation

sAA = Salivary Alpha-Amylase

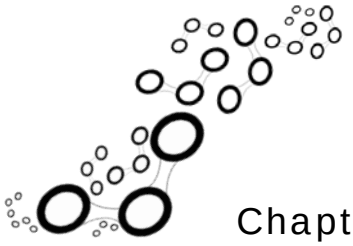
SEM = Standard Error of Means

SES = Subjective Socioeconomic Status

SNS = Sympathetic Nervous System

TSST = Trier Social Stress Test

T2D = Type 2 Diabetes



Chapter 1

From Physiology to Psychology

1. Physiological mechanisms involved in homeostasis

The organism adapts to the challenges of the environment through the adjustment of multiple physiological systems (i.e. metabolic and autonomic nervous system) (McEwen, 2006). This process, known as *allostasis*, is needed to preserve its health status. However, repeated or sustained stimulation results in *allostatic load*, which increases the risk of disease development. In fact, greater *allostatic load* has been associated with increased risk of chronic disease development (Mattei et al., 2010).

It has been suggested that *allostatic load* disrupts the dynamic responses to acute challenges or stress, resulting in an impaired reactivity and recovery capability (McEwen, 1998). In addition to the adaptive role of the acute stress-induced activation of the autonomic nervous system (ANS) and the hypothalamus-pituitary-adrenal (HPA) axis, a chronic deregulation of the ANS and/or HPA axis leads to an increased risk of physical and mental health problems (Chida & Steptoe, 2010; McEwen, 2006, McEwen & Gianaros, 2011). Therefore, the correct functioning of the ANS and the HPA axis is needed for health maintenance.

1.1. The Autonomic Nervous System

The ANS is one of the most important systems in our organism involved in homeostasis, which innervates the smooth musculature of all organs, the heart and glands. Its actions are mainly not under direct voluntary control, and its functions are: (i) to keep the internal milieu of the body constant, that is, homeostasis; (ii) to adjust the body's functioning to changing external or internal circumstances (e.g. food intake, water deprivation, etc.); and (iii) to control the organs and systems involved in homeostasis (Jänig, 1989). This complex system is composed of three branches: The Sympathetic Nervous System (SNS), the Parasympathetic Nervous System (PNS), and the Enteric Nervous System (ENS).

The ENS is a system focused on the gastrointestinal tract, functioning independently from the inputs of the spinal cord and the brainstem. The SNS

and PNS work in a complementary balance, with the SNS being involved in the mobilization of energy, whereas the PNS acts on the conservation of resources. The heart muscle is innervated by the SNS and PNS, and it offers a clear example of the antagonistic actions of these branches: the SNS increases heart rate (HR), atrioventricular conduction, and the contractility of the cardiac muscle, and it dilates coronary arteries, whereas the PNS reduces HR and heart contractility (Gabella, 2001).

The major part of the axons of the neurons that compose the SNS are myelinated, which confers them conduction velocities between 1 to 20 m/s. The SNS preganglionic neurons arise from the ventral roots and the white rami communicants of the thoracic and lumbar sections (from T1 to L3) of the spinal cord (thoracolumbar system). These preganglionic neurons synapse in the paraventral ganglia, a chain of ganglia connected to each other and located in parallel on either side of the vertebral column. From the paraventral ganglia, postganglionic unmyelinated axons innervate the organs throughout our body. Additionally, the SNS preganglionic neurons also synapse in the prevertebral abdominal ganglia, from which postganglionar fibers innervate to organs situated in the abdomen and pelvis (Jänig, 1989).

The preganglionic neurons of the PNS emerge from the brainstem (cranial nerves III, VII, IX and X) and the sacral portion (S2-S4) of the spinal cord (thoracolumbar system). Interestingly, some of the axons of the PNS are myelinated, but a large portion of them are unmyelinated. In the PNS, the axons of the preganglionic are longer than the axons of the postganglionic neurons, given that preganglionic and postganglionic PNS neurons synapse in the PNS ganglia located close to or inside the effector organ (Jänig, 1989).

The autonomic centres of the ANS are located in specific nuclei in the hypothalamus, brainstem, and spinal cord. The hypothalamus is the largest brain structure involved in ANS functioning, exerting the highest and most direct control over the ANS and endocrine system (Gabella, 2001). The hypothalamus is connected to brain structures such as the thalamus,

pituitary gland, and forebrain. Additionally, hypothalamic neurons are connected to the autonomic cardiovascular centres (parabrachial nucleus, nucleus ambiguous, nucleus of the solitary tract, dorsal motor nucleus of the vagus) and to the preganglionic neurons of the SNS and PNS in the spinal cord (Gabella, 2001).

1.2. The Hypothalamus-Pituitary-Adrenal Axis

The HPA axis is a complex physiological system that can be activated by physical and psychological inputs. Initially, the hypophysiotropic neurons of the paraventricular nucleus (PVN) of the hypothalamus are activated by internal or external stimuli of a physiological or psychological nature. The CRH neurons of the PVN synthesize corticotrophin-releasing hormone (CRH), whereas the magnocellular neurons of the PVN, the magnocellular neurons of the supraoptic nucleus (SON), and the CRH neurons in the parvocellular PVN synthesize arginine-vasopressin (AVP). Both hormones, CRH and AVP, are released into the portal circulation system of the median eminence. These hormones activate the anterior pituitary gland, inducing the release of the adrenocorticotropic hormone (ACTH) through the corticotroph cells into the bloodstream. The ACTH moves through the bloodstream, stimulating the cortex of the adrenal glands. This stimulation induces the synthesis and release of glucocorticoids into the bloodstream by the zona fasciculata cells of the adrenal cortex, with cortisol being the most outstanding glucocorticoid in humans (Jacobson, 2005).

In order to preserve a balanced cortisol concentration, a negative feedback loop is needed to maintain glucocorticoids at tolerable levels (Keller-Wood & Dallman, 1984). The secretion of CRH and AVP is under a corticosteroid feedback control in both the circadian release and in response to stress. This feedback is controlled by two different types of corticosteroid receptors that provide information to the hypothalamic CRH/AVP neurons about the concentration of cortisol in the bloodstream: the mineralocorticoid receptors (MRs) and the glucocorticoid receptors (GRs). The difference in affinity between the MRs and GRs suggests their

different involvement in feedback regulation. The MRs, which have a K_D of ≅0.5 nM or less for corticosteroid or cortisol (Dallman et al., 1994), mediate glucocorticoid feedback in basal or low concentrations. However, the GRs, with a K_D≅2.5-20 nM (Dallman et al., 1994), are the best receptors to detect and regulate the circadian peak or the stress-induced release of glucocorticoids. Both kinds of receptors are located in specific brain structures, making them sensitive to cortisol concentrations. The highest expressivity of these receptors appears in the PVN, hippocampus, amygdala, and prefrontal cortex (de Kloet, Oitzil & Joëls, 1999; Patel et al., 2000).

The control of circulating ACTH is the key factor in the regulation of glucocorticoid release by the adrenal glands. Considering that the glucocorticoids play a central role in the regulation of HPA axis function, the inhibitory feedback provided by the glucocorticoids on the ACTH release makes it possible to regulate their concentrations. Thus, glucocorticoids, in ligand binding with GRs and MRs, inform the PVN neurons in the hippocampus about their concentrations. In the case of high levels of glucocorticoids, the PVN neurons inhibit the release of CRH and AVP (Whitnall, 1993).

In addition, other structures such as the hippocampus and prefrontal cortex mediate in the HPA axis regulation. On the one hand, the hippocampus shows an inhibitory effect on the PVN (Patel et al. 2000; Herman et al., 2005). This structure is involved in the circadian rhythm of the HPA axis function, as well as in the response to stress of the HPA axis (Fedler et al., 1961; Fischette et al., 1980). The glutamatergic fibers of the hippocampus contact with the GABAergic neurons in the medial preoptic area, the bed of the stria terminalis, the dorsomedial hypothalamus, and other hypothalamic nuclei, which inhibit the PVN (Cullian et al., 1993; Herman et al., 2003).

On the other hand, the medial prefrontal cortex (MPFC) modulates the stress response of the HPA axis. This structure is involved in the processing of stressful information, which could explain its role in the HPA axis function

(Cullian et al., 1993; Patel et al., 2000). The glutamatergic fibres of the MPFC have contact with the GABAergic neurons of the nucleus solitary tract, the preoptic area, and the bed of the stria terminalis (among others), inducing the inhibition of the PVN neurons. Additionally, the MPFC is strongly linked to the amygdala, a structure with an excitatory effect on the HPA axis (Ulrich-Lai & Herman, 2009). Thus, the coordinated activity of the MPFC and amygdala highlights them as main coordinators of the limbic and physiological stress response (Ulrich-Lai & Herman, 2009).

Under non-stressful conditions, the HPA axis follows a circadian rhythm. In fact, the release of CRH and AVP by the PVN neurons follows a circadian cycle and pulsatile fashion, with a usual frequency of two or three secretion episodes per hour (Liu et al., 1994). Cortisol concentrations change throughout the day, showing higher cortisol concentrations in the morning, with a marked increase (from 50% to 160%) that peaks between 30 and 45 min just after awakening. This phenomenon is known as the cortisol awakening response (CAR), and it can be observed in approximately 70-80% of healthy adult people (Clow et al., 2010a, 2010b; Fries et al., 2009; Elder et al., 2014; Stalder et al., 2016). After that, cortisol concentrations show a progressive decrease during the day, reaching their lowest point during the first half of the sleep period (Pruessner et al., 1997). However, after the second half of the night, cortisol concentrations begin to rise again until awakening (Elder et al., 2014).

It has been stated that the suprachiasmatic nucleus (SCN) of the hypothalamus plays a key role in CAR regulation through two pathways. The first one follows the PVN route described above (HPA-axis cascade through CRH and ACTH), and the second one comprises the stimulation of the adrenal glands via the direct sympathetic connection of the SCN with the splanchnic nerve (Fries et al., 2009). The main brain structures that have been related to CAR regulation are the frontal cortex, the hippocampus, and the amygdala (Buchanan et al., 2004; Wolf et al., 2005; Herman et al., 2005; Pruessner et al., 2007; Fries et al., 2009).

The first description of the CAR suggested that it could be a good index of adrenocortical activity (Pruessner et al., 1997). However, recently, a large number of studies have highlighted the importance of studying the relationship between its deregulation and health (for more detail see: Chida & Steptoe, 2009; Stalder et al., 2016). Unfortunately, the purpose of the CAR is not well understood yet, although it has been shown that it is independent from (i) the cortisol levels during the rest of the day (Edwards et al., 2001; Maina et al., 2009) and (ii) the cortisol reactivity to experimentally-induced psychological stress (Bouma et al., 2009).

2. The psychophysiological stress response

Throughout life, people are exposed to a wide range of circumstances that involve the activation of the ANS and the HPA axis. In situations where a stimulus is considered to be a stressor, both systems are activated.

2.1. How and what can elicit a stress response?

When a potential stressful stimulus is perceived, the first areas activated are the thalamus and prefrontal cortex, with the aim of integrating the information about the stimulus or the context. Then, the evaluation of the significance and meaning of the stimulus occurs. Depending on the results of this evaluation, an emotional response to a specific stimulus or to the context can be elicited. The prefrontal cortex plays a role in the limbic system, which is responsible for emotional outputs. At the same time, other structures in the limbic system (i.e. amygdala, hippocampus) are connected to the hypothalamus, one of the main structures involved in the ANS and HPA axis functioning, which finally induces or does not induce the physiological stress response (Gabella, 2001).

According to *social self-preservation theory,* the motivation to preserve the social-self induces a physiological response to threatening

stimuli (Dickerson & Kemeny, 2004). This kind of *self* reflects the social esteem, value and status mainly based on society's perception of one's worth (Gilbert, 1997; de Waal, 1989). Situations that produce a threat to the social-self induce a psychological, physiological, and behavioural response to cope with them. The magnitude of this response may depend on the intensity of the threat, the context, or whether there are protective factors in the individual or the surrounding society. Thus, different strategies have been used to elicit psychosocial stress in research.

For decades, Selye's (1956) statement that all kinds of stressors could provoke a similar physiological response was accepted. However, a large variability has been found in the physiological response evoked by psychological stimuli, indicating the importance of the specific emotional reactions (Mason, 1968a; 1968b; 1975). Whereas the ANS is easily activated, HPA axis activation appears in specific conditions. Extensive literature supports the sensitivity of the HPA axis to physical and psychological factors (for more detail, see Dickerson & Kemeny, 2004), and has been focused on the description of the characteristics of psychological stimuli that are able to produce an HPA axis response. The main findings suggest that stimuli perceived as uncontrollable (Henry & Grim, 1990; Sapolsky, 1993), unpredictable (Mason, 1968a; b), or threatening (Blascovich & Tomaka, 1996) are able to elicit an HPA axis response. In an effort to delimitate which characteristics are needed in the psychological stimuli, Dickerson and Kemeny (2004) conducted a meta-analysis, concluding that the main factors were coping with a motivating performance task that provokes feelings of uncontrollability, unavoidable negative outcomes, or the impossibility of succeeding despite one's efforts. In fact, coping with stress that involves a social-evaluative threat is powerful enough to elicit an HPA axis response. In sum, tasks that include a perception of uncontrollability and social-evaluative threat are better to elicit larger cortisol releases. The Trier Social Stress Test (TSST) is one of the best and most widely tested protocols to elicit a stress response (Kirschbaum, Pirke, & Hellhammer, 1993).

2.2. The psychological stress response

Dealing with stressful situations involves the mobilization of resources to successfully overcome any difficulties. Along these lines, Cannon (1932) stated that negative emotions are key factors in initiating the physiological resources needed to cope with stress. In fact, the first step in developing a psychophysiological stress response is the appraisal of the situation, that is, its evaluation as stressful or not.

Several theories have emerged to explain how the stress perception induces changes in psychological and physiological states. One of the most important theories is the biopsychosocial model (BPS) of challenge and threat (Blascovich & Tomaka, 1996). This model raises the importance of appraisal in the stress response, indicating that depending on the way it is perceived and evaluated, the situation has a huge impact on the physical and psychological response to it, as well as on the performance (in cognition and behaviour) to successfully overcome it. The BPS model establishes two pathways to appraise the situation: as a threat or as a challenge. This perception depends on the individual's consideration that the situation surpasses his/her resources to cope with it or not. Although both perceptions have been associated with a physiological response, the perception of the situation as a challenge involves more efficient autonomic reactivity and better performance.

In sum, the exposure to a threatening stressful stimulus involves the generation of a negative perception of the experience (Allen, 2014). At the same time, it increases anxiety feelings (von Dawans et al., 2011), stress perception (Sugaya et al., 2012), and negative mood (Yim et al., 2010; Firk & Markus, 2009).

2.3. The ANS stress response

When a stimulus is perceived as stressful, the first physiological system activated is the ANS to promote the "fight or flight" response, as ANS activation is a preparatory function to cope with stress (Selye, 1936; Cannon, 1932). The information about the stressful stimulus is integrated and

evaluated by a circuit that includes the PVN, which interacts with the nucleus of the solitary tract, the vagus nerve (X cranial nerve), the thoracolumbar section of the spinal cord, and the locus coeruleus (Kyrou & Tsigos, 2009). As a result, the SNS is activated, producing the release of large amounts of catecholamines: adrenaline from the adrenal medulla and noradrenaline from the sympathetic nerves. Specifically, noradrenaline is released by the postganglionar sympathetic neurons in all the innervated tissues. Meanwhile, the preganglionar sympathetic neurons activate the adrenal medulla, which mainly releases adrenaline and, to a lesser extent, noradrenaline (Granger et al., 2007; Iversen et al., 2000).

Moreover, as a result of the SNS activation, there is an increase in heart rate (HR), blood flow, and blood pressure, and a reduction in vegetative functions, among other changes, thus reflecting the predominance of the SNS over the PNS activity (Chrousos, 2009; Chrousos & Gold, 1992). The increasing influence of the sympathetic branch activity can be observed through HR and heart rate variability (HRV) (for review: Stein & Kleiger, 1999; Steptoe et al., 2009). Interestingly, in recent years, it has been suggested that salivary alpha-amylase (sAA) could be considered an indirect biomarker of the ANS stress response (Granger et al., 2007; Nater & Rohleder, 2009; Rohleder & Nater, 2009). sAA (a-1,4-a-D-glucan 4-glucanohydrolase; EC 3.2.1.1) is an important salivary enzyme described for the first time by Leuchs in 1831 (Zakowski & Bruns, 1985). The release of the sAA oral enzyme (mainly by parotid glands) occurs as a result of the activation by the noradrenaline released from the SNS neurons of beta-adrenergic receptors in the salivary glands. Its main functions are the digestion of carbohydrates (Baum, 1993) and the prevention and elimination of bacteria (Scannapieco et al., 1993). However, recent research considers it a potential non-invasive biomarker of autonomic activity.

2.4. The HPA axis stress response

Second, the HPA axis is activated, inducing a sharp increase in cortisol levels (Kudielka & Wüst, 2010; Dickerson & Kemeny, 2004). This activation is due to the integration of the activity of three structures: the hypothalamus, the pituitary gland, and the adrenal glands. As mentioned above, when the stimulus is assessed as stressful, the PVN in the hypothalamus produces and releases large amounts of CRH and AVP. These hormones are released to the portal system, arriving to the pituitary gland and, once there, stimulating the release of ACTH into the bloodstream. The ACTH travels through the bloodstream until it stimulates the adrenal glands, which are responsible for the release of glucocorticoids into the bloodstream (Ulrich-Lai & Herman, 2009). In humans, the main final product of the HPA axis is cortisol, which can be measured through blood or saliva and is considered one of the most important biomarkers of the stress response. The cortisol concentration peaks around 10-30 min after the stress stimulus and takes about 60 min to return to baseline concentrations (Ulrich-Lai & Herman, 2009; Dickerson & Kemeny, 2004).

2.5. The stress response as an integrated function

It should be taken into account that the stress response is a complex phenomenon that has to be understood as a unit (see Figure 1). Although not formally part of the neuroendocrine HPA axis, the PVN has descending projections into the brainstem and spinal cord areas that express CRH, vasopressin, and oxytocin (Palkovits, 1999). These descending projections terminate monosynaptically in parasympathetic and sympathetic preganglionic neurons, giving a potential indirect influence of the adrenocortical activity to adrenal sympathetic innervation (Jacobson, 2005). Consequently, there is a connection between the HPA axis and the central nervous system (see Figure 1). For this reason, previous studies have focused on the effects of stress response dysregulation, that is, the dissociation between the ANS and HPA axis systems when facing stress. It has been suggested that a coordinated response of both systems is considered more adaptive than uncoordinated functioning (Bauer et al.,

2002). Moreover, the dysregulation of the stress response would be associated with a greater number of health problems (Ali & Pruessner, 2012).

In recent years, several ways have been employed to examine the associations between the ANS and the HPA axis response to stress. Originally, Bauer et al. (2002) studied the integration between the ANS and the HPA axis response in children, suggesting two models: The *additive* model and the *interactive* model. The *additive* model assumes that there is a symmetrical activity across the ANS and the HPA axis systems that could result in hypoarousal (a blunted response in both systems) or hyperarousal (a heightened response in both systems), with non-symmetrical responses (e.g. high ANS response and low HPA axis response) leading to adjustment problems. Meanwhile, the *interactive* model suggests that the HPA axis functioning acts primarily to suppress the SNS activity, and so symmetrical responses would result in medium levels of arousal. Regardless of the perspective, and in line with the inverted "U" model of arousal and performance (Kagan et al., 1994), both models assume that the most adaptive response is a medium level of arousal.

In the past decade, Gordis et al. (2006; 2008) and El-Sheik et al. (2008) analysed the ANS and HPA axis dissociation using regression methods, providing evidence for the additive model. Recently, Ali and Pruessner (2012) highlighted the relevance of studying the interaction between the two biomarkers. They proposed a method to analyse the ANS and HPA axis dysregulation by estimating the ratio between the areas under the curve with respect to the ground (AUCg) of the variations in sAA, controlling for cortisol variations (AOCg); and the cortisol variations, controlling for sAA variations (COAg). These authors concluded that the best marker for stress dysregulation in people with early life adversities is the COAg. However, further research is needed to establish a standardized and general method to study the ANS and HPA axis functioning coordination.

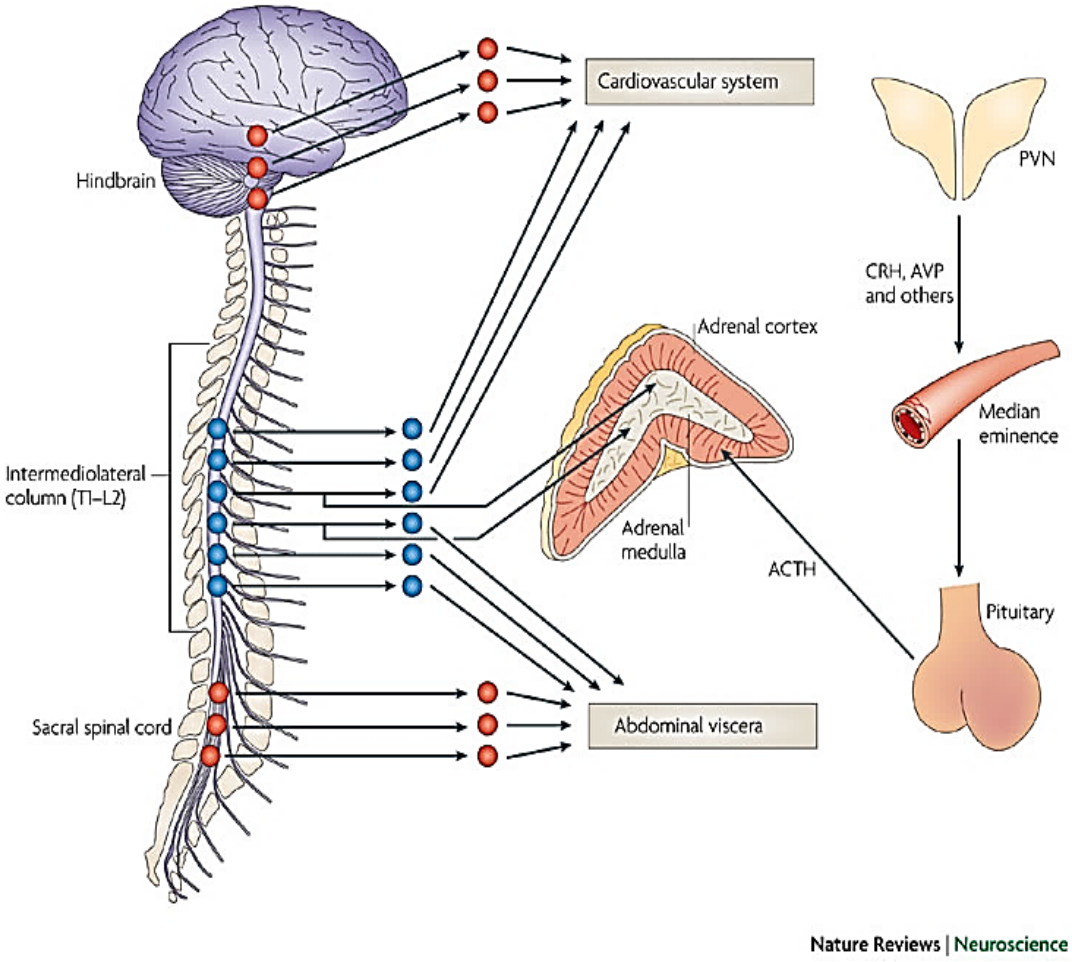


Figure 1. Integrated diagram of the ANS and HPA axis stress response, extracted from Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*, *10*(6), 397-409.

3. Modulatory factors of the ANS and the HPA axis

Large individual differences in the cortisol and autonomic response to acute stress have been found. Thus, in-depth knowledge about the mechanisms involved in HPA axis and ANS regulation would help to understand this variability. In turn, the study of modulatory factors of the HPA axis and autonomic function leads to a better approach to knowledge

about HPA axis and ANS functioning, as well as their involvement in health and disease. Here we will review some of these moderating factors related to our research.

3.1. Age

Aging has been defined as the gradual loss of the body's ability to (i) maintain homeostasis and (ii) adjust itself to changing conditions (e.g. stressors) (Masoro, 2005; Pardon, 2007). This progressive loss could be due to an age-related change in the psychological perception of the situation, and to the loss of the correct functioning of the ANS and HPA axis.

As people age, an attenuation has been observed in the perception of stressful situations (Aldwin et al., 1996). Older people tend to report less negative emotions than younger people (Almela et al., 2011b; Labouvie-Vief et al., 1987; Lawton et al., 1993). Additionally, they perceive less frustration than younger adults on acute laboratory challenges (Hidalgo et al., 2015).

Important changes have also been observed in ANS functioning regulation across the lifespan. This could be explained by the loss of autonomic regulation in aging (Laitinen et al., 2004; Nicolini et al., 2012). Structural and functional changes have been reported in organs linked to the SNS and the PNS, such as a decrease in heart muscle mass and contractibility strength (Lakatta, 1993). Additionally, most of the HRV parameters decrease in advanced ages, resulting in a predominance of the SNP over the PNS; in fact, the change in HRV parameters in aging has been used to determine the prognosis and mortality in the elderly (for more details, see Nicolini et al., 2012). Reduced HRV has been described as an independent predictor of mortality (Kleiger et al., 2005). Meanwhile, a specific pattern in frequency-domain parameters of HRV, such as high frequency (HF) and low frequency (LF) and their ratio (LF/HF), has been related to higher longevity; thus, lower LF, higher HF and a higher LF/HF ratio has been observed in centenarians (Piccirillo et al., 1998; Paolisso et al., 1999; Shimizu et al., 2002).

However, the aging effect on the ANS stress response is still controversial due to mixed results reported in previous studies: no changes (Esler et al., 1995; Wood et al., 2002; Almela et al., 2011b), a decreased response (Kudielka et al., 2004b, c; Strahler et al., 2010), or an increased response (Pascualya et al., 1999; Uchino et al., 1999).

As in the case of cardiovascular parameters, there is research on the age effects on sAA. In basal conditions, no age effect has been found (Aguirre et al., 1987; Pajukoski et al., 1997; Salvolini et al., 1999). Nevertheless, in a recent study, Nater et al. (2013) investigated the circadian sAA release in 185 participants aged 21 to 81, finding greater sAA output and an attenuated wake-evening slope as people age. Likewise, an attenuated response in older adults (59-61 years), compared to young adults (20-31, years) has been observed (Strahler et al., 2010). By contrast, Almela et al. (2011b) reported a higher sAA increase and global output in older people exposed to stress compared to young people.

Regarding the HPA axis, it should be noted that age is one of the most important factors to take into account in hormonal system functioning (Otte et al., 2005). Age-related changes in the HPA axis regulation have been explained by the glucocorticoid cascade hypothesis (Sapolsky et al., 1986). The exposure to cumulative glucocorticoids across the life span provokes the degeneration of brain structures responsible for HPA axis regulation. This, in turn, induces higher corticoid secretion, contributing to degenerative changes in hippocampal neurons, which become unable to properly inhibit glucocorticoid release. In sum, the feed-forward cascade changes in aging, increasing the risk of pathophysiology consequences in older people (see Figure 2, extracted from Veldhuis, 2013).

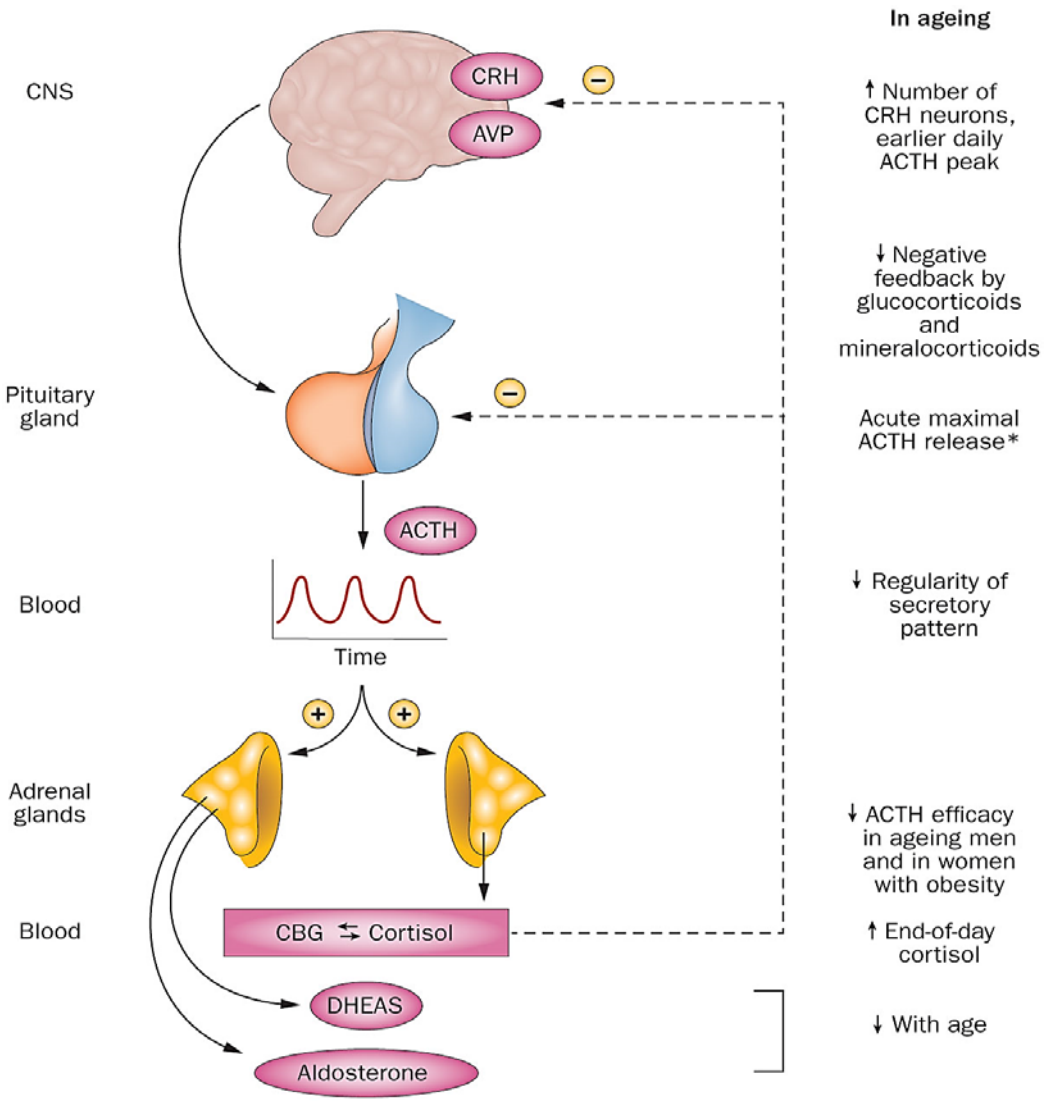


Figure 2. Aging effect on the HPA axis functioning, extracted from: Veldhuis, J. D. (2013) Changes in pituitary function with aging and implications for patient care. *Nature Reviews of Endocrinology.*

Along these lines, previous studies reported elevated stress-induced cortisol release in older people compared to adults (Almela et al., 2011a; Kudielka et al., 2004a, 2004c), although other studies showed a marginal age difference only in men (Kudielka et al., 2000; Rohleder et al., 2002) or did not observe any age effect (Nicolson et al., 1997).

3.2. Sex

As occurred with age, sex is also a very important factor to take into account in the study of the psychophysiological mechanisms involved in the stress response. In fact, sex is one of the key modulatory factors of health, increasing the risk of developing certain diseases due to being a men or a woman (Verbrugge, 1989; Macintyre et al., 1996).

Previous studies analysed the sex differences in the psychological stress response, showing mixed results. Whereas some studies reported higher increases in state anxiety and negative mood after stress exposure in women than in men (Carrillo et al., 2001; Schmaus et al., 2008; Kelly et al., 2008; Childs et al., 2010), others did not find sex differences in mood changes (Kudielka et al., 2004a; Preuß & Wolf, 2009).

Regarding the ANS, previous studies have also shown mixed results of the impact of sex on the stress response. On the one hand, some studies reported heightened HR responses to stress in young women compared to men (Kudielka et al., 2004b; Fichera & Andreasi, 2000). On the other hand, several studies did not find sex differences in the stress response to different kinds of stressors (Earle et al., 1999; Carrillo et al., 2001; Sgoifo et al., 2003; Kelly et al., 2008).

In the case of sAA, sex differences in diurnal and stress responses have previously been studied. Sex differences have not consistently been found in average sAA levels throughout the day in adults (Nater et al., 2007) or under stress conditions in infants, adults, and older people (e.g. Hidalgo et al., 2014, 2012; Thoma et al., 2012; Almela et al., 2011b; Bagley et al., 2011; Davis & Granger, 2009; Rohleder & Nater, 2009).

Although sex differences in the psychological and ANS response have not systematically been found, the sex effect on HPA axis functioning has been clearly stated. First, only men showed a significant increase in the cortisol response before facing stress, even when they only perceived an upcoming stressor or threat and were not actually confronted with it (Kirschbaum et al., 1992). Second, extensive evidence showed higher

cortisol release in response to stress in men compared to women (Goel et al., 2014; Otte et al., 2005). However, the characteristics of the psychological stressor are key factors in these sex differences. In fact, in social interactions, women show higher cortisol responses than men (Stroud et al., 2002), but if the participants are exposed to arithmetic, verbal, or psychological challenges, (i.e. TSST), men are more respondent than women (*for a meta-review,* see Goel et al., 2014). Interestingly, these sex differences change in aging, which could be explained: (i) given the age-related changes in the hypothalamic-pituitary-gonadal axis and HPA axis functioning, and (ii) because of the link between the two hormonal axes. For example, the menstrual cycle and menopause in women may modulate the changes in the ANS and HPA axis functioning (Kajantie & Phillips, 2006). In fact, older women showed an enhanced HPA negative loop dysregulation, compared to young women (Otte et al., 2005), which has been associated with lower concentrations of oestrogens (Gordon et al., 1978). The stress response to social stress is not different between young and older women in terms of cortisol release (Kudielka et al., 1999; Hidalgo et al., 2014) or HR (Kudielka et al., 1999), but older women tend to recover more slowly to stress than young women in the follicular phase (Pattachioli et al., 2006; Kudielka et al., 2004a).

3.3. Psychological factors

Extensive research has studied the effect of several psychological factors on HPA axis and ANS functioning in basal and stress conditions. Specifically, an increased interest in the relationship between personality and health has arisen in the past few decades (Rasmussen et al., 2009). It has been stated that individual personality differences are related to stress perceptions (Connor-Smith & Flachsbart, 2007), which, in turn, may be associated with the stress-involved biological systems (Carver & Connor-Smith, 2010; Dickerson & Kemeny, 2004). Therefore, personality can predispose one to better or worse ANS and HPA axis functioning, which, in turn, could decrease or increase, respectively, the risk of disease development. In this line, in their meta-analyses, Chida and Hamer (2008)

found that negative psychological states, such as anxiety and hostility, are related to heightened CV response and poorer recovery after stress. Meanwhile, positive psychological states or traits, such as happiness, show a protective capability, given that they have been related to a reduction in the HPA axis reactivity to stress.

In the following part of the present thesis, I will focus on the effect of certain psychological factors that previous research has highlighted as strongly related to health, the ANS, and HPA axis functioning.

4. Why do psychological factors matter?

During the past decade, several research studies have analysed the associations of some negative psychological factors with pathogenesis, as well as the protective effects of positive attitudes on health (for more detail see Tindle et al., 2010; Chida & Hamer, 2008; Steptoe et al., 2009). In the following sections, we will revise main findings on damaging and protective health factors before considering some more specific aspects related to the development of this thesis.

4.1. Damaging health factors

The most widely studied personality dimension is neuroticism, which has been related to multiple maladaptive behaviours and psychophysiological health problems (for reviews see Kotov et al., 2010; Lahey, 2009). A large body of evidence considered neuroticism the most robust personality trait predicting psychopathology (for review see Ormel et al., 2013; Lahey et al., 2009). In fact, neuroticism has been related to all causes of mortality (Weis & Costa, 2005) and to diseases associated with HPA axis dysregulation, such as depressive and anxiety disorders (Ormel et al., 2013), mild cognitive impairment or even Alzheimer's disease (Dar-Nimrod et al., 2012; Kuzma et al., 2011), and diabetes and metabolic syndrome (Mommersteeg & Pouwer, 2012), among others.

First attempts to establish the biological basis of neuroticism were inspired by Eysenck's and Gray's theories, in which people high in neuroticism were characterized as having an exacerbated over-responsiveness of specific brain structures, which in turn makes them prone to psychopathology (Eysenck, 1967; Eysenck & Eysenk, 1985; Gray & McNaughton, 2000). Advances in neuroimaging have led to a better comprehension of the brain structures that compose a circuit related to neuroticism (for more detail, see Figure 3, extracted from Ormel et al., 2013).

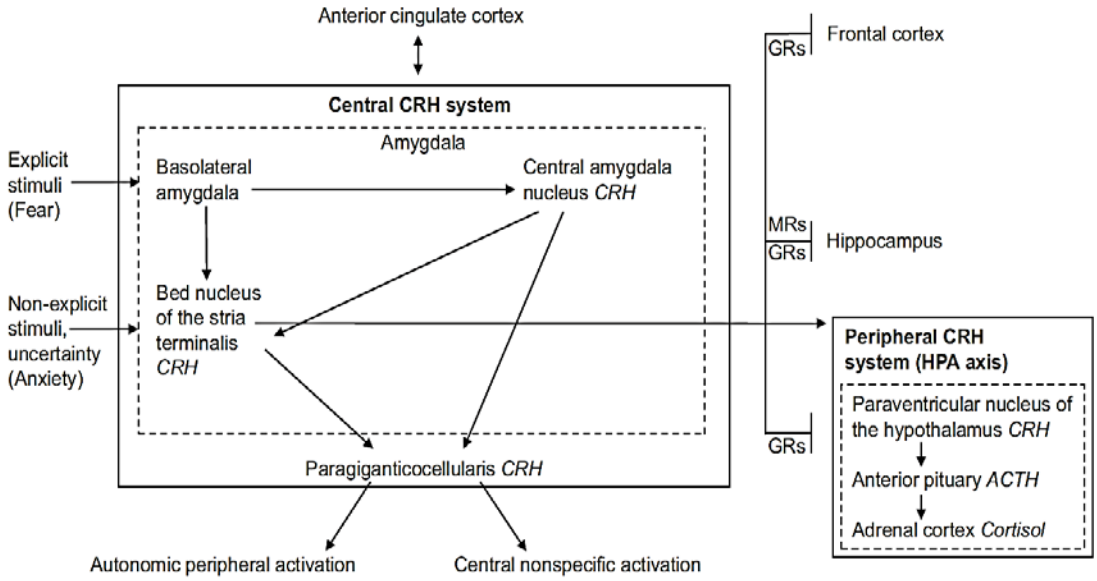


Figure 3. Schematic overview of the neurobiological systems related to neuroticism. Extracted from Ormel et al. (2013). The biological and psychological basis of neuroticism: current status and future directions. *Neuroscience & Biobehavioral Reviews*, *37*(1), 59-72.

The general over-responsiveness to which neuroticism has been related not only refers to brain activity, especially in limbic structures, but also to peripheral activation (the ANS and the HPA axis). It seems that neurotic people become more vulnerable to mental and physical diseases because they are more reactive and less capable of mitigating problematic events and experiences (Bolger & Schiling, 1991; Suls et al., 1998; Suls & Martin, 2005; Lahey, 2009). These aspects have mainly been

studied under stress conditions, but the relationship between neuroticism and stress has not been observed consistently. Neuroticism has been related to higher emotional reactivity to different kinds of stressors and heightened perception of stressful events (Bolger & Schiling, 1991; Suls et al., 1998; Vollrath, 2000; Connor-Smith & Flachbart, 2007). In this regard, previous studies related the neuroticism trait to decreased positive affect and increased negative mood after stress exposure in young people (Gomez et al., 2000; Rusting & Larsen, 1997; Larsen & Ketelaar, 1991; 1989). Interestingly, this relationship was not tested in older people.

Apart from investigating the relationship between neuroticism and the psychological stress response, other studies were interested in studying the relationship between this trait and the main physiological systems involved in the stress response. These studies aimed to clarify the possible underlying mechanisms that relate neuroticism to stress-related pathologies (for a review, see Ormel et al., 2013). Higher neuroticism has been related to heightened autonomic activation, but to lower cortisol release in young people exposed to stress (Oswald et al., 2006; Phillips et al., 2005; Kennedy & Hughes, 2011; Schwebel & Suls, 1999; Kaiser et al., 1997; Kirschbaum et al., 1993; Stemmler & Meinhardt, 1990). By contrast, a considerable body of research did not report significant relationships between neuroticism and the physiological stress response in young people (Verschoor & Markus, 2011; Wirtz et al., 2007; Knyazev et al., 2002; Schommer et al., 1999; Kirschbaum et al., 1992, 1995; Kirkcaldy, 1984; Hinton & Craske, 1977). To our knowledge, only one study observed the relationship between neuroticism and the stress response in older populations (55 to 59 years old), showing that neuroticism was related to a blunted autonomic and cortisol reactivity to stressful situations (Bibbey et al., 2013).

Some studies investigated the relationship between basal ANS activity and neuroticism, reporting a negative relationship (Bleil et al., 2008; Riese et al., 2007) or no significant relationship (Knyazev et al., 2002; Vassend & Knardahl, 2005). Regarding the HPA axis, several studies investigated the relationship between neuroticism (along with the extraversion trait), the

CAR, and the total morning cortisol concentrations, through the area under the curve with respect to the ground (AUC_G). In adolescents, Hauner et al. (2008) showed that higher neuroticism and introversion were related to reduced AUC_G. Van Santen et al. (2011) reported similar results for extraversion, but they did not find significant results for neuroticism. However, Hill et al. (2013) reported a positive relationship between extraversion and AUC_G, whereas no significant relationship was found with neuroticism in a sample with a large age range (17 to 78 years old). Portella et al. (2005) investigated the relationship between neuroticism, the CAR, and AUC_G in people from 21 to 57 years old, showing significant positive relationships between them. In line with these results, Mandold et al. (2012) showed a positive relationship between neuroticism and AUC_G, mediated by acculturation, in adults. However, other studies did not find any relationship between CAR or AUC_G and neuroticism or extraversion traits (e.g., Chan et al., 2007; Munafo et al., 2006; Laceulle et al., 2015).

4.2. Protective health factors

Also in recent decades, a number of studies have focused on what factors could prevent disease development. In fact, positive factors, such as resilience, are now being considered an interesting research topic (Crump et al., 2016; Steptoe, 2016).

Among these protective factors, optimism emerges as one of the most important factors studied. The most widely used questionnaire to measure it is the "Life Orientation Test" (LOT) (Scheier & Carver, 1985), which was later revised (LOT-R) (Scheier et al., 1994). The LOT-R measures dispositional optimism through two subscales: optimism and pessimism. They are considered generalized versions of confidence and doubt that are part of life instead of a specific context (Carver et al., 2010; Scheier & Carver, 1992). Accordingly, people high in optimism tend to hold positive generalized expectancies about their future, whereas people high in pessimism tend to hold negative expectancies (Carver et al., 2010; Scheier & Carver, 1992; Scheier et al., 1994).

Interestingly, aging has been suggested as an important factor to take into account in the independence of the two dimensions, optimism and pessimism. Originally, they were considered opposite poles of one unique dimension, that is, dispositional optimism. In fact, they showed a strong negative correlation in young people (Scheier & Carver, 1985), but they were uncorrelated in middle-aged and older people (Plomin et al., 1992; Mroczek et al., 1993; Robinson-Whelen et al., 1997). It has been proposed that age-related changes in optimism and pessimism (from opposite poles of a unique trait to two separate dimensions) may be due to age-related changes in metacognitive beliefs about optimism and pessimism, allowing older people to adaptively use both of them to cope with different situations (Herzberg et al., 2006). In this line, theoretical formulations (Diener et al., 1999; Ryff & Singer, 1998) have supported the independence of positive and negative mental states based on empirical evidence (e.g. Lai, 1994, 1997; Lai et al., 2005).

One of the most important reasons to study optimism is the lack of studies focused on the underlying mechanisms involved in its relationship with the health status. There is a large body of knowledge about the relationship between optimism and health status. Optimism has been related to metabolic syndrome (Cohen et al., 2010; Roy et al., 2010), cancer (Friedman et al., 1992), anxiety and depression (Rajandram et al., 2011), pain (Ramírez-Maestre et al., 2012), immune functioning (Brydon et al., 2009; Roy et al., 2010) or cardiovascular diseases (Nabi et al., 2010; Tindle et al., 2010), among others. Given this relationship between optimism and health, there has been an increased interest in explaining how this trait could have a protective effect on health (Rasmussen et al., 2009).

Previous studies suggested that optimism might protect against disease through the engagement in health-protective behaviours (Carver et al., 2010). For example, research suggests that having an optimistic disposition predicts lower consumption of saturated fat, increased vitamin intake, increased exercise, and lower body fat levels (Shepperd et al., 1996; Scheier & Carver, 1992). On the other hand, pessimism has been related to a higher risk of substance abuse (Ohannessian et al., 1993; Park et al., 1997)

and reduced physical activity (Brenes et al., 2002). Interestingly, a generalized positive point of view makes optimistic people more confident and persistent when facing life challenges (Carver et al., 2010). In line with the behavioural self-regulation theory (Carver & Scheier, 2000), the way people face challenges or difficulties could impact the way they cope with stress (Carver et al., 2010). People high in optimism are likely to use active coping when faced with a challenge, and they are less likely to adopt an avoidant coping style, which has been associated with poorer long-term health outcomes (Carver & Connor-Smith, 2010; Taylor & Stanton, 2007; Solberg Nes & Segerstrom, 2006).

Despite the recognised contribution of health behaviours and coping styles to the protective effects of optimism, findings from most studies persist after controlling for these factors (e.g. Giltay et al., 2006; Nabi et al., 2010). This suggests that other pathways may be involved, leading to research focused on studying the relationship between optimism and the main underlying physiological mechanisms involved in age and stress-related diseases (the ANS and HPA axis).

Research focused on the relationship between optimism and the psychophysiological stress response has shown mixed results. On the one hand, increased cortisol and HR stress responses (Solberg Nes et al., 2005), as well as faster cortisol recovery after stress, have been observed in young adults with higher optimism compared to those with lower optimism (Brydon et al., 2009). However, optimism was not related to cortisol responses to stress in a large study with healthy older people (Endrighi et al., 2011).

Taking into account that people with high optimism tend to show proactive coping and more engagement on laboratory stress tasks (Carver & Connor-Smith, 2010; Solberg Nes & Segerstrom, 2006), it is possible that this higher engagement induces increased arousal when coping with challenges, resulting in heightened cardiovascular and endocrine responses (Solberg Nes et al., 2005). However, at the same time, an optimistic perspective is expected to protect the person from exacerbated responses to stress, thus having a health-protective effect.

A few studies have investigated the relationship between optimism, circadian cortisol release, and the CAR. Jobin et al. (2014) showed that older people with low optimism had higher daily cortisol values and a lower CAR, but only on days when participants reported higher subjective stress. By contrast, studies with older (Endrighi et al., 2011) and middle-aged adults (Lai et al., 2005) reported a negative relationship between optimism and CAR, but no significant relationship with cortisol decline, the cortisol profile throughout the day, or total cortisol output during the day.

Currently, the pathways underlying the potential protective or damaging effect of psychological factors associated with age on health remain unclear. For this reason, in recent decades there has been an increased interest in clarifying the possible psychophysiological mechanisms involved in the relationship between psychological factors and health (Rasmussen et al., 2009).

Regarding psychological mechanisms, previous literature suggested that a positive re-evaluation of past life is important given its role in psychological well-being in later life (Löckenhoff & Carstensen, 2004; Carstensen et al., 1999) and death acceptance (Cappeliez et al., 2007; Webster, 1993). The *socioselectivity theory* (Carstensen et al., 1999) defends the idea that a positive bias characterizes older people. In fact, a large body of evidence shows that older people increase their attention and recall of more positive facts (for review see Mather & Carstensen, 2005). Considering all this, we have considerable interest in investigating whether people who hold positive expectations re-evaluate their past in a more positive way, thus contributing to greater well-being. Interestingly, there is evidence about the overlapping underlying physiological mechanisms involved in future expectations and autobiographical memories (Addis et al., 2007; Buckner & Carroll, 2007; Spreng & Grady, 2010). Following Seligman's assumption (1991), the generation of future expectations stems from past life perceptions. Thus, it would be reasonable to consider that having positive future expectations stems from a positive re-evaluation of past life experiences. The opposite would be expected in the case of negative future expectations. In this regard, higher neuroticism has been

related to higher negative emotions reported when people narrate personal events (Webster, 1994). Additionally, negative mood can increase the use of more negative emotional valence memories, as observed in depressed people (Fromholt et al., 1995; Mathews & MacLeod, 1994; Mat et al., 1992).

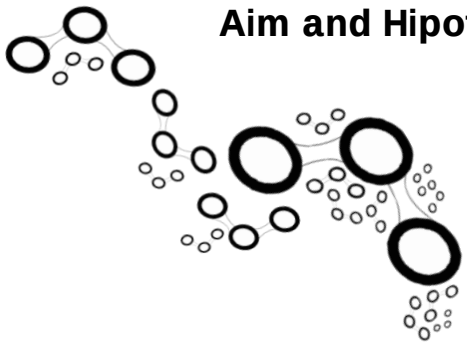
Regarding the potential physiological mechanisms involved in the relationship between psychological factors and health, Tindle et al. (2010) suggested a conceptual model to explain how positive or negative attitudes can respectively reduce or increase the risk of cardiovascular diseases (CVD). The Tindle model suggests that optimism and pessimism may affect health indirectly through health behaviours, and directly through psychophysiological processes (including the activation of stress response systems). Both of these pathways can influence the development of CVD or other chronic diseases such as Type 2 Diabetes (T2D) (e.g. Tinker et al., 2007; Matthews et al., 2004). T2D is one of the most prevalent diseases in people aged 50 years or more (8-10% prevalence) (Scully, 2012). In fact, T2D comprises around 90% of the doctor-diagnosed diabetes cases in the world in the adult population (Zimmet et al., 2001). T2D is considered a heterogeneous metabolic disease that has been characterized by reduced insulin sensitivity and relative insulin deficiency (McCrimmon et al., 2012). Unfortunately, lifestyle (i.e. a sedentary lifestyle, alcohol consumption, or overweigh) and socioeconomic factors related to the way of life are raising the expected T2D prevalence for the coming decades (Zimmet et al., 2001). Extensive evidence has been reported on the relationship between T2D and coexisting disorders, such as retinopathies (Samiec et al., 1998), cognitive dysfunction (Kloppenborg et al., 2008; van Harten et al., 2007), and Alzheimer's disease and vascular dementia risk (Biessels et al., 2008). However, the risk of CVD in T2D is the most worrisome because up to 80% of deaths in T2D patients are caused by CVD (Sowers et al., 2001). In fact, manifestations of CVD, coronary heart disease, stroke, and peripheral vascular disease are common in T2D patients (Laakso, 1999). Moreover, it is important to note that T2D is often a manifestation of the metabolic syndrome, a constellation of CVD and diabetes risk factors

such as hyperglycaemia, hypertension, dyslipidaemia and central obesity (Zimmet et al., 2001). Interestingly, heightened interleukin-6 (IL-6) has been related to higher risk of CVD (Danesh et al., 2008) and T2D (Steptoe et al., 2014). All this together suggests that T2D patients are characterized by disruptions in autonomic, neuroendocrine, and immune systems, making them prone to diseases.

In summary, the study of the factors that contribute to health damage or that help older people to reduce the risk of disease development increases in importance, especially in older people, given their higher vulnerability. It is possible that the factors that affect the ANS and HPA axis functioning play a role in stress-related disease development, as in the case of T2D. In turn, it is possible that people diagnosed with diseases related to the ANS and HPA axis dysregulation could preserve better physical and mental health, given the effect of protective psychological factors. Therefore, increasing knowledge about this topic could contribute to facilitating a better understanding of the process of healthy aging and to reducing the impact of certain extended diseases in the older population, such as T2D.

Chapter 2

Aim and Hipotheses



Aims and Hypotheses

As we mentioned previously, only a few studies have investigated the role of damaging and protective factors that influence the main systems involved in the stress response in older people. Considering the importance of these main systems in health and the enhanced vulnerability of older people, the main objective of this thesis was to investigate possible psychological factors that protect from or contribute to ASN and HPA axis malfunctioning. We had several objectives focused on negative traits, such as neuroticism, and on positive personality dimensions, such as dispositional optimism.

Objective 1. Our aim was to investigate the relationship between the neuroticism trait, depressive mood, and the psychophysiological stress response in healthy older people. Taking previous studies into account, we expected a dysregulation between the ANS and HPA axis activity under stress in people with high neuroticism or depressed mood, as well as heightened anxiety and negative affect after stress.

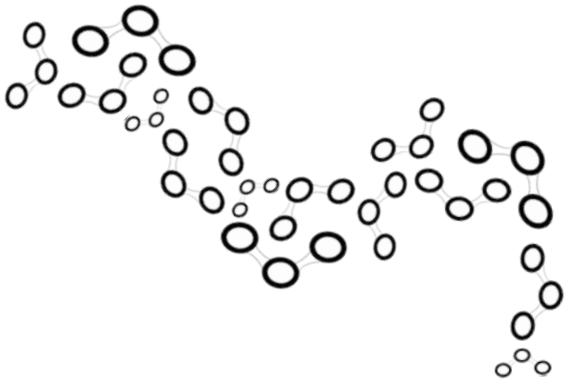
Objective 2. The second aim was to investigate the moderator effect of neuroticism and extraversion on the CAR and the total morning cortisol release in healthy older people. Based on previous literature, we expected to find positive relationships among the CAR, total morning cortisol release (AUC_G), and extraversion, but given the contradictory results reported on neuroticism, we did not have any specific directional hypotheses. Additionally, the moderator effect of sex was explored in these relationships.

Objective 3. We focused on possible protective factors of the ANS and HPA axis, and we aimed to explore the protective effect of dispositional optimism and its subscales on the psychophysiological stress response in healthy older people. In line with previous studies, we expected a positive relationship between optimism and HPA axis and ANS reactivity,

but also faster recovery; the opposite relationships were expected for pessimism. Moreover, we explored the effects of optimism and pessimism on appraisal.

Objective 4. This aim focused on the role of dispositional optimism and its subscales in the awakening cortisol response (CAR) and past life re-evaluation in healthy older people, with a positive re-evaluation being related to higher well-being. In line with previous studies, lower CAR was expected in people higher in optimism, compared to those lower in optimism. Regarding the way optimistic people re-evaluate past events from their lives, we expected a higher focus on positive events, cognitions, and emotions in people with high optimism.

Objective 5. The last aim of this thesis was to analyse the effect of dispositional optimism on the psychophysiological stress response, as well as on the diurnal HPA axis activity, in older people with doctor-diagnosed diabetes mellitus. Given the blunted physiological stress response of T2D people, we expected that optimism would help them to re-align their physiological stress response. Therefore, we expected a higher physiological stress response in T2D people with higher optimism. Additionally, in line with previous studies, lower cortisol throughout the day was expected in T2D older people with higher optimism levels.



Chapter 3

The relationship between neuroticism, depressive mood and the psychophysiological stress response.

Main results of the present chapter have been published in Puig-Perez, S., Villada, C., Pulopulos, M. M., Hidalgo, V., & Salvador, A. (2016). How are neuroticism and depression related to the psychophysiological stress response to acute stress in healthy older people? *Physiology & behavior* (156), 128-136.

1. INTRODUCTION

Throughout life, people are exposed to a wide range of circumstances that can be experienced as stressful. Some of these situations are related to psychosocial stimuli that induce physiological and psychological changes that can damage long-term health (Karatsoreos & McEwen, 2011).

Stress exposure involves the activation of the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal axis (HPA). Physical and/or psychological stress events first affect the autonomic nervous system (ANS) (Xhyheri et al., 2012), increasing the influence of the sympathetic branch in young and older people (Almela et al., 2011b; Appelhans & Luecken, 2006). This activity can be observed through Heart Rate (HR) (for review: Stein & Kleiger, 1999; Steptoe et al., 2009), and through salivary alpha-amylase (sAA) (for review see: Nater & Rohleder, 2009; Rohleder & Nater, 2009). When coping with stress, young and older people show an increase in HR and sAA (Engert et al., 2011; Almela et al., 2011b; van Stegeren et al., 2008; Nater et al., 2005, 2006; Rohleder et al., 2004). Furthermore, to cope with the situation, the HPA axis is activated, triggering a sharp increase in cortisol levels (Kudielka & Wüst, 2010; Dickerson & Kemeny, 2004).

Recent investigations have suggested that the dysregulation of the stress response, i.e. the dissociation between the ANS and HPA systems, would be associated with a greater number of health problems (Ali & Pruessner, 2012). In fact, a coordinated response by both systems is considered more adaptive than uncoordinated functioning (Bauer et al., 2002). Previous research has employed several ways to examine the associations between these two systems (Ali & Pruessner, 2012; El-Sheikh et al., 2008; Gordis et al., 2006, 2008), with Ali and Pruessner (2012) highlighting the relevance of studying their interaction through the ratio of the two salivary biomarkers, cortisol and alpha-amylase.

Aging involves changes in the ability to regulate the systems involved in the stress response, leading to a poorer control of HPA and autonomic regulation (Laitinen et al. 2004; Nicolini et al., 2012; Otte et al., 2005). These changes can be reflected in physiological disruptions in the ability to respond to stress. In fact, previous studies report that older people show higher sympathetic and cortisol response to stress (Almela et al. 2011a, 2011b; Otte et al., 2005). Additionally, there is a large body of evidence about the influence of stable and non-stable psychological factors on the stress response (Chida & Hamer, 2008), and on wellbeing and life satisfaction over the years (Gale et al., 2013). Given that this would lead to health consequences in later life (Lahey, 2009), studying the moderating variables in the stress response is especially relevant in older people.

One of the most important variables affecting the stress response is neuroticism, which has been considered a basic personality dimension that involves emotional lability and over-responsiveness to stimuli (Eysenck & Eysenck, 1975). Higher neuroticism has been associated with increased emotional reactivity to stressors (Bolger & Schilling, 1991; Suls et al., 1998) and heightened perception of stressful events (Vollrath, 2000; Connor-Smith & Flachbart, 2007). Neuroticism seems to increase the vulnerability to mental and physical diseases because people with more neuroticism usually show higher reactivity and are less capable of mitigating problematic events and experiences (Bolger & Schiling, 1991; Suls et al., 1998; Suls & Martin, 2005; Lahey, 2009). However, the link between neuroticism and the stress response in advanced stages of the life cycle is still not completely understood (Lahey, 2009). In young people, some studies have shown no relationship between neuroticism and autonomic or cortisol responses in stressful situations (Verschoor & Markus, 2011; Wirtz et al., 2007; Knyazev et al., 2002; Schommer et al., 1999; Kirschbaum et al., 1992; Kirschbaum et al., 1995; Kirkcaldy, 1984; Hinton & Craske, 1977), while others have reported that neuroticism is related to higher autonomic response and lower cortisol release after stress (Kennedy & Hughes, 2011; Oswald et al., 2006; Phillips et al., 2005; Schwebel & Suls, 1999; Kaiser et al.,

1997; Stemmler & Meinhardt, 1990). To the best of our knowledge, only one study (Bibbey et al., 2013) has analyzed the middle-aged population (55 to 59 years old), showing that neuroticism is related to blunted autonomic and cortisol reactivity to stressful situations. However, no previous studies have been carried out with older people, increasing the need to test this relationship in aging.

In addition to neuroticism, other state dimensions have been related to the stress response. Thus, depression has also shown an important effect on the stress response. On the one hand, studies in a clinical population showed a higher cortisol response to stress in depressed women with and without a history of childhood abuse (Heim et al., 2000, 2002). On the other hand, previous studies (Morris et al., 2012; Rooij et al., 2010; Burke et al., 2005) showed lower cortisol release in depressed and remitted depression patients compared to healthy participants under stress conditions, regardless of sex. Other studies suggested different patterns depending on sex, with women showing an increased cortisol response to stress (Chopra et al., 2009) and men showing a decreased peak percentage of change (Chopra et al., 2009; Trestman et al., 1991). These results coincide with Brooks and Robles (2009), who observed that healthy young men with high depression showed lower cortisol responses to psychosocial stress. Nevertheless, Young et al. (2000) showed elevated baseline cortisol, but normal stress response, in adult depressed patients exposed to the TSST. In sum, depressive symptoms seem to have a different effect on the cortisol response depending on sex and symptomatology severity.

Regarding autonomic function, depression has been related to higher sympathetic activity in response to stress (Hamer et al., 2007; Heim et al., 2000; Light et al., 1998). However, other studies have reported negative associations between depression and HR and/or blood pressure reactivity to stress (Carroll et al., 2007; Ehrenthal et al., 2010; Schwerdtfeger & Rosenkainer, 2011; Rooij et al., 2010; Salomon et al., 2009; Burke et al., 2005), or no relationship between depression and HR (Matthews et al., 2005).

Except for the Carroll et al. (2007) and Burke et al. (2005) studies, which had large mixed samples of young, adult and older people, all the studies mentioned above explored the effect of depressive symptomatology on the ANS and HPA axis in young people, adults or middle-aged people. In older people, lower cardiovascular reactivity to stress has been reported in patients with coronary artery disease with high scores on depression (York et al., 2007) Thus, further research in older people is needed to understand more in depth the pathways through which depression affects physiological adjustment to stress in this period of life.

Apart from a physiological change, coping with a stressor involves psychological changes. People exposed to stress experience it as a negative experience (Allen, 2013), with increases in anxiety (von Dawans et al., 2011), stress perception (Sugaya et al., 2012), and negative mood (Yim et al., 2010; Firk & Markus, 2009). Some studies reported that neuroticism predicts higher negative affect and lower positive affect after a stressful task or negative mood induction in young people (Gomez et al., 2000; Rusting & Larsen, 1997; Larsen & Ketelaar, 1991, 1989). However, to the best of our knowledge, no previous research has studied this relationship in older people, highlighting the need to explore it in this population due to age-related changes in emotional regulation (Carstensen et al., 1999).

Taking into account the impact of age (Nicolini et al. 2012; Otte et al. 2005) and psychological factors such as neuroticism and depression in the HPA and ANS stress response (e.g. Hamer et al., 2007; Oswald et al. 2006; Phillips et al. 2005; Kennedy & Hughes, 2004; Heim et al., 2000, 2002), the aim of the present study was to investigate the effects of these psychological factors on the psychophysiological response to stress and non-stress in a healthy older population from an integrative perspective, including several measures of different systems in order to more closely examine this relationship. Based on previous literature (Almela et al., 2011a; 2011b; Kudielka & Wüst, 2010; Dickerson & Kemeny, 2004), we expected to find a higher response in these main systems involved in the stress response (HR,

sAA and cortisol) in older people exposed to a stress situation compared to those exposed to a non-stress situation. Moreover, considering the literature on healthy middle-aged and older people (Bibbey et al., 2013; Rooij et al., 2010; Carroll et al., 2007; York et al., 2007), we expected neuroticism and depression to be related to a blunted physiological stress response. We also explored the relationship between neuroticism and depression, and the dysregulation of the HPA and ANS response, using the approach employed by Ali and Pruessner (2012), in order to improve our comprehension of the effects of neuroticism and depression in the coordination of these two systems in healthy older people facing stress. Finally, we studied the relationship of neuroticism and depression with negative or positive affect after stress or non-stress exposure in older people. Previous results showed that both adult and middle-aged people with high neuroticism and depressed people perceived the stressful task as more stressful, difficult and demanding (Bibbey et al., 2013; Rooij et al., 2010; Salomon et al., 2009). Therefore, we expected higher negative affect and anxiety after stress in people with higher neuroticism and depression (Gomez et al., 2000; Rusting & Larsen, 1997; Larsen & Ketelaar, 1991, 1989).

2. MATERIAL & METHODS

2.1. Participants

A total of 71 participants from 55 to 76 years old were recruited using informative advertisements. Participants were randomly assigned to two conditions: 36 to the stress condition (16 men) and 35 to the non-stress condition (17 men). The exclusion criteria were: smoking more than 10 cigarettes a day, consuming alcohol or other drugs of abuse, having had surgery under general anesthesia during the past year, severe vision or hearing problems, presence of severe cardiovascular disease, illness that involves a disturbance of the HPA, and neurological or psychiatric disorders. In addition, participants were excluded if they took drugs related to cognitive or emotional function, psychotropic substances, beta-blockers, benzodiazepines, asthma medication, or drugs capable of influencing HPA

function such as glucocorticoids. All the female participants were postmenopausal, and none of them were receiving hormonal replacement therapy. Subjects were contacted by telephone and invited to participate in the study.

The study was carried out according to the Declaration of Helsinki, and the Ethics Committee of the University approved the protocol. All participants received verbal and written information about the study and signed an informed consent. At the end of the study, the participants received a gift worth 15€.

2.2. Procedure

The participants were invited to two sessions on two consecutive days. The first session consisted of a neuropsychological assessment (results published in Pulopulos et al. 2014). In this report we focus on the results from the second session, which took 1h and 30 min to complete and was always carried out in the afternoon between 16h and 20h. Participants were asked to sleep as long as usual, refrain from heavy activity the day before, and not consume alcohol since the night before. Additionally, they were instructed to drink only water and not eat, smoke or take any stimulants 2h prior to both sessions.

The protocol started with a 30-min introduction phase to allow the participants to adapt to the laboratory setting. In this phase, the heart rate recording system was placed on the participant, the first saliva sample for cortisol and sAA was provided, and state anxiety and positive and negative affect were assessed. After the introduction phase, participants were exposed to a stress task or non-stress task, and a saliva sample was collected during exposure to the task. Immediately after the task, state anxiety and positive and negative affect were assessed again. Finally, subjects had 45 min to recover while they answered several questionnaires. During the recovery period, saliva samples were collected every 10 minutes

from the termination of the task. The participants completed personality (EPQ-RS) and depression (BDI) questionnaires.

2.2.1. Stress Condition The participants were exposed to a standardized psychosocial stressor (Trier Social Stress Test – TSST, Kirschbaum et al., 1993; Kudielka et al., 2007) that is able to provoke cortisol, sAA and cardiovascular responses in older people (Almela et al., 2011a; 2011b; Pulopulos et al., 2013). The participants performed a 5-min free speech task (job interview) and a 5-min arithmetic task (serial subtraction), both in front of a committee composed of a man and a woman. They remained standing at a distance of 1.5m from the committee in a room where a video camera and a microphone were clearly visible. The committee member of the opposite sex engaged in all the interactions with participants: (i) ask a set of standardized questions about the participant's characteristics if he/she did not use up the 5 minutes of free speech; (ii) and interrupt and urge the participant to start the subtraction again after each mistake in the arithmetic task. The participants were informed that the task was recorded in order to analyze their performance later. Thus, the TSST is a social-evaluative stress task whose main stress source stems from lack of control and being evaluated by others (committee).

2.2.2. Non-Stress Condition An ad hoc laboratory task was designed following Kudielka et al.'s (2007) criteria, in order to make it similar to the stress condition in physical global activity and mental workload, except for the lack of evaluative threat and uncontrollability, the main stress-producing components of the TSST (Dickerson & Kemeny, 2004). This task consisted of free speech and an arithmetic task without an audience. For the free speech, the participants talked aloud for 5 min about a neutral non-emotional experience, while the arithmetic task consisted of counting by five aloud. Before the task, the participants were informed that they would not be recorded, and their performance would not be evaluated later. None of the stressful elements were present (video camera, microphone and committee). This control task has been used in previous

studies (see Pulopulos et al., 2015; Hidalgo et al., 2015) and can be considered a non-social evaluative stress task.

2.3. Physiological measurements

2.3.1. Heart Rate (HR) It was continuously recorded in the stress and non-stress conditions using a Polar©RS800cx watch (Polar CIC, USA). This device comprises a chest belt placed on the solar plexus and a Polar watch. The Polar watch records R-R intervals with a sampling frequency of 1000 Hz, providing a time resolution of 1ms for each R-R interval. After eliminating the artifacts, the HR means were computed using the software Kubios Analyses (Biomedical Signal Analysis Group, University of Kuopio, Finland). We analyzed HR in periods of 5 minutes. We selected minutes 7 to 12 (baseline) as the baseline, minutes 35 to 40 (free speech), and minutes 43 to 48 (arithmetic task) to measure the response to the stress or non-stress task, and minutes 50 to 55 (recovery) to measure the capability to recover baseline levels after the tasks. All the minutes mentioned above were measured from the beginning of the session.

2.3.2. Salivary Stress Markers The participants provided 7 saliva samples using salivettes (Sarstedt, Nümbrecht, Germany) to measure cortisol and sAA levels during the session. The timing of the saliva sampling was 15 min before the onset of the stress or non-stress task (-15), between the speech and arithmetic tasks (+5), 5 min after the stress or non-stress task (+15), and then in periods of 10 min until the end of the experimental session (+25, +35, +45 and +55 min). Both cortisol and sAA were analyzed from the same saliva samples, and so the time points were identical.

2.3.2.1. Cortisol. Samples were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was frozen at -80°C until the analysis took place. The samples were analyzed by a competitive solid phase radio immune assay (tube-coated), using the commercial kit Spectria Cortisol RIA (cat. Nu 06119) from Orion Diagnostica (Espoo, Finland). All the samples were analyzed in the same trial, assay sensibility

was 0.8 nmol/L, and the inter- and intra-assay variation coefficients were all below 10%.

2.3.2.2. Alpha-Amylase. sAA was measured by an enzyme kinetic method according to the protocol specified in Rohleder et al. (2006) using a commercial kit analysis of sAA (Cat. No. 1-1902, 1-1902-5) by Salimetrics (USA). All the samples from the same individual were analyzed in the same run, the inter- and intra-assay variation was about 10%, and test sensitivity was 0.4 U/ml.

2.4. Psychological measurements

2.4.1. Eysenck Personality Questionnaire-Revised (Eysenck & Eysenck, 1975). To obtain the scores for neuroticism, we used the Spanish version of the Eysenck Personality Questionnaire-Revised, short form (EPQ-RS) (Eysenck & Eysenck, 1997). The EPQ-RS contains a total of 48 items to which participants are asked to respond "yes" or "no". The questionnaire provides four factors: psychoticism, extraversion, neuroticism and lie. The alpha values for the Spanish version range from 0.65 to 0.82 for men, and from 0.67 to 0.82 for women, and the correlations among scales are below 0.16 for men and 0.22 for women (Eysenck & Eysenck, 1997). In our study, we focused only on neuroticism. Of the 48 items, 12 of them measured neuroticism (e.g. "Do you often worry about things you should not have done or said?" or "Are you an irritable person?").

2.4.2. Beck Depression Inventory (BDI) (Beck et al., 1961). The Spanish version of the BDI is used to assess depressive symptomatology. It is a 21-item questionnaire scored from 0 to 3 (Conde & Useros, 1975). It measures cognitive, somatic and behavioral symptoms of depression in the previous two months, with high scores indicating more severe depression. This questionnaire showed good internal consistency (α=.91) and good construct validity.

2.4.3. Positive and Negative Affect (PANAS) They were evaluated by the Spanish version (Sandín et al., 1999) of the PANAS (Positive and

Negative Affect Scale - PANAS; Watson et al., 1988). This 20-item questionnaire assesses affect according to two dimensions: Positive affect (PA) and Negative affect (NA), with 10 items measuring each of them (interested, upset excited, scared, etc.). Participants gave their answers based on how they felt at that particular moment, just after and just before the stress or non-stress task. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). The Spanish version of the PANAS has high internal consistency (Sandín et al., 1999), with a Cronbach's alpha for PA ranging from 0.87 to 0.89, and for NA from 0.89 to 0.91.

2.4.4. State Anxiety The Spanish version (Spielberger et al., 1982) of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970), with a Cronbach's alpha ranging from 0.90 to 0.93 (Spielberger et al., 1982), consists of two scales containing 20 items each, rated on 4-point scales (1-Not at all to 4-Very), to measure individual differences related to the trait and state anxiety constructs. For this study, we only used the state scale values.

2.5. Statistical Analyses

One-way ANOVAs were performed to investigate condition and sex differences on demographic, anthropometric and psychological tests (neuroticism and depression), with condition (stress vs. non-stress) and sex (men vs. women) as between-subject factors.

Given that salivary cortisol and sAA did not show normal distributions, they were square root transformed. To investigate the stress response, we performed ANCOVAs and ANOVAs for repeated measures with condition as between-subject factor and time (for PA, NA and state anxiety: pre-task and post-task; for HR: habituation, stress/non-stress task -average of the speech and arithmetic- and recovery; for sAA: -15, +5 and +15; and for cortisol: -15, +5, +15, +25, +35, +45 and +55 min) as a within-subject factor. Sex was introduced as a covariate for cortisol analyses to control for its effect on the physiological stress response. Moreover, baseline levels for HR

(p=0.037) and cortisol (p=0.053) were different in the stress and non-stress conditions; thus, they were added as covariates. Based on Ali and Pruessner (2012), we calculated the COAg ($AUCg_{CORTISOL}/AUCg_{sAA}$) and AOCg ($AUCg_{sAA}/AUCg_{CORTISOL}$) ratios to obtain two possible markers of physiological stress dysregulation. Regarding HR response, $HR_{Reactivity}$ (average for the speech and arithmetic stress or non-stress task minus habituation period) and $HR_{Recovery}$ (recovery period minus habituation period) indexes were calculated. For psychological response, we calculated the change for PA, NA and state anxiety (ΔPA, ΔNA, ΔAnxiety: post-task minus pre-task).

Correlation analyses were performed between neuroticism and depression, with psychological (PA, NA and anxiety change) and physiological response to stress (COAg, AOCg and HR reactivity and recovery indexes) split by condition. To test the relationships between neuroticism and depression and autonomic and endocrine responses to stress or non-stress, we performed a moderation regression analysis. Neuroticism and depression were set as predictors of COAg, AOCg and HR reactivity and recovery indexes. Moreover, we analyzed the relationship between neuroticism and depression and COAg, AOCg, and HR reactivity and recovery indexes, but taking into account the condition as a moderator variable (neuroticism × condition and depression × condition interactions). We established sex, age, BMI and SES as covariates due to their effect on cardiovascular (Nicolini et al., 2012; Liao et al., 1995), cortisol and sAA responses (Castro-Diehl et al., 2014; Almela et al., 2011a, 2011b; Tilbrook & Clarke, 2006). Except for sex (men = 0, women = 1) and condition (stress = 0, non-stress = 1), all variables were z-transformed prior to their entry in the analysis to facilitate the interpretation of first-order terms.

One woman in the non-stress condition was excluded because her cortisol concentrations differed more than 3 S.D. from the sample mean.

We used the Greenhouse-Geisser procedure when the requirement of sphericity in the repeated-measures ANOVAs was violated. Post hoc

planned comparisons were performed using the Bonferroni adjustments for the *p*-values < 0.05. All *p*-values reported are two-tailed, and the level of significance was marked at $p < 0.05$. When not otherwise specified, results shown are means ± standard error of means (SEM). We used SPSS 19.0 to perform the statistical analyses.

3. RESULTS

3.1. Preliminary analyses

The mean age of the sample was 64.20 years (from 56 to 76 years old), and 52.9% of the participants had an educational level beyond high school. Participants showed a medium subjective socioeconomic status (SES) ($M = 5.51$, $SEM = 0.130$) and normal body mass index (BMI) ($M = 26.901$, $SEM = 0.396$). There were no significant differences between conditions and sex on age, SES or educational level (all $p > 0.359$). BMI did not differ between conditions ($F_{(1, 69)} = 0.466$, $p = 0.497$), but men showed a higher BMI than women ($F_{(1, 69)} = 4.376$, $p = 0.040$). Finally, there were no condition or sex differences for depression and neuroticism (all $p > 0.090$) (*Table 1*).

Table 1	Total (n=71)			Stress (n=36)			Non-Stress (n=35)		
	Total	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*	*Men*	*Women*
Age	64.20 (0.495)	64.12 (0.784)	64.27 (0.633)	64.66 (0.697)	64.94 (1.043)	64.42 (0.959)	63.74 (0.705)	63.35 (1.163)	64.11 (0.844)
SES	5.51 (0.130)	5.61 (0.208)	5.42 (0.161)	5.46 (0.180)	5.69 (0.299)	5.26 (0.214)	5.56 (0.190)	5.53 (0.298)	5.59 (0.243)
BMI	26.901 (0.396)	27.770 (0.465)	26.146 (0.599)	27.16 (0.604)	27.53 (0.771)	26.88 (0.909)	26.62 (0.514)	27.99 (0.557)	25.332 (0.742)
BDI	4.96 (0.504)	4.06 (0.684)	5.74 (0.714)	4.53 (0.686)	4.06 (1.160)	4.90 (0.833)	5.40 (0.742)	4.06 (0.793)	6.67 (1.177)
Neuroticism	4.01 (0.204)	4.18 (0.284)	3.86 (0.293)	3.91 (0.297)	3.75 (0.382)	4.05 (0.449)	4.11 (0.283)	4.59 (0.403)	3.67 (0.379)

3.2. Physiological Response

3.2.1. HR Significant effects of condition ($F_{(1, 66)} = 35.178$, $p < 0.001$) and the interaction between time and condition ($F_{(1, 66)} = 21.199$, $p < 0.001$) were found. Post hoc analyses showed higher HR in the stress condition

during the exposure to stress and after it than in the non-stress condition (all $p < 0.001$) (see *Figure 1*).

3.2.2. Cortisol The repeated-measures ANCOVA showed a main effect of condition ($F_{(1, 66)} = 33.337$, $p < 0.001$), and the interaction between condition and time ($F_{(2.265, 149.510)} = 20.195$, $p < 0.001$). The time factor approached significance ($F_{(2.265, 149.510)} = 2.790$, $p = 0.058$). Post hoc analyses showed higher cortisol concentrations in the stress condition than in the non-stress condition in all post-task samples (+5, +15, +25, +35, +45 and +55) (all $p<.016$) (*Figure 1).*

Table 2

	Neuroticism		Depression	
	Stress	**Non-Stress**	**Stress**	**Non-Stress**
ΔPA	r=-.064, p=.714	r=.299, p=.081	r=.418, p=.139	r=.085, p=.629
ΔNA	r=.121, p=.490	r=-.014, p=.938	r=.343, p=.163	r=.213, p=.216
ΔAnxiety	r=-.035, p=.841	r=-.136, p=.435	r=-.006, p=.974	r=.229, p=.187
$HR_{Reactivity}$	r=.048, p=.784	r=.161, p=.363	r=-.275, p=.105	r=.282, p=.106
$HR_{Recovery}$	r=.125, p=.474	r=-.039, p=.827	r=-.003, p=.985	**r=.405, p=.018**
AOCg	r=.063, p=.718	**r=-.329, p=.054**	r=-.189, p=.271	r=-.165, p=.344
COAg	r=.096, p=.583	r=.280, p=.104	**r=.354, p=.034**	r=-.189, p=.276

ΔPA= post-task PA minus pre-task PA; ΔNA= post-task NA minus pre-task NA; ΔAnxiety= post-task state anxiety minus pre-task state anxiety.

Table 2. Correlation analyses between neuroticism and depression and psychophysiological response to stress and non-stress exposure (psychological: change of PA, NA, state anxiety; physiological: HR reactivity and recovery indexes, and AOCg and COAg).

3.2.3. sAA The ANOVA for repeated-measures showed a main effect of time ($F_{(1.742, 282.101)} = 23.755$, $p < 0.001$) and the interaction between time and condition ($F_{(1.742, 282.101)} = 3.377$, $p = 0.044$. The condition effect was not significant ($F_{(1, 69)} = 0.049$, $p = 0.825$)(*Figure 1)*. Post hoc analyses showed that both the stress and non-stress conditions increased sAA from -15 to +5 (both $p < 0.001$); however, the stress condition maintained its sAA values high at

+15 (-15 to +15: $p = 0.004$), while the non-stress condition rapidly recovered its baseline values (-15 to +15: $p > 0.99$).

3.3. Psychological response

3.3.1. State Anxiety The repeated measures ANCOVA showed a main effect of condition ($F_{(1, 68)} = 4.957$, $p = 0.029$) and the interaction between time and condition ($F_{(1, 68)} = 19.034$, $p < 0.001$), but time was not significant ($F_{(1, 68)} = 0.001$, $p = 0.970$). Post hoc analyses showed a significant increase in state anxiety only in the stress condition ($p < 0.001$), but not in the non-stress condition ($p = 0.608$). Higher state anxiety was found after the task in the stress group compared to the non-stress group ($p = 0.001$), but there were no significant differences before the task ($p = 0.999$).

3.3.2. Positive and Negative Affect The repeated measures ANCOVA did not show effects of time, condition or the interaction between time and condition (all $p > 0.104$) on positive affect. Regarding negative affect, ANCOVA showed a significant effect of the time-condition interaction ($F_{(1, 68)} = 21.344$, $p < 0.001$). Condition approached significance ($F_{(1, 68)} = 3.750$, $p = 0.057$), but time was not significant ($F_{(1, 68)} = 0.068$, $p = 0.795$). Post hoc analyses showed a significant increase in negative affect only in the stress condition ($p < 0.001$), but not in the non-stress condition ($p = 0.189$). There was higher state anxiety after stress in the stress group compared to the non-stress group ($p = 0.002$), but there were no significant differences before stress ($p = 0.952$).

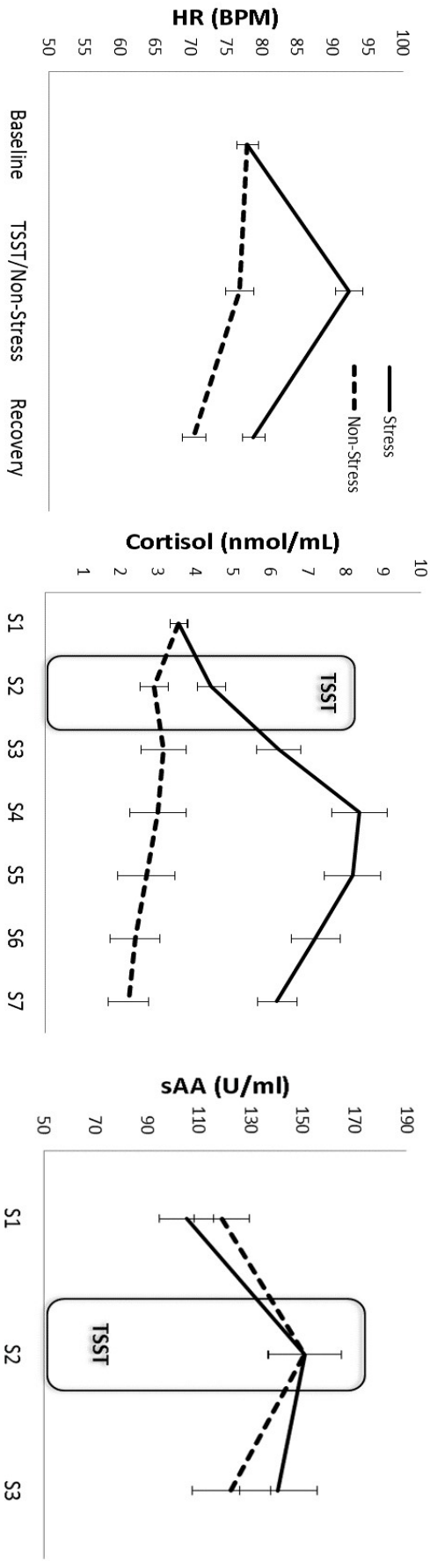


Figure 1. Mean values and ±SEM of the physiological response to the stress and non-stress tasks in HR, cortisol and alpha-amylase.

3.4. Correlation analyses

3.4.1. Neuroticism Correlation analyses showed that neuroticism was marginally related to lower AOCg (r = -0.329, *p* = 0.054). No other significant relationships were found related to neuroticism in the stress or non-stress conditions (see Table 2).

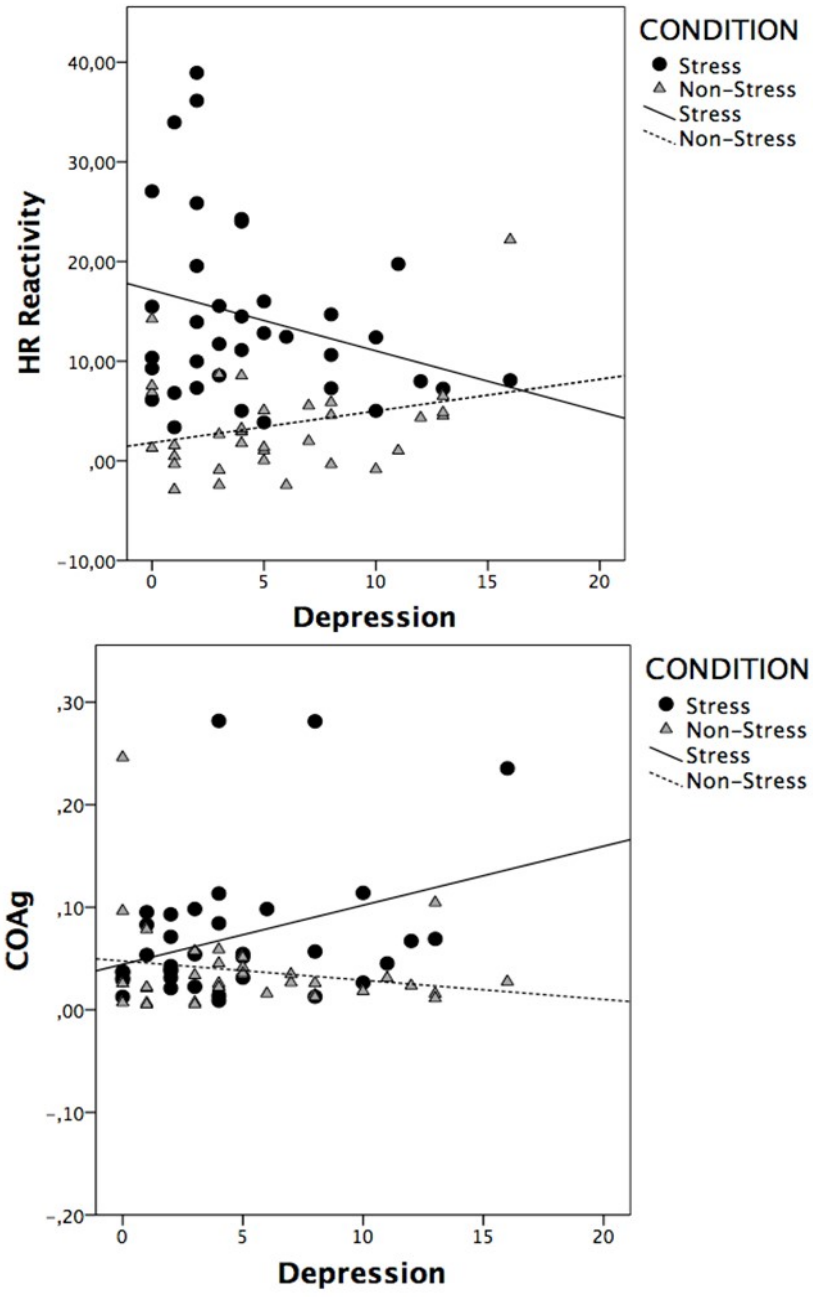


Figure 2. Scatter plots of direct relationship with depression as predictor of HR reactivity and COAg, all of them without covariates

Table 3

Model:

AdjR²= 0.273, ΔR^2= 0.087, $F_{(1, 60)}$ = 7.176, p = 0.010

	β	SE	t	*p*	CI
Constant	0.351	0.200	1.756	0.084	[-0.049, 0.752]
Sex	-0.598	0.234	-2.552	0.013	[-1.066, -0.129]
Age (*Z*)	-0.172	0.117	-1.467	0.148	[-0.406, 0.062]
SES (*Z*)	0.018	0.118	0.155	0.877	[-0.218, 0.255]
BMI (*Z*)	0.148	0.121	1.224	0.226	[-0.094, 0.390]
BDI (Z_X)	0.423	0.172	2.456	**0.017**	[0.078, 0.768]
Condition (*M*)	-0.079	0.221	-0.356	0.723	[-0.521, 0.364]
BDI × Condition (Z_XM)	-0.664	0.248	-2.679	**0.010**	[-1.159, -0.168]
Effect BDI on COAg					
Stress Condition	0.423	0.172	2.456	**0.017**	[0.078, 0.768]
Non-Stress Condition	-0.241	0.174	-1.384	0.172	[-0.589, 0.107]

Model:

AdjR²= 0.185, ΔR^2 < 0.001, $F_{(1, 60)}$ = 0.021, p = 0.884

	β	SE	t	*p*	CI
Constant	-0.307	0.212	-1.447	0.153	[-0.731, 0.117]
Sex	0.565	0.248	2.276	0.026	[0.068, 1.061]
Age (*Z*)	0.124	0.124	1.002	0.321	[-0.124, 0.372]
SES (*Z*)	-0.102	0.125	-0.813	0.420	[-0.353, 0.149]
BMI (*Z*)	-0.172	0.128	-1.341	0.185	[-0.428, 0.084]
BDI (Z_X)	-0.218	0.183	-1.196	0.237	[-0.584, 0.147]
Condition (*M*)	0.026	0.234	0.112	0.911	[-0.442, 0.495]
Neuroticism × Condition (Z_XM)	0.038	0.263	0.146	0.884	[-0.487, 0.564]
Effect BDI on AOCg					
Stress Condition	-0.218	0.183	-1.198	0.237	[-0.549, 0.189]
Non-Stress Condition	-0.180	0.184	-0.976	0.333	[-0.549, 0.189]

Table 3. Moderation analyses where depression scores predict COAg and AOCg moderating by condition with 95% confidence intervals (n = 70).

3.4.2. Depression Correlation analyses showed that depression was related to COAg (r = 0.354, p = 0.034) in the stress condition and to $HR_{Recovery}$ (r = 0.405, p = 0.018) in the non-stress condition. No other significant relationships were found related to depression in the stress or non-stress condition (see Table 2).

3.5. Moderation analyses

3.5.1. Neuroticism There were no significant direct relationships or relationships moderated by condition between neuroticism and $HR_{Reactivity}$, $HR_{Recovery}$, AOCg or COAg, (all p > 0.198).

3.5.2. Depression Regardless of the condition, higher scores on depression were directly related to higher COAg (p = 0.017), but not to AOCg (p = 0.237). The interaction between depression and condition was significant for COAg (p = 0.010), but not for AOCg (p = 0.884) (see Table 3). Post hoc analyses showed that higher scores on depression were positively related to higher COAg only in the stress condition (p = 0.017) (see Figure 2), but not in the non-stress condition (p = 0.333), which is an indicator of physiological stress dysregulation, showing a predominance of cortisol response over sympathetic response (see Table 3).

Regarding HR, depression was negatively related to $HR_{Reactivity}$, regardless of condition (p = 0.029), and marginally positively related to $HR_{Recovery}$ (p = 0.064). Post hoc analyses showed that depression was negatively related to $HR_{Reactivity}$ in the stress condition (p = 0.029) (see Figure 2), but not in the non-stress condition (p = 0.203) (see Table 4). No significant relationships were found in the post hoc analyses for $HR_{Recovery}$ (all p > 0.127).

Table 4

Model:

AdjR^2 = 0.439, ΔR^2 = 0.058, $F_{(1, 60)}$ = 6.160, p = 0.016

	β	SE	t	*p*	CI
Constant	0.672	0.177	3.797	< 0.001	[0.318, 1.027]
Sex	-0.242	0.210	-1.153	0.253	[-0.663, 0.178]
Age (Z)	0.019	0.103	0.183	0.856	[-0.187, 0.224]
SES (Z)	0.057	0.105	0.544	0.588	[-0.153, 0.268]
BMI (Z)	0.086	0.108	0.800	0.427	[-0.129, 0.301]
Depression (ZX)	-0.337	0.151	-2.237	**0.029**	[-0.639, -0.036]
Condition (M)	-1.157	0.193	-5.985	**< 0.001**	[-1.544, -0.770]
Depression × Condition (ZXM)	0.528	0.213	2.482	**0.016**	[0.103, 0.954]
Effect Depression on $HR_{Reactivity}$					
Stress Condition	-0.337	0.151	-2.237	**0.029**	[-0.639, -0.036]
Non-Stress Condition	0.191	0.149	1.286	0.203	[-0.106, 0.489]

Model:

AdjR^2 = 0.392, ΔR^2 = 0.036, $F_{(1, 60)}$ = 3.573, p = 0.064

	β	SE	t	*p*	CI
Constant	0.490	0.185	2.648	0.010	[0.120, 0.861]
Sex	-0.139	0.220	-0.631	0.530	[-0.578, 0.301]
Age (Z)	0.318	0.107	2.966	0.004	[0.104, 0.533]
SES (Z)	0.062	0.110	.560	0.577	[-0.158, 0.282]
BMI (Z)	0.192	0.113	1.711	0.092	[-0.033, 0.418]
Depression (ZX)	-0.180	0.158	-1.142	0.258	[-0.495, 0.135]
Condition (M)	-0.902	0.202	-4.460	**< 0.001**	[-1.306, -0.497]
Depression × Condition (ZXM)	0.421	0.223	1.890	0.064	[-0.025, 0.866]
Effect Depression on $HR_{Recovery}$					
Stress Condition	-0.180	0.158	-1.142	0.258	[-0.495, 0.135]
Non-Stress Condition	0.241	0.155	1.549	0.127	[-0.070, 0.552]

Table 4. Moderation analyses where depression scores predict $HR_{Reactivity}$ and $HR_{Recovery}$ moderating by condition with 95% confidence intervals (n = 70).

4. DISCUSSION

The main purpose of the present study was to analyze the effects of neuroticism and depression on the psychophysiological response of healthy older people exposed to stress or non-stress. As we expected, higher response of HR, sAA and cortisol was found in those participants exposed to stress, compared to those exposed to a non-stress condition. We also observed that neuroticism was not related to the psychophysiological stress response. A positive relationship was found between depression and COAg in the stress condition, which is an indicator of physiological stress dysregulation. However, no associations were found between depression and cortisol and sAA response in the non-stress condition. Finally, depression was related to a blunted HR response.

Agreeing with previous research, we observed higher HR, sAA and cortisol release in the stress condition than in the non-stress condition (Almela et al., 2011a; 2011b; Kudielka & Wüst, 2010; Otte et al., 2005; Dickerson & Kemeny, 2004). Additionally, as previous studies showed, we observed an increase in NA and anxiety after stress exposure (von Dawans et al., 2011; Yim et al., 2010; Firk & Markus, 2009).

Our results showed that neuroticism was not related to the stress response or recovery after TSST. These results coincide with previous studies in young people that found no effect of neuroticism on autonomic function (Hutchinson & Ruiz, 2011; Knyazev et al., 2002; Kirkcaldy, 1984; Hinton & Craske, 1977) or on the cortisol response to stress (Kirschbaum et al., 1992, 1995; Schommer et al., 1999; Verschoor & Markus, 2011; Wirtz et al., 2007). In contrast to this, some studies have found that neuroticism was related to higher cardiovascular and cortisol reactivity in young people (Kennedy & Hughes, 2011; Kaiser et al., 1997; Schwebel & Suls, 1999), and to a worse return to baseline after exposure to stress in middle-aged people (Bibbey et al., 2012). We have to take into account that, in the present research, we studied healthy older people with scores around the mean. This restricted range may limit the effect of this trait in stressful situations because

neuroticism's effect on ANS lability has been observed in stressful events, especially in the case of extreme neuroticism scores (Eysenck & Eysenck, 1985).

Regarding depression, our results showed a positive relationship between depression scores and higher COAg in the high stress condition, which is an indicator of a dysregulation of the HPA and ANS response. These results agree with previous studies that showed heightened total cortisol release in response to stress in depressed people (Chopra et al., 2009; Heim et al., 2000; 2002), while others have related depression to a blunted cortisol response (Morris et al., 2012; Brooks & Robles, 2009; Trestman et al. 1991), or did not find a relationship (Young et al., 2000). Our results did not reveal any significant relationship with AOCg, i.e. predominance of sAA release over cortisol variations. Alli and Pruessner (2012) showed higher levels of AOCg in people with higher depression scores in a mixed sample (healthy and with early life adversities), and they highlighted AOCg as the best indicator of stress system dysregulations in a population with early life adversities. However, given the closer relationship between depression and the HPA axis (for review see Watson & Mackin, 2006) than between depression and sAA, we consider that COAg could be a better indicator, at least for healthy older people. A decrease in the HPA regulation capability appears, even before the symptomatic phase of depression, which suggests that HPA dysregulation precedes depression (Holsboer, 2001). In fact, an increased cortisol release in response to high stress and low stress predicts an increase in depressive symptomatology (Morris et al., 2012). Thus, our results extend the knowledge about the effects of depressive symptomatology on the physiological stress response in the healthy older population.

Interestingly, our results showed that depression was related to a blunted cardiovascular response to stress, which partially confirms our results obtained through COAg, showing a predominance of the HPA response over ANS. As in our findings, previous studies showed a decreased

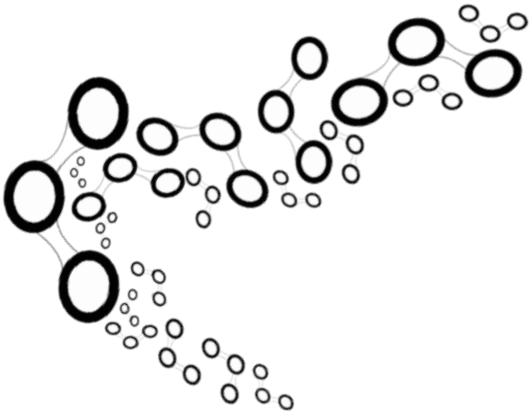
cardiovascular response in young (Schwerdtfeger & Rosenkaimer, 2011), adult or middle-aged (Rooij et al., 2010; Salomon et al., 2009), and older people with depression (York et al., 2007), as well as in large age samples (Carroll et al., 2007; Burke et al., 2005). These results agree with York et al. (2007), who argued that a blunted cardiovascular response in older depressed people would be due to a decrease in the sensitivity and density of adrenergic receptors. Along these lines, Ehrenthal et al. (2010) showed altered cardiovascular adaptability to stress in depressed people. By contrast, Matthews et al. (2005) showed no relationship between depression and parasympathetic shifts in a healthy population, and other studies have shown a greater parasympathetic decrease in people with more depressive symptoms (Light et al., 1998) and a greater sympathetic response in healthy people with higher depressive symptomatology (Hamer et al., 2007) or in depressed women with a previous history of childhood abuse (Heim et al., 2000). However, we have to take into account that healthy older people are characterized by showing a flattened sympathetic response (Carroll et al., 2007; Laitinen et al., 2004). It is possible that the effects of age on cardiovascular function (Nicolini et al., 2012; Laitinen et al., 2004) would be exacerbated by depression symptoms (York et al., 2007).

Surprisingly, we did not find a significant effect of neuroticism or depression on state anxiety or negative or positive affect changes after the task in older people. There is a large body of evidence of the relationship between neuroticism and negative mood in young people (Gomez et al., 2000; Rusting & Larsen, 1997; Larsen & Ketelaar, 1991, 1989). In this line, previous studies (Brindle et al., 2013; Salomon et al., 2009; Rooij et al., 2010) showed that depression could facilitate a heightened negative perception of a stressor in young, adult and middle-aged people. In our results, neuroticism and depression were not associated with stress- and non-stress-related changes in negative mood. We think age is an important factor to take into account because it may affect this relationship. The socioemotional selectivity theory argues that the perception of positive and

negative emotions and its regulation is age dependent (Carstensen et al., 1999). In fact, older people experience less negative emotions and improve their emotional regulation with age (Carstensen et al., 1999). Considering this explanation, and the fact that the population explored is non-clinical, we conclude that the improved emotional regulation in healthy older people may affect the relationships among neuroticism, depression, state anxiety and negative affect.

Finally, the present study has some limitations. First, the sample used is a healthy population; thus, we cannot extrapolate our conclusions to the clinical population, although the study of a sub-clinical population can help us to clarify the pathogenesis of affective disorders (Brooks & Robles, 2009). Moreover, a confounding effect of some unmeasured variable can never be completely ruled out (Christenfeld et al., 2004). Nevertheless, we tried to statistically account for an extensive range of potential confounders and carry out a conservative sample selection. Consequently, the number of participants was low, which limits the generalization of the results of our study, but the strict control of confounding factors provides a homogeneous healthy sample that allows us to observe the relationships among the variables more clearly. Moreover, our focus on older people extends previous knowledge explored only in young populations or poorly in older people. Finally, the study of behavioral responses and their relationship with neuroticism and the psychobiological response could be of interest in future studies on this topic, given the relevance of behavior in understanding coping strategies to deal with stressors (Sgoifo et al., 2003; Pico-Alfonso et al., 2007; Villada et al., 2014a).

In sum, in the present study we contributed to increasing the knowledge about the relationship between neuroticism, depression and the physiological stress response in older people, identifying a possible indicator of HPA and ANS response dysregulation in this population.



Chapter 4

Neuroticism and extraversion traits and their relationship with morning cortisol release.

Main results of the present chapter have been published in Puig-Perez, S., Pulopulos, M. M., Hidalgo, V., & Salvador, A. (2016). Are Neuroticism and extraversion related to morning cortisol release in healthy older peole? *International Journal of Psychophysiology.*

1. INTRODUCTION

There is a physiological response to waking characterized by an increase in cortisol concentrations, peaking between 30 and 45 minutes post-awakening (Stalder et al., 2016; Fries et al., 2009). This response is considered to be an indicator of cortisol rhythm regulation, as a part of normal healthy human circadian physiology. The sharp increase in cortisol after awakening has been defined as the dynamic of a post-awakening increase in cortisol levels (the cortisol awakening response, here in after CAR), usually measured through the area under the curve with respect to the increase (AUC_I) or through the difference in cortisol concentrations between the moment of awakening and 30-45 minutes later. Additionally, the overall morning cortisol production combines information about the cortisol levels just after awakening and the CAR, which can be assessed through the area under the curve with respect to the ground (here in after, AUC_G) (see Pruessner et al., 2003).

Some brain structures, such as the hippocampus, amygdala and prefrontal cortex, contribute to the regulation of the HPA-axis activity due to the high levels of expression of Glucocorticoid receptors there (Fries et al., 2009; Herman et al., 2005; Patel et al., 2000). Interestingly, neuroticism and extraversion have been considered important moderators of the age-related loss of volume and structural connectivity in the prefrontal cortex in older people, with higher neuroticism and lower extraversion being related to a greater age-related decline (Jackson et al., 2011).

For instance, higher neuroticism has been related to disorders and diseases associated with HPA-axis dysregulation, such as mild cognitive impairment (Kuzma et al., 2011), depressive and anxiety disorders (Ormel et al., 2013), Alzheimer's disease (Dar-Nimrod et al., 2012), chronic pain (Ramirez-Maestre & Esteve, 2013), and diabetes and metabolic syndrome (Mommersteeg & Pouwer, 2012). Given the importance of personality traits for health and their relationship with HPA-axis functioning (Lahey, 2009), several studies have investigated the role of neuroticism and extraversion in

CAR and AUC_G, showing mixed results. Hauner et al. (2008) reported a reduced AUC_G in adolescents with high neuroticism and introversion compared to those with low neuroticism and introversion. Hill et al. (2013) reported that higher extraversion predicted heightened AUC_G in people from 18 to 78 years old, whereas van Santen et al. (2011) found that a reduced CAR was associated with higher extraversion. However, no significant relationships were found between neuroticism and the CAR in the Hill et al. (2013) and van Santen et al. (2011) studies. By contrast, Portella et al. (2005) reported heightened CAR and AUC_G in people from 21 to 57 years old with high neuroticism compared to those with low neuroticism. These results agree with Mangold et al. (2012), who observed that people with low neuroticism and acculturation showed increased CAR compared to other groups (high neuroticism and acculturation, high neuroticism and low acculturation, low neuroticism and high acculturation) in adults from 18 to 38 years old. Finally, other studies reported no relationships between neuroticism or extraversion and CAR or AUC_G (e.g., Chan et al., 2007; Munafo et al., 2006; Laceulle et al., 2015).

Methodological issues may have contributed to these discrepancies in the results. None of the aforementioned studies used electronic devices to control for adherence in the cortisol measurements (Kudielka et al., 2003). Nor did they consider the variation in cortisol profiles (increase or decrease in cortisol levels immediately after awakening) (Almela et al., 2012; Thorn et al., 2006) or measure the CAR and AUC_G for at least two days, as recommended (Stalder et al., 2016). Thus, the lack of control over adherence to the protocol could result in a non-reliable CAR measurement, affecting the results (Stalder et al., 2016; Clow et al., 2010a, 2010b; Kudielka et al., 2003). Recently, no significant relationships between neuroticism and AUC_G and CAR were found in healthy young people when an electronic device was used to control saliva sampling times (Garcia-Banda et al., 2014). Another aspect to be considered is that these studies investigated the relationship between personality and CAR in adolescents, young people and/or samples with a broad age range (i.e., including

young adults and older people). Important changes have been reported in both neuroticism and extraversion (Eysenck, 1987) and CAR in older ages (see Fries et al., 2009; Clow et al., 2010a, 2010b); thus, age differences might affect the relationship between neuroticism, extraversion and CAR. However, no previous studies have analyzed this relationship specifically in older people.

Therefore, the present study aimed to investigate how neuroticism and extraversion traits are related to CAR and AUC_G in people aged 55-78 years old. To do so, 160 older participants collected three saliva samples during the first 45 min after awakening on two consecutive weekdays, and they completed the Eysenck Personality Questionnaire-Revised, short form (EPQ-RS). Based on previous literature, we expected to find positive relationships between AUC_G, CAR and extraversion (Hill et al., 2013; Hauner et al., 2008). Regarding neuroticism, we did not have any specific directional hypotheses, due to the contradictory results found by previous studies (Garcia-Banda et al., 2014; Hill et al., 2013; Hauner et al., 2008; Chan et al., 2007; Portella et al., 2005). Additionally, we aimed to explore the importance of sex in the relationship between neuroticism, extraversion and HPA-axis function (CAR and AUC_G), in order to add evidence to the reported data on sex differences (DeSoto & Salinas, 2015; Fries et al., 2009; Lynn & Martin, 1997).

2. MATERIAL & METHODS

2.1 Participants

People aged 55-78 years old were recruited through informative advertisements. The exclusion criteria were: smoking more than 10 cigarettes a day, consuming drugs of abuse, having surgery under general anesthesia during the past year, the presence of neurological or psychiatric disorders, the use of drugs that affect cognitive or emotional functions, or that influence HPA function (e.g. glucocorticoids, benzodiazepines, etc.). All the female participants were postmenopausal and not receiving hormonal replacement therapy.

The final sample was composed of 160 native Spanish speakers (81 men) from 55 to 78 years old (Total sample: 64, SEM = 0.353; Men: M = 64; SEM = 0.553; Women: M = 64; SEM = 0.439) with a medium subjective socioeconomic status (measured using the MacArthur Scale of Subjective Social Status; Adler et al., 2000; from 1: lowest, to 10: highest; Total sample: 5.99, SEM = 0.095; Men: M = 6.11, SEM = 0.149; Women: M = 5.87, SEM = 0.117). Most of them had an educational level beyond high school (84.4%) and were retired (88.8%). Regarding marital status, 66% were married, 10.1% single, 11.3% divorced and 12.6% widowed.

2.2 Procedure

The study was performed according to the Declaration of Helsinki, and the Ethics Committee of the University approved the protocol. All the participants received verbal and written information about the study and signed an informed consent.

Participants completed the Spanish version of the Eysenck Personality Questionnaire short form (EPQ-RS; Eysenck & Eysenck, 1997) to obtain scores on neuroticism and extraversion. Moreover, they provided 3 saliva samples on two consecutive weekdays. The samples were taken immediately after awakening (0) and 30 min (+30) and 45 min (+45) post-awakening. Additionally, they recorded in a log their awakening time and the time of each saliva collection. The participants were thoroughly instructed about how to provide the saliva samples, and they were also given detailed written instructions (for more details, see Almela et al., 2012).

2.3 Measures

2.3.1 Eysenck Personality Questionnaire-Revised (Eysenck & Eysenck, 1975)***.*** We used the Spanish version of the Eysenck Personality Questionnaire-Revised, short form (EPQ-RS; Eysenck & Eysenck, 1997). The EPQ-RS comprises a total of 48 items to which participants are asked to respond “yes” or “no”. It makes it possible to obtain scores for the three personality dimensions: neuroticism, extraversion and psychoticism. The

scales range from 0 to 12, with higher scores indicating more neuroticism, extraversion or psychoticism. The scales range from 0 to 12, with higher scores indicating more neuroticism, extraversion or psychoticism. The alpha values range from 0.65 to 0.82 for men, and from 0.67 to 0.82 for women.

2.3.2 ***Cortisol analysis*** Saliva was centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity. After that, the saliva was stored at -80°C until the assay was performed in duplicate by competitive solid phase radioimmunoassay (tube coated) with the commercial kit Spectria Cortisol RIA from Orion Diagnostica (Espoo, Finland). All the samples from each subject were analyzed in the same assay and in duplicate, with within- and inter-assay variation coefficients below 8%.

2.4 Data management and statistical analyses

Cortisol data were log transformed because they did not show normal distribution. The areas under the curve with respect to the ground (AUC_G: $(((S2_{+30}+S1) \times time_{S2-S1})/2) + (((S3_{+45}+S2_{+30}) \times time_{S3-S2})/2)$) and with respect to the increase (AUC_I: $AUC_G - (S1 \times \Sigma time)$) were calculated as measures of the AUC_G and CAR, respectively (see Pruessner et al., 2003).

Previous studies have reported the importance of ensuring the accuracy of CAR sampling, with it being crucial to take the first saliva sample right after awakening (Stalder et al., 2016; Clow et al., 2010a). Additionally, it has been indicated that self-reported sampling accuracy cannot be relied upon (Almela et al., 2012; Broderick et al., 2004; Kudielka et al., 2003), and that a lack of increase in cortisol levels after awakening might be due to a delay in the first saliva sample (Thorn et al., 2006), although undiagnosed pathologies unknown to the participant might also contribute to CAR disruptions (for more details see Stalder et al., 2016; Clow et al., 2010a). As the exclusion of participants with suspected inaccurate saliva sampling could result in a selection bias that would reduce the generalization of the results (Stalder et al., 2016), we considered the possibility of confirming the results for the complete sample in a subsample of participants who showed a positive CAR (> 0) on both days, following the

method described in Almela et al. (2012) and Thorn et al. (2006). A total of 98 participants (45 men) showed a positive CAR ($AUC_I > 0$) on two days (the 2-Day CAR group), 49 participants (26 men) showed a positive CAR on only one day, and 13 participants (10 men) did not show a positive CAR on any day (the 1 or 0-Day CAR group). ANOVAs were used to test age, SES, BMI, extraversion, neuroticism, and time of awakening between groups (2-Day CAR and 0 or 1-Day CAR). ANOVA for repeated measures was used to analyze the cortisol profile, with Time (0, +30 and +45) as a within-subject factor and group (2-Day CAR and 0 or 1-Day CAR) as between-subject factor. The average for each day was performed before analyzing the cortisol profiles through ANOVA for repeated measures. We used the Greenhouse-Geisser procedure because the requirement of sphericity in the ANOVA for repeated measures was violated. *Post-hoc* comparisons were performed using Bonferroni adjustments.

We tested the relationship between neuroticism and extraversion and awakening time through correlation analyses with (partial correlations) and without (bivariate correlations) controlling for sex and age. Linear regressions were conducted to explore whether neuroticism and extraversion traits were related to awakening cortisol concentrations (S1), CAR and AUC_G average of the two sampling days, with and without covariates. In step 1 we added sex, age and time of awakening as covariates due to their influence on the CAR (Fries et al., 2009), and in step 2 we added neuroticism or extraversion. In step 3 we added the interaction terms of neuroticism × sex or extraversion × sex in order to explore the possible moderator effect of sex in the relationship between neuroticism or extraversion and S1, CAR or AUC_G. With the aim of reducing multicollinearity, the regression analyses were performed separately for each personality dimension. Additionally, following Thorn et al. (2006) and Almela et al. (2012), the regression analyses were repeated in the subsample of participants who showed the CAR on both sampling days (2-Day CAR).

For analyses with AUC_G, two women (one in the 2-Day CAR group and one in the 0 or 1-Day CAR group) were excluded due to heightened cortisol values (> 3 S.D.). Regarding the CAR, one man in the 0 or 1-Day CAR group was excluded due to elevated cortisol values (> 3 S.D.). All *p*-values reported are two-tailed. SPSS 22 was used to perform the statistical analyses.

3. RESULTS

3.1. Cortisol awakening response profiles

There were no differences in age (2-Day CAR: *M* = 64.39, S.D. = 4.39; 0 or 1-Day CAR: *M* = 64.11, S.D. = 4.64), SES (2-Day CAR: *M* = 5.91, S.D. = 1.16; 0 or 1-Day CAR: *M* = 5.95, S.D. = 1.28), BMI (2-Day CAR: *M* = 27.10, S.D. = 3.99; 0 or 1-Day CAR: *M* = 27.26, S.D. = 3.42), extraversion (2-Day CAR: *M* = 6.28, S.D. = 1.65; 0 or 1-Day CAR: *M* = 6.36, S.D. = 1.82) or neuroticism (2-Day CAR: *M* = 3.84, S.D. = 1.97; 0 or 1-Day CAR: *M* = 4.17, S.D. = 1.85) between the 2-Day CAR and 0 or 1-Day CAR groups (all $p > 0.254$). Time of awakening was similar in the 2-Day CAR group on the two sampling days (Day 1 *M* = 7:13, S.D. = 0:55; Day 2 *M* = 7:16, S.D. = 0:54) and in the 0 or 1-Day CAR (Day 1 and 2 *M* = 7:19, S.D. for Day 1= 1:03 and for S.D. Day 2 = 1:02). There were no differences in time of awakening between the 2-Day CAR group and the 0 or 1-Day CAR group on any of the sampling days (both $p > 0.540$).

ANOVA showed Time ($F_{(1.4,\ 232.6)} = 144.691$, $p < 0.001$) and Time × Group ($F_{(1.4,\ 232.6)} = 133.133$, $p < 0.001$) effects. In the 2-Day CAR group, cortisol increased from awakening to 30 min later ($p < 0.001$) and maintained its levels 45 min after awakening (+30 vs. +45: $p > 0.999$). In the 0 or 1-Day CAR group, there were no significant differences between awakening cortisol (S1) and 30 min or 45 min after awakening (all $p > 0.292$), but there was a significant cortisol decrease from the +30 to +45 saliva samples ($p < 0.001$). In the 0 or 1-Day CAR group, S1 showed larger cortisol concentrations than in the 2-Day CAR group ($p < 0.001$), whereas in the +30 and +45 samples, the cortisol concentrations were lower than in the 2-Day CAR group (both $p < 0.001$) (see Table 1).

3.2. Relationships between personality traits and cortisol awakening response

Neuroticism and extraversion were not significantly related to time of awakening on any sampling day, with or without controlling for age and sex, for both the entire sample and the 2-Day CAR group alone (all p > 0.212).

Table 1	S1	+30	+45	AUC_I	AUC_G
2-Day CAR	7.46 (.46)	15.21 (.58)	14.87 (.52)	224.83 (128.09)	561.89 (212.43)
0 or 1-Day CAR	11.56 (.57)	12.18 (.73)	10.91 (.65)	13.406 (98.45)	543.43 (227.29)
Total Sample	9.10 (5.10)	13.94 (6.01)	13.22 (5.57)	143.72 (156.17)	557.77 (217.76)

Table 1. Mean and S.D. for S1, +30, +45, AUC_I and AUC_G values for 2-Day CAR group, 0 or 1-Day CAR group, and total sample. Values reported are expressed in raw data.

Results of linear regression analyses with neuroticism and extraversion as predictors of S1, AUC_I (CAR) and AUC_G after controlling for age and sex, as well as the moderator effect of sex in these relationships, are reported in Table 2[1].

[1] Additional regression analyses were performed in a sample composed of people who showed a positive CAR (AUC_I > 0) on both days (average of two days) and those who showed a positive CAR only one day (only with data of the day with positive CAR) (n = 147). These analyses were performed in order to reduce the bias of completely removing the suspected non-adherent saliva samplings (Stadler et al., 2016). Regression analyses with 2-Day CAR and 1-Day CAR (only with AUC_I > 0 data) showed that higher extraversion was marginally related to higher cortisol concentrations at S1 (β = 0.145, p = 0.077), whereas higher neuroticism was marginally related to lower cortisol at S1 (β = -0.158, p = 0.053). Sex did not moderate these relationships (both p > 0.142). As in results performed with the total sample and with the 2-Day CAR group, higher neuroticism was related to lower AUC_G regardless of sex (β = -0.219, p = 0.007), but extraversion was not related to AUC_G (β = 0.128, p = 0.121). Sex did not moderate these relationships (p = 0.435). Regarding CAR, neuroticism was not related to CAR (β = 0.036, p = 0.662), whereas higher extraversion was related to reduced CAR (β = -0.184, p = 0.024). Sex did not moderate the relationship between extraversion and CAR (p = 0.717), and although the Neuroticism × Sex interaction was significant (p = 0.049), in post hoc analyses it was not (both p > 0.096).

Table 2		**S1**				**AUC_G**				**CAR**			
		Total Sample		**2-Day CAR**		**Total sample**		**2-Day CAR**		**Total Sample**		**2-Day CAR**	
		(n = 160)		(n = 98)		(n = 158)		(n = 97)		(n = 159)		(n = 98)	
Step 1		**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***
	Awakening Time	0.239	**0.002**	0.125	0.222	0.195	**0.015**	0.089	0.389	-0.203	**0.010**	-0.166	0.106
	Sex	-0.164	**0.033**	-0.114	0.270	-0.032	0.686	-0.041	0.692	0.152	0.052	0.102	0.317
	Age	-0.029	0.707	-0.042	0.683	-0.082	0.301	-0.066	0.527	-0.038	0.627	-0.047	0.646
Step 2	Extraversion	R^2 = 0.098		R^2 = 0.043		R^2 = 0.055		R^2 = 0.021		R^2 = 0.073		R^2 = 0.041	
		ΔR^2 = 0.009		ΔR^2 = 0.013		ΔR^2 = 0.006		ΔR^2 = 0.006		ΔR^2 = 0.006		ΔR^2 = 0.001	
		$F_{(1, 155)}$ = 4.210		$F_{(1, 93)}$ = 1.035		$F_{(1, 153)}$ = 2.233		$F_{(1, 92)}$ = 0.491		$F_{(1, 154)}$ = 3.033		$F_{(1, 93)}$ = 0.987	
		β	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***P***
		0.095	0.215	-0.115	0.266	0.079	0.318	-0.081	0.438	-0.078	0.318	-0.038	0.711
Step 3	Sex × Extraversion	R^2 = 0.098		R^2 = 0.049		R^2 = 0.057		R^2 = 0.021		R^2 = 0.078		R^2 = 0.046	
		ΔR^2 < 0.001		ΔR^2 = 0.007		ΔR^2 = 0.001		ΔR^2 < 0.001		ΔR^2 = 0.005		ΔR^2 = 0.006	
		$F_{(1, 154)}$ = 3.361		$F_{(1, 92)}$ = 0.954		$F_{(1, 152)}$ = 1.820		$F_{(1, 91)}$ = 0.396		$F_{(1, 153)}$ = 2.755		$F_{(1, 92)}$ = 0.895	
		p = 0.794		p = 0.424		p = 0.643		p = 0.848		p = 0.357		p = 0.462	
		β	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***
	Men	0.118	0.308	-0.023	0.879	0.039	0.735	-0.102	0.503	-0.158	0.177	-0.122	0.428
	Women	0.077	0.455	-0.189	0.175	0.113	0.297	-0.062	0.667	-0.014	0.897	0.307	0.462
Step 2	Neuroticism	R^2 = 0.109		R^2 = 0.063		R^2 = 0.104		R^2 = 0.065		R^2 = 0.067		R^2 = 0.049	
		ΔR^2 = 0.020		ΔR^2 = 0.033		ΔR^2 = 0.055		ΔR^2 = 0.051		ΔR^2 < 0.001		ΔR^2 = 0.010	
		$F_{(1, 155)}$ = 4.764		$F_{(1, 93)}$ = 1.569		$F_{(1, 153)}$ = 4.420		$F_{(1, 92)}$ = 1.610		$F_{(1, 154)}$ = 2.767		$F_{(1, 93)}$ = 1.193	
		β	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***
		-0.144	0.061	-0.185	0.071	-0.235	**0.003**	-0.228	**0.028**	-0.006	0.934	0.099	0.337
Step 3	Sex × Neuroticism	R^2 = 0.113		R^2 = 0.074		R^2 = 0.104		R^2 = 0.066		R^2 = 0.092		R^2 = 0.112	
		ΔR^2 = 0.004		ΔR^2 = 0.010		ΔR^2 < 0.001		ΔR^2 = 0.001		ΔR^2 = 0.025		ΔR^2 = 0.063	
		$F_{(1, 154)}$ = 3.940		$F_{(1, 92)}$ = 1.461		$F_{(1, 152)}$ = 2.925		$F_{(1, 91)}$ = 1.287		$F_{(1, 153)}$ = 3.090		$F_{(1, 92)}$ = 2.324	
		p = 0.410		p = 0.313		p = 0.780		p = 0.806		**p = 0.043**		**p = 0.012**	
		β	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***
	Men	-0.072	0.531	-0.054	0.744	-0.211	0.071	-0.197	0.233	-0.186	0.114	-0.225	0.164
	Women	-0.200	0.052	-0.269	0.043	-0.255	**0.016**	-0.069	0.806	0.135	0.196	0.308	**0.019**

Table 2. Regression analyses with neuroticism and extraversion as predictor of AUC_G and CAR for the total sample and only for the 2-Day CAR group.

3.2.1. Awakening cortisol (S1) Regression analyses without controlling for covariates showed a negative relationship between neuroticism and S1 that was statistically significant for the entire sample (β = -0.160, p = 0.044) and marginally significant for the 2-Day CAR group (β = -0.198, p = 0.051).

After controlling for covariates, this negative relationship is marginally significant for both the entire sample (p = 0.061) and the 2-Day CAR group (p = 0.071) (see Table 2). Sex did not moderate these relationships (all p > 0.313) (see Table 2). Regression analyses did not show a significant relationship between extraversion and S1, with and without controlling for covariates, in the entire sample or in the 2-Day CAR group (all p > 0.213), and sex did not moderate this relationship (all p > 0.424) (see Table 2).

3.2.2. CAR Regression analyses with the entire sample and with the 2-Day CAR group showed no significant relationships between neuroticism, extraversion and the CAR (total sample: all p > 0.295; 2-Day CAR: all p > 0.245). These relationships remained non-significant after controlling for age, sex and time of awakening covariates (all p > 0.318) (see Table 2). Sex moderated the relationship between neuroticism and the CAR in both the total sample (p = 0.043) and the 2-Day CAR sample (p = 0.012). Regardless of the significance of the interaction, in the total sample, post hoc analyses did not show a significant relationship between neuroticism and the CAR in women (p = 0.196) or men (p = 0.114) (see Table 2). However, in the 2-Day CAR sample, neuroticism was positively related to CAR in women (p = 0.019), but not in men (p = 0.164). Regarding extraversion, sex did not moderate the relationship with CAR in the total sample or in the 2-Day CAR sample (all p > 0.357).

3.2.3. AUC_G Results showed a negative relationship between neuroticism and AUC_G in the entire sample and only in the 2-Day CAR group, without controlling for covariates (all p < 0.025) (see Table 2). These relationships remained significant when the analyses were performed controlling for covariates (both p < 0.028). Sex did not moderate any relationship between neuroticism and AUC_G in the total sample or in the 2-Day CAR group (all p > 0.780). Finally, extraversion was not related to AUC_G in the entire sample or the 2-Day CAR group analyses, with or without controlling for covariates (all p > 0.356). Sex did not moderate any relationship between extraversion and AUC_G in the total sample or the 2-Day CAR group (all p > 0.643) (see Table 2).

4. DISCUSSION

In the present study, we aimed to investigate the relationships between neuroticism, extraversion, the CAR and AUC_G in healthy older people. Our results showed that neuroticism was negatively related to cortisol concentrations just after awakening (S1) and to AUC_G, regardless of sex, but not to the CAR. However, after exploring the possible moderator effect of sex, our results showed a positive relationship between neuroticism and the CAR in women, but not in men. Finally, no significant relationships were found between the CAR or AUC_G and extraversion, where sex did not moderate any relationship.

Our results showed that higher neuroticism was associated with decreased AUC_G regardless of sex/gender, but not to the CAR. Agreeing with Pineles et al. (2013), neuroticism was also related to reduced cortisol concentrations immediately after awakening (S1). Thus, the relationship between neuroticism and decreased AUC_G is supported by the negative correlation between neuroticism and cortisol concentrations just after awakening, with these lower cortisol concentrations in the first sample driving the association between neuroticism and AUC_G. Thus, beginning with lower concentrations and maintaining a standard increase would result in a reduced AUC_G. In this line, Hauner et al. (2008) found decreased AUC_G in male adolescents with high neuroticism. Thus, the current results extend these findings to older people and are consistent, regardless of whether participants showing a negative CAR on one or both days were included in the analyses. In contrast to our study, a positive relationship was reported between neuroticism and AUC_G in people from 21 to 57 years old (Portella et al., 2005) and in adults from 18 to 38 years old, mediated by acculturation (Mangold et al., 2012). Together, these results may indicate that the association between neuroticism and AUC_G is negative in adolescents and older people, but positive in young-middle adults. This could be due to important changes in the prefrontal cortex in more extreme periods of the life-cycle, but not at young and middle ages. In adolescence, the prefrontal cortex is still developing, while at older ages

the prefrontal cortex is declining (for reviews, see Raz & Rodrigue, 2006; Sisk & Zehr, 2005). Specifically, we consider that age-related changes in the brain may explain the negative relationship between AUC_G and neuroticism observed in older people. Recent research has shown that a higher age-related decline in prefrontal regions is associated with greater neuroticism in middle-aged and older people (Jackson et al., 2011). Thus, because the prefrontal cortex is a key structure in HPA and CAR regulation (Fries et al., 2009), a greater age-related prefrontal cortex decline in older people with higher neuroticism could be related to a different regulation of cortisol secretion after awakening, that is, an overall lower cortisol secretion.

Coinciding with previous studies (Garcia-Banda et al., 2014; Hill et al., 2013; Laceulle et al., 2015), we did not observe a significant relationship between neuroticism and the CAR for men and women together. However, after considering sex as a possible moderator in this relationship, our results showed that neuroticism was positively related to the CAR in women, but not in men. In additional results performed with those who showed a positive CAR on two days and on only one day, sex also moderated the neuroticism and CAR relationship, but this relationship was only close to significance in women. Previous studies linked an enhanced CAR to increased physical and mental health problems, such as the rate of healing (Ebrecht et al., 2004), some types of depression (Dedovic & Ngiam, 2015), the progression of subclinical atherosclerosis (Eller et al., 2005) or coronary artery disease (Bhattacharyya et al., 2008). Taking all of the above into account, sex may moderate the damaging relationship between neuroticism and HPA functioning, with this relationship accentuated in women. Agreeing with DeSoto and Salinas (2015), our results confirmed a different sex pattern in the relationship between neuroticism and HPA functioning. It has been proposed that differences in HPA functioning between men and women could occur because of the sex differences in the negative feedback loop, which is stronger in women, and due to the possible interference of testosterone and estrogen in HPA functioning (DeSoto & Salinas, 2015). Moreover, sex-related differences in the

relationship between neuroticism and steroid hormones may also contribute to these differences. Along these lines, higher neuroticism has been related to lower testosterone in men (Obmiński et al., 2016), but higher concentrations in women (Barry et al., 2011). However, this relationship has not always been observed (Ekholm et al., 2014; Sellers et al., 2007; Conrad et al., 2002). Further research is clearly needed to better understand the neuroendocrinological mechanisms that underlie sex differences in the way neuroticism is related to HPA axis functioning.

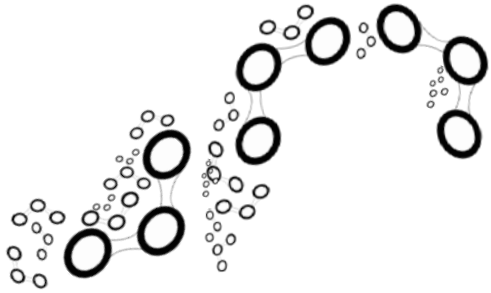
In line with previous literature (García-Banda et al., 2014; Munafo et al., 2006; Laceulle et al., 2015), in our study extraversion was not associated with the CAR or AUC_G for the complete sample or for the 2-Day CAR group. However, these results contrast with other data (Hill et al., 2013; Hauner et al., 2008). Two possible explanations may account for discrepancies in the literature. First, none of the previous studies focused specifically on older people (Hill et al., 2013; Hauner et al., 2008; Chan et al., 2007; Munafo et al., 2006; Laceulle et al., 2015; Portella et al., 2005); and age-related changes in the brain, HPA axis regulation (Clow et al., 2010b; Fries et al., 2009) and personality (Eysenck, 1987) could affect these associations. Another possible explanation is related to methodological differences. These studies measured cortisol levels only one day, an issue that could affect CAR measurements (Hellhammer et al., 2007). Along this line, a negative relationship between CAR and extraversion is observed in our sample when the analyses are performed including the CAR data only for days with positive CARs (2-Day CAR and 1-Day CAR; see footnote 1), in which, for part of the sample, only the data of one day of CAR measurement is included. However, this relationship did not remain significant for the complete sample or for the 2-Day CAR group. Thus the relationship observed between extraversion and CAR in our study and previous studies may be due to state confounders in CAR measurements (Hellhammer et al., 2007; Stalder et al., 2016). Moreover, our results for the complete sample and, especially, for the 2-Day CAR group, agree with Garcia-Banda et al., (2014), who employed good strategies for checking participants' adherence by using

an objective measurement of the saliva sampling. Although we did not use this procedure in our study, we made an effort to control for possible non-adherence to the salivary sampling protocol and increase the reliability of the CAR measurement. To do so, we employed strategies to increase adherence to the protocol (e.g. flexibility in choosing the days of saliva collection). Based on Almela et al. (2012) and Thorn et al. (2006), we collected saliva samples on two consecutive days (Clow et al., 2010a; Hellhammer et al., 2007), and we controlled for participants' possible non-adherence to the salivary sampling protocol by analyzing the cortisol secretion profile on both days (Almela et al., 2012; Thorn, et al., 2006). Most of these strategies were not used in previous studies (Hill et al., 2013; Van Sanden et al., 2011; Hauner et al., 2008; Chan et al., 2007; Munafo et al., 2006; Portella et al., 2005). Thus, non-adherence to the protocol may have resulted in a non-reliable CAR measurement that affected the results of these studies (Stalder et al., 2016; Clow et al., 2010a, 2010b).

A limitation of the current study is that we did not use an objective registering of the awakening time. Future studies using electronic devices to objectively register the awakening time can improve the reliability of awakening cortisol measurements and make it possible to delve into their relationship with personality traits, as suggested recently (Stalder et al., 2016). Instead, we employed different strategies that have been recommended in the recently published guideline consensus to improve CAR measurement reliability. First, we compared the results for the total sample to the results for participants suspected of adhering to the saliva sampling (2-Day CAR), as other studies have done (Almela et al., 2012; Thorn et al., 2006). Although it is true that this exclusion strategy only discards extreme cases of non-compliance (Stadler et al., 2016), it could help to reduce the bias. Additionally, to increase adherence to the protocol, we considered a large number of issues recommended in the recent guidelines for CAR assessment (Stadler et al., 2016), i.e. we used a self-report diary on saliva sampling, we tried to engage the volunteers with the goals of the research study, we emphasized the importance of the S1 sampling, we

gave detailed oral and written information about saliva sampling clearly explained what "the moment of awakening" means, and we provided full oral and written instructions about the sampling days and times and t undesired morning behaviors, among others. The CAR measure is still being clarified, and the physiological function of the CAR remains unclear (Clow et al., 2010b). Both reduced and heightened AUC_G and CAR have been related to health problems (Clow et al., 2010a; Fries et al., 2009), and so we still do not know what a heightened or reduced morning cortisol release really means. However, the development of studies that increase the knowledge about factors affecting the AUC_G and CAR could advance their understanding. In addition, this study is limited by its cross-sectional design; thus, causality cannot be inferred.

In conclusion, our results contribute to providing a clearer picture of the relationship between personality traits and the CAR and AUC_G in older people. Moreover, they confirm the moderator role of sex in the relationship between CAR and neuroticism, as recently reported (DeSoto & Salinas, 2015), and extend it to the older population. We found that, in this age group, neuroticism, but not extraversion, is related to less AUC_G secretion after awakening, regardless of sex, and to a heightened CAR only in women. These results contribute to furthering the knowledge about how neuroticism is related to HPA-axis functioning, which helps to better understand the underlying biological mechanism that relates neuroticism to health problems in the long and short term (Lahey, 2009).



Chapter 5

Optimism and the psychophysiological stress response in healthy older people.

Main results of the present chapter have been published in Puig-Perez, S., Villada, C., Pulopulos, M. M., Almela, M., Hidalgo, V., & Salvador, A. (2015). Optimism and pessimism are related to different components of the stress response in healthy older people. *International Journal of Psychophysiology*, *98*(2), 213-221.

1. INTRODUCTION

Throughout life, people have to deal with psychosocial stressors from many sources and with different durations. The sympathetic nervous system (SNS) and the hypothalamus–pituitary–adrenal (HPA) axis, which regulates cortisol release, are activated in response to physical and psychological stressors (Dickerson & Kemeny, 2004; Kudielka et al., 2004b). Moreover, psychological (e.g., increases in state anxiety and negative affect) and behavioral (such as displacement and submissive behaviors) changes are also associated with acute stress exposure (Villada et al., 2014a, 2014b). In their meta-analysis, Chida and Steptoe (2010) concluded that greater cardiovascular (CV) reactivity to and poor recovery from acute stress are related longitudinally to unwell/unsatisfactory CV status. They reported that higher future blood pressure was associated with greater reactivity and poor recovery, although other CV alterations were related differently, thus contributing to an increased future CV risk status.

Some age-related changes in the stress response have been reported, as older people show higher stress-induced cortisol release (Almela et al., 2011b; Otte et al., 2005), higher sympathetic tone (Almela et al., 2011b), a decline in autonomic regulation of cardiac dynamic capacity (Laitinen et al., 2004) and changes in emotional regulation (Carstensen, 1999; Mather & Carstensen, 2005), all of which may affect the ability to cope with stress (Pardon, 2007). However, aging is only one of many factors that can affect the stress response. In fact, consistent individual differences in stress systems have been reported (Lovallo, 2011).

Individual personality differences are related to differences in stress perceptions (Connor-Smith & Flachsbart, 2007), which can also affect the biological stress systems (Carver & Connor-Smith, 2010; Dickerson & Kemeny, 2004). Chida and Hamer (2008) concluded that negative psychological states, i.e. anxiety and hostility, are related to higher CV response and poorer recovery after stress, while positive psychological states or traits (e.g. happiness, self-enhancement) are associated with

reduced HPA axis reactivity. Given the important relationship between the acute stress response and health, the study of stress-related factors that can modulate this relationship acquires great relevance, especially in the most vulnerable life periods, such as old age. Specifically, interest in the relationship between personality characteristics and physical health has increased considerably in the past few decades (Rasmussen et al., 2009).

Optimism, a very influential trait in the way people perceive and conduct their lives (Carver et al., 2010), has been related to wellbeing and several stress-related diseases, such as metabolic syndrome (Cohen et al., 2010; Roy et al., 2010), cancer (Friedman et al., 1992; Rajandram et al., 2011) and cardiovascular diseases (Nabi et al., 2010; Tindle et al., 2010). One of the most widely used questionnaires to measure optimism is the «Life Orientation Test» (LOT) (Scheier & Carver, 1985), which was later revised (LOT-R) (Scheier et al., 1994). The LOT-R measures dispositional optimism, which involves generalized outcome expectancies. Originally, it was considered to be one-dimensional, with optimism and pessimism at opposite poles that showed a strong negative correlation in young people (Scheier & Carver, 1985). Thus, optimistic people tend to have positive generalized expectations about their future, while pessimists have negative expectations (Scheier & Carver, 1992; Scheier et al., 1994). However, other studies in middle-aged and older people revealed low shared variance between optimism and pessimism (Mroczek et al., 1993; Plomin et al., 1992; Robinson-Whelen et al., 1997), leading to their consideration as independent factors. Some theoretical formulations (Diener et al., 1999; Ryff & Singer, 1998) defended the independence of positive and negative mental states, based on several studies that reported this independence (e.g. Lai, 1994, 1997; Lai et al., 2005). Other authors have proposed that this age-related difference may be due to changes in metacognitive beliefs about optimism and pessimism, allowing people to adaptively use either of them to cope in different situations (Herzberg et al., 2006).

Research focusing on the relationships between optimism and the physiological stress response to acute stress is sparse and has shown mixed

results. In young adults, Solberg Nes et al. (2005) reported an increased cortisol and HR response in participants who scored higher on dispositional optimism and self-consciousness. Moreover, Brydon et al. (2009) observed faster cortisol recovery after stress in young men with more dispositional optimism. These results suggest that the presence of optimistic beliefs affects HPA and sympathetic activity, along with self-consciousness, highlighting the protective role of optimism, given that slower recovery after stress can have negative health consequences (McEwen, 1998; Sapolsky et al., 2000). Therefore, optimism has been considered positive in the long run, given that, as behavioral self-regulation theory establishes (Carver & Scheier, 2000), an optimistic assessment of the situation produces confidence and facilitates the capacity to maintain an effort to achieve goals, increasing positive affect and wellbeing (Solberg Nes et al., 2005). By contrast, negative evaluations would increase a sense of doubt and facilitate the removal and disengagement impulse, reducing behaviors of effort (Carver & Scheier, 2000). Previous studies showed that optimism is related to better goal-readjustment (Aspinwall & Richter, 1999; Duke et al., 2002), which raises the hypothesis that the relationship between optimism and a person's quality of life depends on reengaging in new goals when valuable goals become unreachable (Rasmussen et al., 2006). Thus, the way people generate expectations would affect the perception of the situation, which in turn would have an influence on the psychophysiological response to acute stress.

However, this possible effect of optimism on the acute stress response has hardly been studied in older people. To the best of our knowledge, only one study has examined this relationship. Endrighi et al. (2011) analyzed the relationship between dispositional optimism and the cortisol awakening response (CAR), daily cortisol release and stress-induced cortisol release after exposure to two laboratory stress tasks (computerized color-word and mirror tracing task) in older people. These authors observed lower CAR in optimists, suggesting that dispositional optimism may have a protective role. However, unlike the studies with

young people mentioned above, they did not observe significant relationships between dispositional optimism and acute stress-induced and daily cortisol release in older people. Thus, optimism seems to have a relationship with HPA-axis activity in older people, but not specifically with the stress-induced cortisol response. However, it should be noted that in this study, the authors considered dispositional optimism, and not optimism and pessimism separately, which seem to be different dimensions in older people (Herzberg et al., 2006). Thus, it would be advisable to consider them as two separate dimensions in order to more closely examine the relationships among optimism, pessimism and the stress response in older people.

With this in mind, the purpose of this study was to investigate how optimism and pessimism are related to the HPA and HR response, as well as situational appraisal, in a situation of acute social stress in older people. Healthy volunteers from 55 to 76 years old were exposed to a psychosocial stressor (Trier Social Stress Test, TSST) or a control task in order to study their stress-induced cortisol, heart rate (HR) response and situational appraisal. Based on previously mentioned results, we expected a positive relationship between optimism and HPA and HR reactivity (Solberg Nes et al., 2005), but also faster recovery (Brydon et al., 2009) after the stressful task, given that more optimistic people are better able to manage stress and overcome it successfully (Carver et al., 2010). On the other hand, we expected a negative relationship between pessimism and HR and cortisol recovery from the acute stress. Some authors have suggested that the stress response depends greatly on the way the event is interpreted (Salvador & Costa, 2009), so that the situational appraisal gains importance. Coinciding with Endrighi et al. (2011) we expected lower stress perceptions in optimistic people exposed to stress, as well as higher stress perceptions in pessimistic people. Additionally, in order to better understand the effect of these personality traits on the perception of the situation, we explored the effect of optimism and pessimism traits on the perception of how difficult, frustrating or important the stress task was, and how much effort it required.

2. MATERIAL & METHODS

2.1. Participants

For subject recruitment, informative advertisements were displayed on the University campus, especially directed to students of La Nau Gran, a study program for people over 55 years old. The exclusion criteria were: smoking over 10 cigarettes a day, abuse of alcohol or other drugs of abuse, severe vision or hearing problems, presence of severe CVD, an illness that involves HPA disturbance, and neurological or psychiatric disorders. Subjects were also excluded if they were being medicated with drugs related to cognitive or emotional functions, or with an influence on hormonal levels or cardiovascular function (such as glucocorticoids or b-blockers), or if they consumed psychotropic substances. All the female participants were postmenopausal, and none of them were receiving estrogen replacement therapy. Subjects who met the criteria were contacted by telephone and asked to participate in the study.

The final sample was composed of 72 participants randomly assigned to two conditions: 38 to the stress condition (19 men) and 34 to the control condition (17 men). 87.5% of the participants were students in the La Nau Gran program, and 12.5% were referred by these students (acquaintances, relatives or friends).

The study was performed according to the Declaration of Helsinki, and the Ethics Committee of the university approved the protocol. All participants received verbal and written information about the study, signed an informed consent form, and received a gift worth 15€ for their collaboration.

2.2. Procedure

In this study, each subject participated in an individual session lasting approximately 1h and 30 minutes (between 16 and 20h) in a laboratory at the School of Psychology. Upon their arrival at the laboratory, the height

and weight of the participants were measured in order to calculate the Body Mass Index (BMI), and the experimenter verified that they had followed the instructions given previously: sleep as long as usual, refrain from heavy activity the day before, and not consume alcohol since the night before. Additionally, they were instructed to drink only water and not eat, smoke or take any stimulants 2h prior to the session.

2.2.1. Stress Condition We employed the Trier Social Stress Test (TSST, Kirschbaum et al., 1993; Kudielka et al. 2007) to provoke cortisol and cardiovascular responses (Almela et al., 2011a, 2011b; Hidalgo et al., 2012). The task consisted of 5 min of free speech (job interview) and a 5 min arithmetic task (serial subtraction), performed in front of a committee composed of a man and a woman. Interactions with participants were always performed by the committee member of the opposite sex. During the 5 minutes of free speech, the participants had to report what characteristics make them the best candidate for a fictitious position as a representative in the university. If the participant did not use up the 5 minutes, the committee asked a set of standardized questions about the participant's characteristics. In the case of the arithmetic task, the participant performed a serial subtraction, and the committee interrupted and urged the participant to start the subtraction again after each mistake. The participants remained standing at a distance of 1.5m from the committee. Moreover, a video camera and a microphone used to film both tasks were clearly visible.

As *figure 1* shows, the protocol started with a *baseline period* of 25 min to allow the participants to adapt to the laboratory setting. At the beginning of this phase, the experimenter placed the HR recording system on the participant, who completed the LOT-R, and the first salivary cortisol sample was taken. After the baseline, the *introduction phase* began (5 min), where participants were informed about the procedure for the stress task (instructions) in front of the committee in the same room where the task would take place. Next, the participants had 3 minutes to prepare the speech task (*preparation phase*). Then, the *stress phase* (TSST, speech plus

arithmetic tasks) was carried out, and a saliva sample was collected. Finally, during the *recovery phase*, subjects had 55 min to recover, during which they answered several questionnaires, including the situational appraisal, and collected 5 saliva samples at 10 min intervals after the termination of the task.

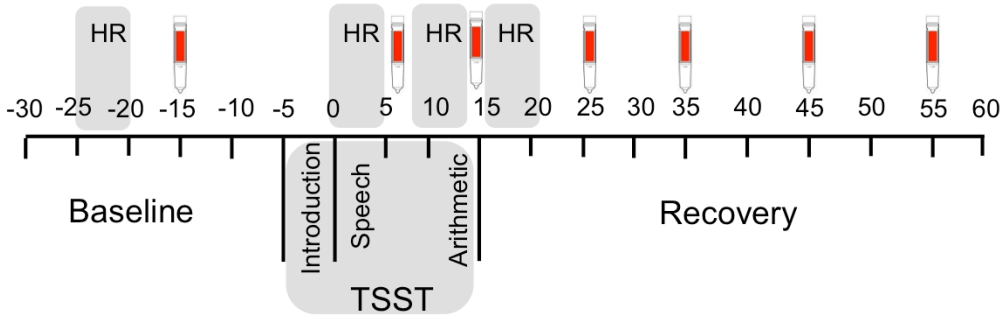


Figure. 1. Timeline of the stress and control conditions, with the 7 saliva samples for cortisol measurements, and the 5 min periods for HR measurements.

2.2.2. Control condition This condition followed the same schedule as the stress condition, but the TSST was replaced by an ad hoc control task that included a free speech task (5 min) and an arithmetic task (5 min), but without a committee. During the free speech, the participant talked aloud about a recent non-emotional experience, while the arithmetic task consisted of a counting by five aloud. The participants performed the free speech and arithmetic tasks in the same room as the stress task, but none of the stressful elements were present (video camera, microphone and committee), and the participants were informed before the task that their performance would not be recorded. This control task has been used in previous studies (see Pulopulos et al., 2013, 2015; Hidalgo et al., 2015), and was designed to maintain similar overall physical activity and mental workload, but without evaluative threat and uncontrollability, the main components capable of provoking stress (Dickerson & Kemeny, 2004).

2.3. Assessment and Measures

2.3.1. Life Orientation Test Revised *(LOT-R;* Scheier et al., 1994*).* This is a 10 item questionnaire answered on a 5-point Likert scale. Three items measure optimism (i.e., "In uncertain times, I usually expect the best"), and three other items measure pessimism (i.e., "If something can go wrong for me, it will"); the remaining items are distractors. This questionnaire provides a measure of optimism and pessimism or a total score of dispositional optimism, depending on whether it is considered as a two-dimensional or one-dimensional measure, respectively. We employed the Spanish version (Otero et al., 1998), which has shown adequate reliability (α = 0.75) (Ferrando et al., 2002).

2.3.2. Perceived Stress Scale (PSS, Cohen et al., 1983). This is a 14-item scale that measures the degree to which life situations are evaluated as stressful during the past month; the scores range from 0 to 40, with higher scores indicating more perceived stress. A Spanish version of the PSS shows an adequate internal consistency (α = 0.81), and it was used to adjust for background stress (Remor, 2006).

2.3.3. Situational Appraisal Participants completed five questions on a 5-point Likert scale (not at all = 1 to extremely = 5) after the stress/control task, concerning: stress, difficulty, frustration, effort and how important the task had been to them (e.g. How much effort did the task require?). These questions were developed based on previous studies (Baggett et al., 1996; Gonzalez-Bono et al., 2002), taking into account the Lazarus coping model (Lazarus & Folkman, 1984) and behavioral self-regulation theory (Carver & Scheier, 2000), which have been employed in previous studies (Costa & Salvador, 2012; van der Meij et al., 2010).

2.3.4. Heart Rate (HR) Data for HR were continuously recorded throughout all the sessions using a Polar©RS800cx watch (Polar CIC, USA), which consists of a chest belt and a Polar watch. The transmitter is located on the chest belt, which is placed on the solar plexus and transmits HR information to the receiver (Polar watch). The Polar watch records R-R

intervals with a sampling frequency of 1000 Hz, providing a time resolution of 1ms for each R-R interval. The data collected by the Polar watch were downloaded and stored in the Polar ProTrainer5™ program in the computer, and they were analyzed using Kubios Analysis (Biomedical Signal Analysis Group, University of Kuopio, Finland). We analyzed the HR in 5-minute periods during baseline (-25 to -20 min), speech (0 to +5 min), arithmetic (+7 to +12 min) and recovery (+15 to +20), all with respect to the beginning of the stress or control task. In the HR analysis, one woman (control condition) was excluded due to technical problems.

2.3.5. Cortisol A total of 7 saliva samples were collected using salivettes (Sarstedt, Nümbrecht, Germany) to measure cortisol levels during the session. The timing of the saliva sampling was 15 min before the participants were exposed to the stress or control task (-15), between the speech and arithmetic task (+5), and at intervals of 10 min after the termination of the task (+15, +25, +35, +45 and +55 min). Participants were instructed to introduce the cotton swab of the salivette in their mouths for exactly 2 minutes, not chew the cotton, and move the swab in a circular pattern around in their mouths to collect saliva from all the salivary glands (see Nater & Rohleder, 2009). Samples were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was frozen at -80°C until the analysis took place. The samples were analyzed in the Central Research Unit at the University by a competitive solid phase radio immune assay (tube-coated), using the commercial kit Spectria Cortisol RIA (cat. Nu 06119) from Orion Diagnostica (Espoo, Finland). All the samples were analyzed in the same trial and in duplicate; assay sensibility was 0.8 nmol/L; and the inter- and intra-assay variation coefficients were all below 8%. One woman (control condition) was excluded because her cortisol values differed more than 3 S.D. from those of the rest of the subjects.

2.4. Statistical Analysis

ANOVA with condition (stress vs. control) and sex (men vs. women) as between-subject factors was used to explore differences in

demographics (socioeconomic status and age), anthropometric characteristics (body mass index), and psychological data (PSS, optimism and pessimism). We used a MANOVA with condition and sex as between-subject factors to explore differences in situational appraisal items (stressful, frustrating, difficult, effort and important). Cortisol data were square root transformed before the analyses because they showed abnormal distribution on the Kolmogorov-Smirnov test. For cortisol analyses, a repeated-measures ANOVA was calculated with condition and sex as between-subject factors, and time (-15, +5, +15, +25, +35, +45 and +55 min) as within-subject factor. HR data were analyzed using ANOVA for repeated measures, with condition and sex as between-subject factors and time (baseline, speech, arithmetic, recovery) as within-subject factor.

For the HR analyses, we also established the index of (i) reactivity$_{HR}$ (maximum value to stressful task minus baseline period) and (ii) recovery$_{HR}$ (recovery period minus baseline period) to measure the increase and the complete return to baseline levels. For cortisol, we calculated two indexes: (i) reactivity$_{C}$ (maximum cortisol levels of +15, +25 or +35 min after the stressful task minus previous -15 min cortisol levels) and (ii) recovery$_{C}$ (maximum cortisol levels of +15, +25 or +35 minus minimum cortisol levels of +45 or +55 min). The latter index is used as a measure of the capacity to reduce cortisol levels in a specified period of time. The recovery$_{C}$ index was not calculated because of time limitations in the return to baseline levels.

To study the relationships among the psychological variables, Spearman's Rho correlation was used to test the relationship between optimism and pessimism, given that the Kolmogorov-Smirnov test revealed abnormal distribution in these variables. Pearson correlations were performed to test the relationship between situational appraisal items and cortisol and HR (reactivity and recovery indexes). Finally, we employed hierarchical regression analysis (Aiken & West, 1991) to investigate whether optimism and pessimism were related to HR and cortisol (reactivity and recovery indexes) and situational appraisal.

We used the Greenhouse-Geisser procedure when the requirement of sphericity in the Repeated-Measures ANOVA was violated. Post hoc planned comparisons were performed using Bonferroni adjustments. All *p*-values reported are two-tailed, and the level of significance was set at $p<.05$. When not otherwise specified, results shown are means (M) ± standard error of means (SEM). We used SPSS 22.0 to perform the statistical analysis.

3. RESULTS

3.1. Sample Characteristics

ANOVA did not show significant effects of condition (stress vs. control) or sex (men vs. women) on age (total sample: $M = 63.99$, SEM = 0.479), socioeconomic status (SES) ($M = 5.58$, SEM = 0.138), body mass index (BMI) ($M = 26.86$, SEM = 0.401) or PSS (M = 16.55, SEM = 0.866) (all $p > 0.05$).

We analyzed optimism and pessimism separately because: i) we did not find a significant correlation between optimism (M = 11.60, SEM = 0.244) and pessimism (M = 7.08, SEM = 0.286) (*rho* = -0.023, $p = 0.849$); and ii) the previously mentioned literature shows that these traits may be independent (Mroczek et al., 1993; Lai, 1994, 1997; Lai et al., 2005; Plomin et al., 1992; Robinson-Whelen et al., 1997), at least in the aging population. No significant effects of condition were found on optimism and pessimism (stress vs. control: all $p > 0.550$). Sex did not show significant effects on optimism either ($F_{(1, 70)} = 0.933$, $p = 0.338$), although women showed higher pessimism than men ($F_{(1, 70)} = 5.273$, $p = 0.025$).

3.2. Situational appraisal

We observed a condition effect on the situational appraisal completed by the participants after the task: people exposed to the TSST perceived it as more stressful ($F_{(1, 66)} = 53.114$, $\eta^2 = 0.446$, $p < 0.001$), frustrating ($F_{(1, 66)} = 52.819$, $\eta^2 = 0.445$, $p < 0.001$), difficult ($F_{(1, 66)} = 72.823$, $\eta^2 = 0.525$, $p < 0.001$) and involving more effort ($F_{(1, 66)} = 40.731$, $\eta^2 = 0.396$, $p < 0.001$) than

those exposed to the control situation. However, the stress task was perceived as being as important as the control task ($F_{(1, 66)} = 2.601$, $\eta^2 = 0.038$, $p = 0.112$).

Table 1

	$Reactivity_{HR}$		$Recovery_{HR}$		$Reactivity_{C}$		$Recovery_{C}$	
	Stress	**Control**	**Stress**	**Control**	**Stress**	**Control**	**Stress**	**Control**
Effort	*r* = -0.025	*r* = -0.149	*r* = -0.056	*r* = -0.210	*r* = -0.195	*r* = 0.113	*r* = -0.207	*r* = 0.088
Frustration	*r* = -0.094	*r* = -0.221	*r* = -0.032	*r* = -0.203	*r* = 0.010	*r* = 0.004	*r* = -0.206	*r* = 0.089
Stressful	*r* = 0.019	*r* = 0.036	*r* = -0.123	*r* = -0.099	*r* = 0.088	*r* = 0.217	*r* = 0.017	*r* = 0.158
Difficulty	*r* = -0.104	*r* = 0.182	*r* = -0.118	*r* = -0.144	*r* = -0.064	*r* = 0.036	*r* = -0.104	*r* = 0.112
Importance	*r* = -0.055	*r* = -0.177	*r* = -0.154	*r* = -0.089	*r* = 0.020	*r* = 0.107	*r* = -0.017	*r* = 0.003

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 1. Correlation analyses performed between situational appraisal items and HR and cortisol reactivity and recovery indexes. All $p > 0.10$.

There were no significant effects of sex on the situational appraisal items (all $p > 0.459$). However, the sex × condition interaction had a significant effect on effort ($F_{(1, 66)} = 4.424$, $\eta^2 = 0.063$, $p = 0.039$). Post hoc analyses showed that women exposed to the TSST perceived that the task required more effort than the women in the control group ($p < 0.001$) and the men exposed to the TSST ($p = 0.045$). No sex differences were found for the control task (all $p < 0.338$).

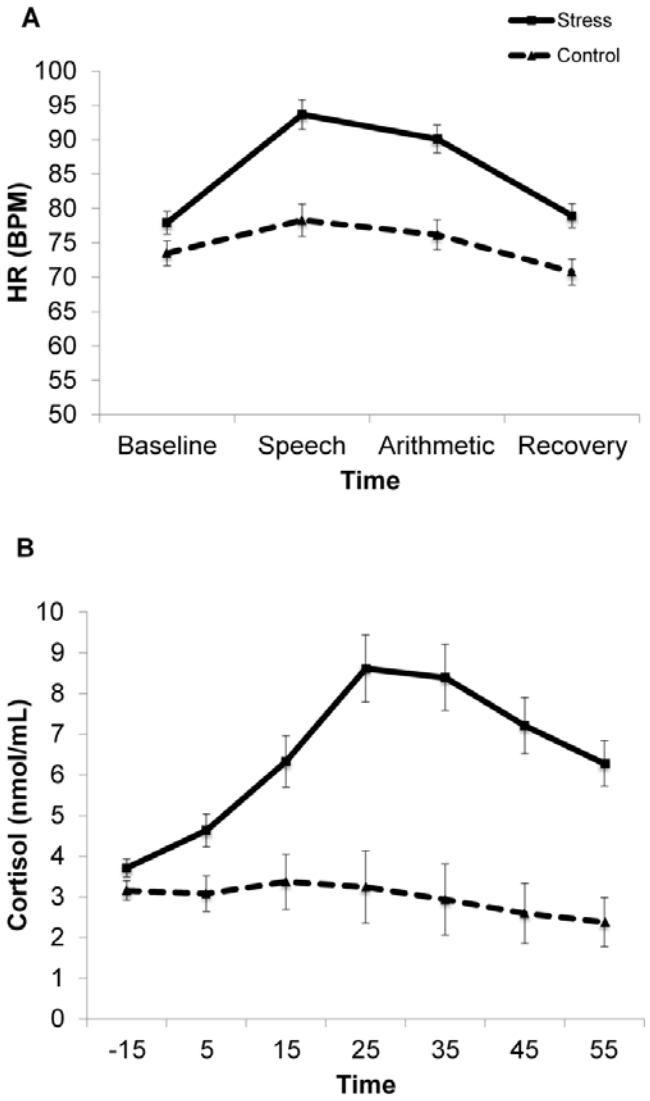


Figure 2. Means of heart rate (A) and salivary cortisol concentration (B) (±SEM) in stress and control conditions.

Correlation analyses did not show any relationship between situational appraisal items and cortisol and HR reactivity or recovery indexes in the stress or control condition (see Table 1, all $p > 0.163$).

3.3. Physiological response

3.3.1. Heart Rate Main effects of time ($F_{(1.63, 109.23)} = 106.116$, $\eta^2 = 0.613$, $p < 0.001$), condition ($F_{(1, 67)} = 15.635$, $\eta^2 = 0.189$, $p < 0.001$) and the time × condition interaction ($F_{(1.63, 109.23)} = 22.755$, $\eta^2 = 0.254$, $p < 0.001$) were found, but there were no significant effects of sex or its interactions (all $p < 0.426$). Post hoc analyses showed that participants in the stress condition showed significantly higher HR during the speech, arithmetic and recovery (all $p < 0.002$) periods, except at baseline ($p = 0.073$) (see Figure 2A).

3.3.2. Cortisol ANOVA for repeated measures showed significant effects of time ($F_{(2.18, 146.21)} = 17.208$, $\eta^2 = 0.204$, $p < 0.001$), condition ($F_{(1, 67)} = 26.731$, $\eta^2 = 0.285$, $p < 0.001$), sex $F_{(1, 67)} = 14.392$, $\eta^2 = 0.177$, $p < 0.001$), time × condition ($F_{(2.18, 146.21)} = 21.799$, $\eta^2 = 0.245$, $p < 0.001$) and time × sex ($F_{(2.18, 146.21)} = 6.754$, $\eta^2 = 0.092$, $p = 0.001$). The time × sex × condition interaction approached significance ($F_{(2.18, 146.21)} = 2.825$, $\eta^2 = 0.040$, $p = 0.058$). Post hoc analyses showed that participants in the stress condition showed significantly higher levels of cortisol in all samples (+5, +15, +25, +35, +45 and +55 min; all $p < 0.004$), except at baseline ($p = 0.061$). Generally, men showed higher cortisol levels than women ($p < 0.001$). In the stress condition, men showed higher cortisol levels than women at baseline and in the rest of the samples (all $p < 0.025$). However, no significant differences between men and women were found in the control condition (all $p > 0.110$). There were no significant differences between the stress and control conditions in baseline cortisol levels for men and women (-15: $p > 0.155$). Higher cortisol levels in the stress condition than in the control condition were found in all samples after the beginning of the task (+5, +25, +35, +45 and +55) for men (all $p < 0.010$). For women, higher cortisol levels in the stress condition than in the control condition were found in +25, +35, +45 and +55 (all $p < 0.035$), but not in +5 ($p = 0.121$) (see Figure 2B).

Table 2

	HR REACTIVITY				HR RECOVERY				
	R^2	F	*p*	β	R^2	F	*p*	β	T
Step 1	0.350	8.892	< 0.001		0.402	11.090	< 0.001		
Age				-0.016			**0.005**	0.284	0.97
Sex				-0.016				0.039	0.95
BMI				0.134			**0.002**	0.318	0.91
Condition			< 0.001	-0.562			< 0.001	-0.407	0.99
Step 2	0.377	7.873			0.408	8.975	0.403		
Pessimism			0.098	-0.176				0.086	0.87
Step3	0.419	7.694	**0.036**		0.427	7.959	0.151		
Optimism				**-0.208**				-0.140	0.97
Step 4	0.420	6.522	0.727		0.450	7.363	0.112		
Condition*Optimism				0.195				0.873	0.03
Stress			0.088	-0.244			**0.033**	**-0.298**	
Control			0.204	-0.175			0.945	0.009	
	CORTISOL REACTIVITY				**CORTISOL RECOVERY**				
	R^2	F	*p*	β	R^2	F	*p*	β	T
Step 1	0.402	11.094	< 0.001		0.295	6.583	< 0.001		
Age				-0.156				-0.116	0.97
Sex			**0.001**	**-0.349**			**< 0.001**	**-0.485**	0.95
BMI				0.069				-0.164	0.92
Condition			**< 0.001**	**-0.502**			**0.013**	**-0.264**	0.99
Step 2	0.403	8.766	0.782		0.295	5.192	0.926		
Pessimism				0.028				-0.010	0.89
Step3	0.405	7.259	0.629		0.309	4.551	0.255		
Optimism				0.048				0.121	0.97
Step 4	0.411	6.268	0.442		0.362	4.696	**0.026**		
Condition*Optimism				-0.434				-1.331	0.03
Stress			0.376	0.126			**0.017**	**0.363**	
Control			0.848	-0.026			0.461	-0.106	

Table 2. Regression analyses performed with optimism as predictor of HR and cortisol response to stress and control task. F values refer to the ANOVA of the model, and T to tolerance values. $p < 0.05$ are marked in bold.

3.4. Relationships among optimism, pessimism and physiological activation[1]

We performed regression analyses to test whether optimism and/or pessimism played a role in HR and HPA reactivity and recovery, and whether these relationships were different for the stress and control conditions. In step 1, we added age, BMI, sex and condition as covariates because of their relationship with HR and cortisol release. In step 2, we added pessimism or optimism when the predictor in the following step was optimism or pessimism, respectively. In step 3, we added the optimism or pessimism score. Based on Aiken and West (1991), in step 4 we added the condition × optimism or condition × pessimism interaction when the predictor was optimism or pessimism, respectively.

Optimism was negatively related to reactivity$_{HR}$ (β = -0.208, p = 0.036) in the entire sample. Therefore, people with high scores on optimism presented less HR reactivity to a stress or control task (*see Table 2*). Regarding recovery$_{HR}$, the condition × optimism interaction approached significance (p = 0.112), showing that higher optimism scores were related to faster recovery$_{HR}$ (β = -0.298, p = 0.033) in the stress condition, but not in the control condition (β = 0.009, p = 0.945).

Regarding cortisol indexes, the condition × optimism interaction was significant for recovery$_{C}$ (p = 0.026), showing a positive relationship between optimism and recovery$_{C}$ (β = 0.363, p = 0.017) in the stress condition. That is, higher scores on optimism were related to faster cortisol

[1] Additional regression analyses with dispositional optimism were performed for the psychological stress response, adding age, BMI, sex and condition as covariates in step 1 and dispositional optimism in step 2. Based on Aiken and West (1991), in step 3 we added the condition × dispositional optimism interaction. Our results did not show significant relationships between dispositional optimism and any of the HR or cortisol indexes, or the condition × dispositional optimism interaction (all p > 0.173).

recovery after exposure to the TSST. This relationship was not significant in the control condition (β = -0.106, *p* = 0.461) (*see Table 2*).

Finally, pessimism did not show any relationships with HR or the cortisol indexes (all *p* < 0.073) (*see* Table 3).

Table 3

	HR REACTIVITY				HR RECOVERY				
	R^2	F	*p*	β	R^2	F	*p*	β	T
Step 1	0.350	8.892	< 0.001		0.402	11.090	< 0.001		
Age				-0.016			**0.005**	0.284	0.97
Sex				-0.016				0.092	0.95
BMI				0.134			**0.002**	0.318	0.91
Condition			**< 0.001**	-0.562			**< 0.001**	-0.407	0.99
Step 2	0.389	8.270	**0.047**		0.422	9.487	0.139		
Optimism				-0.200				-0.143	0.97
Step3	0.419	7.694	0.073		0.427	7.959	0.439		
Pessimism				-0.187				0.079	0.87
Step 4	0.420	6.529	0.698		0.429	6.758	0.680		
Condition*Pessimism				-0.126				0.134	0.09
Stress				-0.152				0.116	
Control				-0.230				0.034	
	CORTISOL REACTIVITY				**CORTISOL RECOVERY**				
	R^2	F	*p*	β	R^2	F	*p*	β	T
Step 1	0.402	11.094	< 0.001		0.295	6.908	< 0.001		
Age				-0.156				-0.116	0.97
Sex			**0.001**	**-0.349**			**< 0.001**	**-0.485**	0.95
BMI				0.069				-0.102	0.92
Condition			**< 0.001**	-0.502			**0.013**	-0.264	0.99
Step 2	0.404	8.815	0.638		0.309	5.825	0.250		
Optimism				0.046				0.122	0.97
Step3	0.405	7.259	0.763		0.309	4.780	0.978		
Pessimism				0.031				-0.003	0.89
Step 4	0.416	6.399	0.289		0.311	4.065	0.696		
Condition*Pessimism				0.346				-0.138	0.09
Stress				-0.063				0.035	
Control				0.153				-0.052	

Table 3. Regression analyses performed with pessimism as predictor of HR and cortisol response to stress and control task. F values refer to the ANOVA of the model, and T to tolerance values. *p* < 0.05 are marked in bold.

Table 4

	Stressful				Frustration				Effort				Difficult				Importance				
	R^2	F	*p*	β	R^2	F	*p*	β	R^2	F	*p*	β	R^2	F	*p*	β	R^2	F	*p*	β	T
Step 1	.444	17.568	<.001		.422	16.316	<.001		.401	14.923	<.001		.528	25.004	<.001		.044	1.016	.391		
Age				-.079				-.022				-.152				.006				-.081	.99
Sex				.046				.063				.054				.064				<.001	.99
Condition			<.001	-.661			<.001	-.646			<.001	-.613			<.001	-.723				.191	.99
Step 2	.461	13.925	.151		.428	12.363	.401		.441	13.041	**.031**		.535	19.001	.322		.265	1.247	.173		
Pessimism				.141				.084				**.215**				.089				.174	.88
Step3	.465	11.140	.499		.432	9.872	.543		.442	10.280	.902		.538	15.141	.531		.301	1.291	.235		
Optimism				-.063				-.058				-.012				-.054				.144	.97
Step 4	.477	9.566	.246		.432	8.115	.823		.447	8.635	.416		.565	13.876	.049		.303	1.082	.728		
C*Optimism				.625				.121				.447				-.949				.238	.03
Stress				.048				-.080				.067			.332	.116				.101	
Control				-.167				-.037				-.087			.067	-.218				.185	
Step 1	.444	17.568	<.001		.422	16.316	<.001		.401	14.923	<.001		.528	25.004	<.001		.044	1.016	.391		
Age				-.079				-.022				-.152				.006				-.081	.99
Sex				.046				.063				.054				.064				<.001	.99
Condition			<.001	-.661			<.001	-.646			<.001	-.613			<.001	-.723				.191	.99
Step 2	.449	13.247	.441		.426	12.245	.509		.416	11.051	.804		.532	18.725	.493		.061	1.068	.274		
Optimism				-.072				-.063				-.024				-.059				.133	.98
Step3	.478	14.658	.136		.432	9.872	.425		.457	10.280	**.034**		.538	15.141	.086		.090	1.291	.151		
Pessimism				.168				.080				**.214**				.344				.183	.88
Step 4	.491	12.702	.286		.434	8.164	.642		.473	9.094	.142		.578	14.632	**.016**		.091	1.072	.797		
C*Pessimism				-.324				-.146				-.453				.674				.102	.09
Stress				.226				.120			**.011**	**.339**			**.021**	**.270**				.155	
Control				.024				.030			.684	.059			.262	-.143				.218	

Table 4. Regression analyses performed with optimism and pessimism as predictors of situational appraisal in stress and control conditions. C ∗ optimism and C ∗ pessimism refer to condition × optimism and condition × pessimism interactions respectively. F values refer to the ANOVA of the model, and T to tolerance values. $p < 0.05$ are marked in bold.

3.5. Relationships among optimism, pessimism and situational appraisal[2]

Regression analyses were used to analyze the relationship between optimism and pessimism and the situational appraisal. In step 1, we added sex, age and condition as covariates. In step 2, we added the score on optimism or pessimism when the predictor in the following step was pessimism or optimism, respectively. In step 3, we added the optimism or pessimism score; and in step 4, we added the condition × optimism or condition × pessimism interaction to analyze the possible effect of condition in the relationship.

Regarding optimism, regression analyses did not show significant relationships between optimism and the perception of the stress task as more stressful, frustrating, difficult, requiring more effort or important when considering both conditions together (all $p > 0.235$). The interactions between condition and optimism were not significant (all $p > 0.246$), except for difficulty ($\beta = -0.949$, $p = 0.049$). However, the post hoc analyses did not show a significant relationship between optimism and difficulty perceived in any condition (both $p > 0.067$) (*see* Table 4).

By contrast, higher pessimism was related to perceiving that the task requires more effort ($\beta = 0.214$, $p = 0.034$). The condition × pessimism interaction was significant for difficulty ($p = 0.016$). Post hoc analyses showed that participants with higher pessimism perceived the stress task as more difficult ($\beta = 0.270$, $p = 0.021$). This relationship was not significant for the control condition ($p = 0.262$) (*see* Table 4). Although the condition ×

[2] Additionally, regression analyses with dispositional optimism as predictor and situational appraisal items (stressful, frustrating, difficult, effort and important) as dependent variables were performed, adding sex, age and condition as covariates in step 1, dispositional optimism in step 2, and the condition × dispositional optimism interaction in step 3. Our results did not show any significant relationships between dispositional optimism and the situational appraisal items, or condition × dispositional optimism interactions (all $p > 0.092$).

pessimism interaction was not significant for effort, participants with higher pessimism perceived that the stress task required more effort (β = 0.339, *p* = 0.011). This relationship was not significant for the control condition (*p* = 0.684) (*see* Table 4).

4. DISCUSSION

The main purpose of this study was to investigate the role of optimism and pessimism in HR and HPA response in a controlled stressful situation. Moreover, we aimed to explore the relationship of these personality traits with the situational appraisal of the situation. To do so, healthy people aged 55 or older were randomly distributed into two groups and exposed to a standardized psychosocial stressor or to a non-stressful situation. First, we confirmed the stressful nature of the procedure, as exposure to the TSST produced changes in HR and cortisol release. At the same time, subjects assessed the TSST as more stressful, frustrating, difficult, and involving more effort than the control task. Second, and more importantly, in the stressful situation, we found that optimism was related to faster recovery of cortisol levels. Finally, pessimism did not affect the HR or HPA function, but it was related to the perception of the stress task as more difficult and requiring more effort.

The exposure to the TSST elicited higher cardiovascular activity, confirming previous results in the older population (Almela et al. 2011b; Kudielka et al., 2004b), but with no sex differences. We also showed that the TSST is potent enough to induce significant increases in cortisol levels in both sexes (Almela et al., 2011b; Kudielka et al., 2004a; Strahler et al., 2010). All these results confirm that the stress task used was able to produce an ANS and HPA-axis response. Finally, higher cortisol baseline and response to stress was found in men than in women, as in previous studies (Almela et al., 2011b; Strahler et al., 2010).

Regarding the relationship between personality and the physiological stress response, we observed a different pattern of relationships depending on whether optimism or pessimism was considered. Recently, Endrighi et al. (2011) reported that high dispositional optimism was related to lower perceived stress, but not to the stress-induced cortisol response in older people. Nonetheless, in the young population, high dispositional optimism was related to higher HR and cortisol reactivity (Solberg Nes et al., 2005) and faster cortisol recovery after stress (Brydon et al., 2009). Agreeing with Endrighi et al. (2011), who studied a similar sample, our results did not show any relationship between dispositional optimism and the physiological stress response in older people. However, when we explored the role of the optimism and pessimism factors separately, following the recommendations of Rasmussen et al. (2009), we found that optimism was related to faster recovery of cortisol levels afterwards, although we did not find significant relationships between pessimism and the physiological stress response. Thus, our results are in line with Carver et al. (2010), who suggested that analyzing optimism and pessimism separately can result in a better prediction of outcomes in some situations. Moreover, previous studies suggest that these traits are independent in older people (Mroczek et al., 1993; Plomin et al., 1992; Robinson-Whelen et al., 1997). For these reasons, we have obtained a clearer picture of their importance in the stress response in older people and in foreseeable health consequences. Furthermore, it should be noted that differences in the nature of the stressor could also contribute to explaining the discrepancies between previous studies and our results. Previous studies employed a cognitive stressor (Endrighi et al., 2011; Solberg Nes et al., 2005), a kind of stress task in which increases in arousal could be adaptive for better performance on the task. Instead, in our study the stressor involves a social threat. Therefore, it is possible that an increase in arousal could be less adaptive and diminish the performance on the task. Thus, the context could play a determinant role in the way optimism affects the response to stress. Further research is needed to test this idea.

It is worth noting that a delayed return to baseline levels of cardiovascular function is currently thought to provoke increases in the risk of developing cardiovascular diseases (Esch et al., 2002; Heponiemi et al., 2007). Additionally, a delayed cortisol recovery is related to an increase in the risk of CVD development (Esch et al., 2002) and other stress-related pathologies, such as depression or type 2 diabetes (Gragnoli, 2012). According to the McEwen model (1998), repeated or sustained stimulation results in *allostatic load,* which disrupts the dynamic responses to acute challenges or stress, resulting in impaired reactivity and recovery. Thus, the exposure to repeated acute stress has adverse consequences for health, due to an inadequate restoration of the stress response. In other words, a slow reduction in cortisol concentrations after stress results in adverse health consequences due to disruptions in the cardiovascular, metabolic, immune and nervous systems (McEwen, 1998). The HPA capacity to turn off after stress is reduced in aging (Aguilera, 2011). Therefore, factors that preserve the correct functioning of the negative-feedback effect of cortisol, such as optimism, become more important in older people.

Consequently, our findings suggest a protective role of optimism on health (Carver et al., 2010). At least in older people, positive expectations may contribute to generating an adaptive physiological adjustment, which would have a protective effect against stress-related diseases. This faster physiological recovery could be due to the fact that people with higher optimism take an active approach to problem solving, which is considered more adaptive coping (Scheier, et al. 1994; *for review*: Solberg Nes & Segerstrom, 2006). Furthermore, further research is clearly needed to more thoroughly investigate whether the relationship between optimism and stress-induced physiological reactivity is different for young and older people.

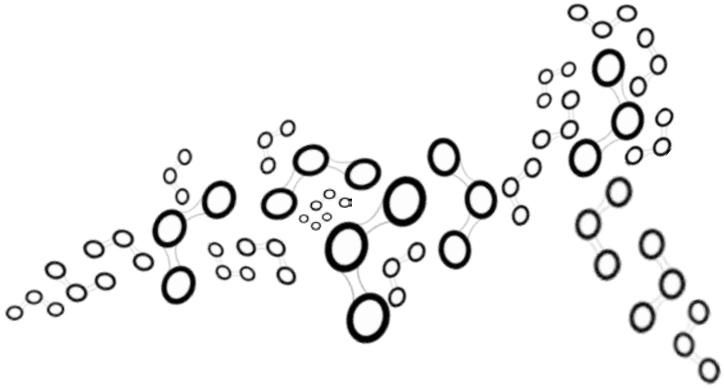
Regarding the relationship between personality and situational appraisal, we observed that pessimism, but not optimism, was related to the situational perception. Endrighi et al. (2011) observed similar results, showing higher stress perception 20 and 45 min after stress in older people

with low scores on dispositional optimism. Although our results showed that dispositional optimism did not predict the situational appraisal, analyzing the role of optimism and pessimism separately we observed that pessimism was related to perceiving more effort, regardless of the condition, and to more perceived difficulty in the stress condition. As we mentioned previously, studying the optimism and pessimism subscales separately might provide greater knowledge about their functions (Carver et al., 2010; Rasmussen et al., 2009), especially taking their independence in middle-aged and older people into account (Mroczek et al., 1993; Robinson-Whelen et al., 1997; Plomin et al., 1992). However, assuming that a one-dimensional perspective considers pessimistic people to be those with lower scores on dispositional optimism, our results agree with Endrighi et al. (2011). Some authors have proposed that erroneous stress perception and the tendency to use maladaptive coping strategies (Chico, 2002) make pessimists more vulnerable to health problems than optimists (Carver et al., 2010).

This study has several limitations that need to be addressed in order to properly interpret its results. In this study, we cannot determine causality, but we have made an effort to obtain a very homogenous sample and eliminate a number of possible confounding factors. However, the strict selection of participants increases the difficulty of generalizing our results to the general population. Moreover, due to the relevance of the return to baseline after a stressful event for health (Esch et al., 2002; Gragnoli, 2012; Heponiemi et al., 2007) and its relationship with optimism (Carver et al., 2010), we think it should be given greater importance in future studies. Clearly, more studies are needed to establish the relationship between optimism and the HPA axis and the way it can change across the life span. To the best of our knowledge, this is the first study to relate HR, cortisol release and situational appraisal to optimism and pessimism separately in stressful situations in healthy older people. Finally, it should be noted that we made an effort to reduce the possible confounding factors in order to have a clearer picture of the relevance of optimism and pessimism in the

psychophysiological response to stress. Unfortunately, we did not measure the coping styles, which could be a mediator in this relationship (see Solberg Nes & Segerstrom, 2006). Another interesting factor to take into account is the socioeconomic position, given its important role in the physiological response (Castro-Diehl et al., 2014). In future studies, it would be interesting analyze the effects of these factors in the relationships between optimism and pessimism and the psychophysiological stress response.

In sum, we conclude that the relationships found contribute to clarifying the different mechanisms underlying stress-related disorders, due to: (i) the role of optimism in the physiological response in stressful situations; (ii) the importance of recovery in pathological processes; and (iii) the prevalence of disorders and pathologies in aging. In conclusion, our results emphasize that the optimism trait plays a significant role in cardiovascular and endocrine function, and that their protective effect on health could be especially relevant in the aging population. This personality trait, and probably the associated coping style, would contribute to an enhanced quality of life, reducing the development of diseases and, in turn, the personal and social costs associated with pathological aging.



Chapter 6

Optimism, morning cortisol release and past life review

Main results of the present chapter are under review in Puig-Perez, S., Pulopulos, M. Hidalgo, V., & Salvador, A. Being an Optimist or a Pessimist and its Relationship with Morning Cortisol Release and Past Life Review in Healthy Older People.

1. INTRODUCTION

The way we imagine future events has an important role in having a successful life. With this in mind, Carver and Scheier (2000) developed the self-regulation behavior theory, which defines optimism and pessimism as generalized versions of confidence and doubt related to future events in life. According to these authors, optimism and pessimism are considered stable dispositions, that is, generalized ways of generating expectations in a positive or negative way, respectively (Scheier & Carver, 1992; Carver et al., 2010). Moreover, the ways we generate our expectations may affect our physical and mental health (for more details see Carver, et al., 2010).

In recent decades, interest in the relationship between the personality and underlying physiological mechanisms that predispose one to health and disease has increased. (Rasmussen et al., 2009). More specifically, influenced by Positive Psychology, the relationship between optimism and pessimism and the functioning of the hypothalamic-pituitary-axis (HPA), one of the most important physiological mechanisms in the response to external demands, has attracted researchers' attention (Puig-Perez et al., 2015; Jobin et al., 2013; Endrighi et al., 2011; Lai et al., 2005).

An index of HPA axis functioning is the cortisol awakening response (CAR), which has been described as the increase in cortisol levels from awakening to 30-45 min after waking (see Stalder et al., 2016). The CAR has been related to psychosocial correlates that are important for health, such as chronic stress, work strain, and loneliness (Chida & Steptoe, 2009), but only a few studies have focused on the relationship between CAR and optimism, showing mixed results. In young people, Lai et al. (2005) found that optimism is related to a lower CAR, whereas Ebrecht et al. (2004) reported no significant relationships. Regarding older adults, Endrighi et al. (2011) confirmed that optimism was related to a lower CAR. However, a more recent study specified that this relationship is significant only in people who reported a high stress perception on the sampling day, but not in those perceiving normal stress (Jobin et al., 2013).

In addition, optimism and pessimism may be related not only to physiological mechanisms, but also to cognitive function. In this regard, Buckner and Carroll (2007) found that episodic and spatial autobiographical memories have common underlying component processes with future prospections. In fact, some evidence reveals an overlap between the brain regions involved in both personal future prospections and autobiographical memory (Spreng & Grady, 2010; Spreng et al., 2009; Addis et al., 2007). These studies indicate that the generation of expectations and the recall of personal information may be closely related. Therefore, it makes sense to expect a relationship between personality, mood states, and the recall of experienced past events. Seligman's assumption (1991) claims that expectations about the future stem from people's view of the past events. That is, the way we perceive our past experiences could affect our perception of how capable we are of coping with present and future events. Following this line of thinking, it is also possible that people who hold positive expectations about their future evaluate their past by focusing on positive happenings from the past and avoiding negative ones. This could contribute in turn to a more positive perception of their ability to cope with present and future life events, and it could partly explain why optimistic people cope and adjust better to life events (for a review, see Carver et al., 2010). In fact, several studies have shown that generating positive expectations is related to an adaptive coping style (Solberg Nes & Segerstrom, 2006) and greater persistence on difficult tasks (Solberg Nes et al., 2005; Segerstrom, 2001). Therefore, we consider that the perception of the past may be another important aspect to take into account in the way optimism and pessimism affect our lives.

Some authors have suggested that there are age differences in the purpose of recalling personal experienced events (Webster & McCall, 1999). However, aging changes not only the purpose of recalling personal experienced events, but also the way this is done. Carstensen et al. (1999) indicated in their socioemotional selectivity theory that aging is characterized by a positive bias that involves an increase in attention to

and recall of positive memories. They suggested that, for older people, the future takes a backseat in their lives, decreasing their interest in unpleasant or future needs and increasing the importance of the present. Moreover, the change in the perception of the remaining time from extensive to limited has a strong impact on one's life review (Carstensen et al., 1999), which may reflect the ability of older people to appreciate life, putting past events in perspective (Webster & McCall, 1999). Along these lines, several studies have shown the close relationship between the process of recollecting personal memories and the evaluation of negative past experiences (life review) as key factors in death acceptance (Cappeliez et al., 2007; Webster & Cappeliez, 1993), which could have a great impact on psychological wellbeing.

In sum, the generation of negative or positive future prospections would affect HPA functioning and past life perception, with both having important roles in physical and mental wellbeing, especially in older people. With all of this in mind, the aim of the present study was to investigate the relationship between optimism and pessimism and the cortisol awakening response (CAR) and the past life review in healthy older people. To do so, optimism and pessimism were measured in 76 healthy older volunteers (from 56 to 77 years old) who were instructed to perform a task that consisted of writing a short story about their life, highlighting the most important events, impressions and experiences. Additionally, the participants provided cortisol saliva samples on two consecutive weekdays to assess their morning cortisol concentrations in the home setting. Several studies have found that people with greater optimism show behavioral and cognitive processes that facilitate resilience, good adjustment to life's adversities, and wellbeing (Carver et al., 2010). We consider that having an optimistic point of view may provide a more positive perception of one's own life, and the opposite would be true for pessimism. Thus, we expected that people with high optimism would describe more positive facts, cognitions, and emotions, whereas higher pessimism would be associated with a more negative life-story. Finally, regarding the relationship between

optimism, pessimism, and HPA functioning, we expected to confirm a lower CAR in people with high optimism (Endrighi et al., 2011; Lai et al., 2005) and a higher CAR in people with high pessimism.

2. MATERIAL AND METHODS

2.1. Participants

In all, 76 participants (40 men) from 56 to 77 years old were recruited using informative advertisements. Exclusion criteria were: consuming alcohol or other drugs of abuse, having severe vision problems, diseases related to HPA disruptions (e.g. diabetes), and neurological or psychiatric disorders. Moreover, the volunteers medicated with drugs related to HPA, cognitive, or emotional function (benzodiazepines, beta-blockers, etc.), or psychotropic substances, were also excluded. All female participants were postmenopausal, and none of them were receiving estrogen replacement therapy.

Subjects were contacted by telephone and invited to participate in the study, which was performed according to the Declaration of Helsinki. The Ethics Committee of the University approved the protocol. All participants received verbal and written information about the study and signed an informed consent. At the end of the study, the participants received a gift worth 15€.

2.2. Procedure

The data from the present paper are part of the MNEME Project. Participants were invited to join a large study in which they participated in one experimental session (described in detail in Puig-Perez et al., 2015) where dispositional optimism (LOT-R) was measured. When the participants finished the session at our laboratory, they were instructed by the

experimenters to write a Life Story and answer a set of questionnaires, including the Perceived Stress Scale (PSS, Cohen et al., 1983).

Additionally, they provided a total of 6 saliva samples on two consecutive weekdays during the morning (just on awakening and 30 and 45 minutes after waking) at home. The participants recorded their awakening time and the time of each saliva collection in a log, they were thoroughly instructed about how to provide the saliva samples, and they were also given detailed written instructions (for more details, see Almela et al., 2012). The salivettes were stored in MEMS TrackCap containers (MEMS 6 TrackCap Monitor, Aardex Ltd., Switzerland) to objectively check the sampling time and participants' adherence to our instructions.

2.3. Assessment and Measures

2.3.1. Life Orientation Test Revised (Scheier et al., 1994). The Spanish version of the LOT-R (Otero et al., 1998), with optimism and pessimism subscales, was used to measure dispositional optimism. LOT-R is composed of 10 items on a 5-point Likert scale. 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. The three items on the pessimism subscale were previously inverted to add them to the three items that measure optimism and obtain a total score, with higher scores indicating higher dispositional optimism. Separate scores from the optimism and pessimism subscales have also been used in previous studies (see Carver et al., 2010). The use of separate scores in addition to the total score was recently recommended (Rasmussen et al., 2009; Ferrando et al., 2002), especially in older people, given the independence of the subscales in aging (Puig-Perez et al., 2015; Herzberg et al., 2006). The total scale has shown adequate reliability and validity ($\alpha = 0.75$), and so have its subscales (Optimism: $\alpha = 0.72$; Pessimism: $\alpha = 0.71$).

2.3.2. Perceived Stress Scale (PSS, Cohen et al., 1983). The Spanish version of the PSS (Remor, 2006) was used as a measure of perceived stress during the last month, and it shows adequate reliability and validity (α=0.81). This scale consists of 14 items with a 5-point Likert scale (0 = *never* to 4 = *very often)*. After reversing items 4, 5, 6, 7, 9, 10 and 13, a total score is obtained by adding the reversed items to the rest of the items on the scale, with higher scores indicating higher perceived stress.

2.3.3. Hormonal Analyses Saliva was centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity. After that, the saliva was stored at -80°C until the assay was performed in duplicate by competitive solid phase radioimmunoassay (tube coated) with the commercial kit Spectria Cortisol RIA from Orion Diagnostica (Espoo, Finland). All the samples from each subject were analyzed in the same assay and in duplicate, with within- and inter-assay variation coefficients below 8%.

2.3.4. Life Story The participants were instructed to write a summary of their lives on a sheet of paper, highlighting the events, cognitions, and emotions that they considered most important to them. They were instructed to do this accurately, relaxing in their own homes, and taking into account that their analyzed data would be completely masked. The number of events, cognitions, and emotions in the Life Stories were counted with regard to emotional valence, according to the following four categories: i) Positive Events (PE), ii) Positive Cognitions-Emotions (PCE), iii) Negative Events (NE), and iv) Negative Cognitions-Emotions (NCE). To avoid false attributions of emotionality, ambiguous or neutral valence items were omitted. The outcomes are the total scores for PE, PCE, NE and NCE registered in each life story.

Three judges (one man and two women, age M = 24.67 SEM = 1.764) were trained to analyze participants' life stories. The judges had had no previous contact with the participants, and they did not know the purpose of the study. Additionally, the instructions for analyzing the stories were

given separately to each judge, and they analyzed all the life stories separately with no contact between them. The agreement between the three judges was measured by an intra-class correlation coefficient (ICC) for each category, showing high agreement in PE (ICC = 0.923), PCE (ICC = 0.835), NE (ICC = 0.963) and NCE (ICC = 0.881).

Given that the judges' agreement was high in each category, we then averaged the judges' scores on PE, PCE, NE and NCE for each participant, obtaining one score for each category per participant. After that, we computed the total number of events reported, regardless of the valence, and then we computed the percentage corresponding to PE and NE from the total number of events reported. Moreover, we computed the total number of cognitions and emotions reported, regardless of the valence, and we estimated the percentage corresponding to PCE and NCE from the total number of cognitions and emotions. These percentages of PE, NE, PCE and NCE were used in the analyses in the present study.

2.4. Statistical Analysis[1]

T-tests were performed to explore sex differences in age, socioeconomic status (SES), perceived stress, optimism, pessimism, and life story outcomes (total events; total cognitions and emotions; and percentages of PE, PCE, NE and NCE reported). Chi-square was used to explore sex differences in educational level, work activity, and marital status.

[1] Additional analyses were performed with dispositional optimism. The entire sample (n = 76) was split in two groups by the mean, based on dispositional optimism scores: low dispositional optimism (n = 36, 19 men), and high dispositional optimism (n = 40, 21 men). To test the effect of dispositional optimism on the percentage of PE and NE, a repeated-measures ANOVA was performed with high and low dispositional optimism groups as between-subject factors, valence (positive vs. negative) as within-subject factor, and age reported as covariate. One-way ANOVAs were performed with high and low dispositional optimism groups as between-subject factors, and S1, AUC_G and AUC_I as within-subject factors.

Some theories (Diener et al., 1999; Ryff & Singer, 1998) defend the independence of positive and negative mental states. Moreover, previous research suggested that optimism scores may be independent from pessimism scores in middle-aged and older people (e.g. Lai, 1994, 1997; Lai et al., 2005; Mroczek et al. 1993; Plomin et al., 1992; Robinson-Whelen, 1997).

For these reasons, and following the recommendations of Rasmussen et al. (2009), we also explored the effect of the optimism and pessimism subscales separately. Therefore, we first divided the entire sample according to optimism, and later according to the pessimism subscale by mean split: high and low optimism (high optimism: n = 45, 21 men; low optimism: n = 31, 19 men), and high and low pessimism (high pessimism: n = 31, 14 men; low pessimism: n = 45, 26 men).

To test the effects of optimism and pessimism on the percentage of PE and NE reported in the life stories, repeated-measures ANOVA were performed, with high and low optimism and pessimism groups as between-subject factors, and valence (positive vs. negative) as within-subject factor. The same analyses were performed using the percentages of PCE and NCE as dependent variables.

Previous literature has highlighted the importance of accurate CAR sampling (Stalder et al., 2016; Clow et al., 2010a). After exploring our sample, two patterns of CAR were identified, and so the participants were classified according to their CAR profile pattern. A total of 26 participants (17 men) showed a positive CAR on 1 or 0 days of sampling (1 or 0 Day-CAR), whereas 50 of them (23 men) showed a positive CAR on both days (2 Day-CAR). A delay in the first saliva sample after awakening could lead to blunted CAR registering (Stalder et al., 2016), but it is also true that undiagnosed pathologies unknown to the participant may contribute to CAR disruptions (for more details see Stalder et al., 2016; Clow et al., 2010a). Thus, the exclusion of participants with suspected inaccurate sampling could result in a selection bias, which would reduce the generalization of the results (Stalder et al., 2016). For these reasons, we decided to employ a method used and discussed recently (Stalder et al., 2016; Puig-Perez, 2016b;

Almela et al. 2012; Thorn et al. 2006), which consists of confirming the results of the complete sample and the subsample of participants who showed the CAR on both days. Repeated-measures ANOVAs were performed to analyze the cortisol profiles, with time (awakening, 30 min and 45 min after awakening) and group (1 or 0 Day-CAR vs. 2 Day-CAR) as within and between-subject factors, respectively. Cortisol data were log transformed because they did not show normal distribution.

Following Pruessner et al. (2003), the areas under the curve with respect to the increase (AUC_I) and with respect to the ground (AUC_G) were calculated, with AUC_I considered as CAR and AUC_G as the total morning cortisol concentrations. One-way ANOVAs were performed with the optimism and pessimism groups as between-subject factors and cortisol concentrations just after awakening (S1), AUC_G and AUC_I as within-subject factors. One woman was excluded from AUC_G measures and one man from AUC_I measures because both showed more than 3 S.D. One woman was excluded from all the cortisol analyses because she showed more than a 5-minute delay between self-reported awakening sampling and the objective measure of awakening sampling.

Finally, Pearson correlations were performed to test the possible relationships between the life story categories (PE, NE, PCE and NCE) and morning cortisol release (S1, AUC_G and CAR). Additionally, partial correlations between these variables after controlling for sex and age were also reported.

Bonferroni adjustments for *p*-values were used for *post hoc* planned comparisons. All *p*-values reported are two-tailed, with the level of significance marked at < 0.05. A 95% Confidence Interval (CI) and η^2 were reported. Results shown are means ± standard error of means (SEM) when not otherwise specified. We used SPSS 22.0 to perform the statistical analyses.

3. RESULTS

3.1. Preliminary Results

3.1.1. General Sample Characteristics.

The mean age of the sample was 65.03 years old (SEM = 0.513), with a medium subjective socioeconomic status (measured using the MacArthur Scale of Subjective Social Status, SES; Adler et al., 2000) of 5.61 (SEM = 0.127) and low perceived stress during the past month of 16.17 (SEM = 0.748). The educational level was beyond high school for 80.2% of the participants, and 82.7 % were retired. Regarding marital status, 72.4 % were married, 6.6% divorced, 11.8 % widowed, and 9.2 % single. No sex differences were found in age ($t_{(74)} = 0.128$, $p = 0.899$), socioeconomic status (SES: $t_{(73)} = 1.464$, $p = 0.147$), educational level ($\chi_5 = 3.381$, $p = 0.641$), marital status ($\chi_3 = 6.534$, $p = 0.088$) or work activity ($\chi_3 = 2.369$, $p = 0.499$).

3.1.2. High and Low Optimism and Pessimism Groups[2]

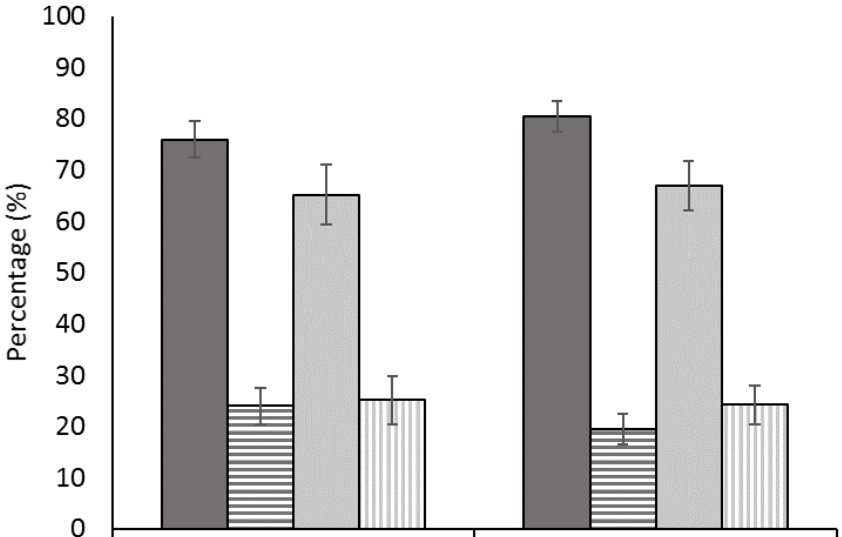
Significant differences in optimism and pessimism were found between the high and low optimism ($t_{(74)} = -13.429$, $p < 0.001$) and pessimism ($t_{(74)} = 11.173$, $p < 0.001$) groups, respectively. The number of men and women in the high and low optimism groups ($\chi_1 = 1.574$, $p = 0.210$) or the high and low pessimism groups ($\chi_1 = 1.172$, $p = 0.279$) did not show significant differences.

The high and low optimism groups did not show differences in age ($t_{(74)} = -0.133$, $p = 0.894$), SES ($t_{(73)} = -1.492$, $p = 0.140$), perceived stress ($t_{(74)} = 1.064$, $p = 0.291$) education ($\chi_5 = 5.257$, $p = 0.385$), marital status ($\chi_3 = 2.164$,

[2] There were significant differences in dispositional optimism between the high and low dispositional optimism groups ($t_{(74)} = -12.190$, $p < 0.001$). People in the low dispositional optimism group were older than those in the high dispositional optimism group ($t_{(74)} = 2.442$, $p = 0.017$). No differences in the number of men and women were found between the high and low dispositional groups ($\chi_2 = 0.920$, $p > 0.99$) and there were no differences in SES ($t_{(73)} = -0.829$, $p = 0.410$), educational level ($\chi_5 = 8.933$, $p = 0.112$), civil status ($\chi_3 = 1.994$, $p = 0.574$), or work activity ($\chi_3 = 4.395$, $p = 0.222$) between groups.

p = 0.539), or work status (χ_3 = 4.540, p = 0.209). People in the high pessimism group were older ($t_{(74)}$ = 3.076, p = 0.003) and reported a higher SES ($t_{(73)}$ = -0.166, p = 0.034) than those in the low pessimism group, but there were no differences between groups in perceived stress ($t_{(74)}$ = 0.274, p = 0.785), education (χ_5 = 7.693, p = 0.174), marital status (χ_3 = 3.239, p = 0.356), or work status (χ_3 = 0.186, p = 0.980).

Figure 1



	Low Optimism	High Optimism
PE	75,957	80,539
NE	24,043	19,461
PCE	65,177	66,927
NCE	25,145	24,184

Figure 1. Percentage of recall of PE, NE, PCE and NCE in High and Low Optimism groups.

3.1.3. Awakening Cortisol Response.

There were no significant differences in age, SES, perceived stress, or pessimism between the 1 or 0 Day-CAR and 2 Day-CAR groups (all p > 0.430), but higher optimism was found in the 2 Day-CAR group ($t_{(74)}$ = -2.316, p = 0.023). ANOVA for repeated measures showed a main effect of time

($F_{(1.439,106.478)}$ = 70.113, η^2 = 0.487, p < 0.001) and time × group ($F_{(1.439,106.478)}$ = 36.651, η^2 = 0.331, p < 0.001). Post hoc analyses showed that only the group with a positive CAR on both sampling days showed an increase from awakening to 30 min after awakening (p < 0.001), and after that, their cortisol concentrations remained higher than on awakening (p < 0.001), with no significant differences from 30 min after awakening (p = 0.739). Meanwhile, the 1 or 0 Day-CAR group did not show significant differences between cortisol concentrations at awakening and 30 or 45 minutes after awakening (all p > 0.127).

3.3. Optimism, Pessimism and Life Stories[3]

3.3.1. Optimism ANOVA for repeated-measures performed with the high and low optimism groups showed a main effect of Valence for events ($F_{(1,74)}$ = 148.121, η^2 = 0.667, p < 0.001), and for cognitions and emotions ($F_{(1,74)}$ = 47.683, η^2 = 0.392, p < 0.001). Thus, more positive events, cognitions, and emotions than negative ones were reported, regardless of pertaining to the high or low optimism group (p < 0.001). Valence × group interactions for both events and cognitions and emotions were not significant (both p > 0.327) (see Figure 1).

3.3.2. Pessimism ANOVA for repeated-measures performed with the high and low pessimism groups showed a main effect of Valence for events ($F_{(1,74)}$ = 150.523, η^2 = 0.670, p < 0.001) and for cognitions and emotions ($F_{(1,74)}$ = 46.099, η^2 = 0.384, p < 0.001). Regardless of the pessimism group, more PE

[3] ANOVA of repeated measures showed a significant main effect of valence and a marginal effect of the valence × group interaction on events (Valence: $F_{(1,74)}$ = 160.473, η^2 = 0.684, p < 0.001; Valence × Group: $F_{(1,74)}$ = 3.597, η^2 = 0.046, p = 0.062) and cognitions and emotions reported (Valence: $F_{(1,74)}$ = 52.241, η^2 = 0.414, p < 0.001; Valence × Group: $F_{(1,74)}$ = 6.585, η^2 = 0.082, p = 0.012). ANOVA showed a higher percentage of PE than NE (p < 0.001, CI = 47.943 to 65.840), and higher percentages of PCE than NCE reported (p < 0.001, CI = 29.606 to 52.141), regardless of the dispositional optimism group. Post hoc analyses showed that the high dispositional optimism group reported marginally more PE (p = 0.062, CI = -0.431 to 17.467) and significantly more PCE (p = 0.028, CI = 1.817 to 30.816). High dispositional optimism showed marginally less NE (p = 0.062, CI = -17.467 to 0.431) and significantly less NCE (p = 0.031, CI = -24.252 to -1.159) than the low dispositional optimism group.

and PCE than NE and NCE, respectively, were reported ($p < 0.001$). The Valence × group interaction was significant for events ($F_{(1,74)} = 5.027$, $\eta^2 = 0.064$, $p = 0.028$) and for cognitions and emotions ($F_{(1,74)} = 8.031$, $\eta^2 = 0.098$, $p = 0.006$). Post hoc analyses showed that the high pessimism group reported higher percentages of NE ($p = 0.028$, CI = 1.128 to 19.147) and NCE ($p = 0.008$, CI = 4.199 to 27.288), but less PE ($p = 0.028$, CI = -19.147 to -1.128) and PCE ($p = 0.028$, CI = -31.266 to -1.798) than the low pessimism group (see Figure 2).

3.4. Optimism, Pessimism and Morning Cortisol[4]

ANOVA with the total sample showed that the high optimism group had higher CAR compared to the low optimism group ($F_{(1,74)} = 6.812$, $p = 0.011$), however, this difference disappeared if the analyses were performed only with people in the 2 Day-CAR group ($p = 0.392$). No differences between optimism groups were found in S1 or AUC_G in the total sample or in the 2 Day-CAR group (all $p > 0.078$). There were no differences in CAR, S1, or AUC_G between the pessimism groups in the total sample, or after repeating the analyses with the 2 Day-CAR people (all $p > 0.116$).

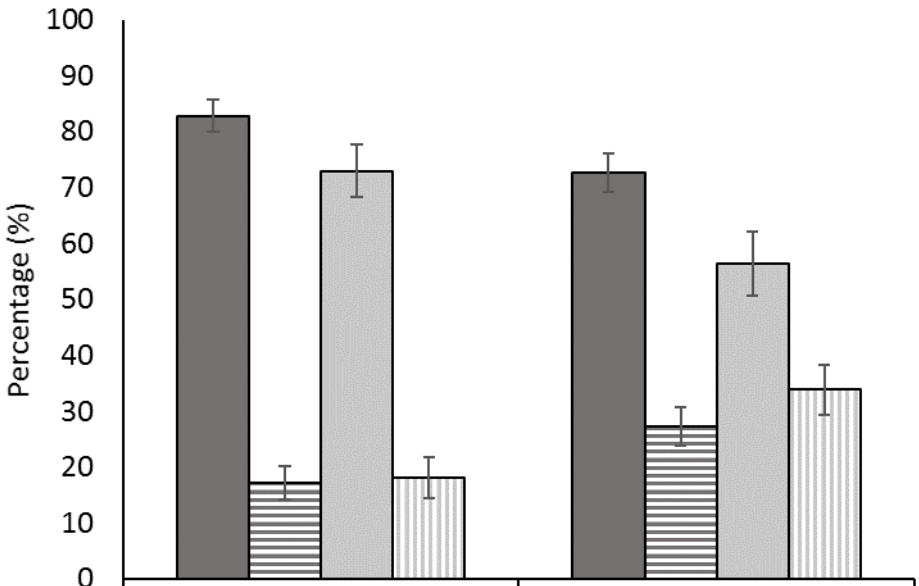
3.5. Life Stories and Morning Cortisol

Correlations performed with percentages of life story categories and AUC_G and CAR showed a marginally negative relationship between the percentage of PE and the CAR ($r = -0.220$, $p = 0.057$), and a marginally positive relationship between NE and the CAR ($r = -0.220$, $p = 0.057$). After controlling for sex and age, these marginal relationships disappeared (both $p = 0.159$). No significant relationships were found between percentages of PCE and NCE and the CAR, with and without controlling for age and sex

[4] ANOVA did not show significant differences in cortisol S1 or AUC_G between high and low dispositional optimism groups with the total sample or only with those people in the 2 Day-CAR group (all $p > 0.115$). Regarding CAR, high dispositional optimism showed higher CAR than the low dispositional optimism group ($F_{(1,74)} = 4.322$, $p = 0.041$) with the total sample. However, after repeating the analyses only with 2 Day-CAR people, these differences between the high and low dispositional optimism groups disappeared ($p = 0.577$).

(all $p > 0.138$). No significant relationships were found between AUC_G or S1 and PE, NE, PCE or NCE, with and without controlling for age and sex (all $p > 0.162$).

Figure 2



	Low Pessimism	High Pessimism
PE	82,805	72,668
NE	17,195	27,332
PCE	72,957	56,425
NCE	18,155	33,898

Figure 2. Percentage of recall of PE, NE, PCE and NCE in High and Low Pessimism groups.

4. DISCUSSION

The aim of this study was to investigate the role of optimism and pessimism in the way healthy older people focus their attention on remembering positive or negative events, cognitions, and emotions. Moreover, we wanted to explore the relationships of optimism and pessimism with morning cortisol concentrations and the CAR in healthy older people. For this purpose, a total of 76 participants with ages ranging

from 56 to 77 years old carried out a brief summary of their lives and provided 6 saliva samples on two consecutive weekdays (3 samples per day) to assess the CAR.

To the best of our knowledge, this is the first study to investigate the impact of optimism and pessimism on past life review. Our analyses suggest that pessimism seems to be more important than optimism in past life review. Likewise, our additional analyses with dispositional optimism showed that people with higher dispositional optimism reported less negative events, cognitions, and emotions in their life stories. Thus, our results agree with previous studies that found a relationship between negative states, such as depression and anxiety, and a negative focus in past life recall (Ricarte et al., 2012; Raes et al., 2003), given that our results highlight the role of pessimism rather than optimism in past recall. It is relevant that the autobiographical memory process and the evaluation of negative past experiences are key factors in death acceptance (Cappeliez et al., 2007; Webster & Cappeliez, 1993). Based on our results, holding negative future expectations is related to focusing on negative aspects of our lives, which may affect our perception of how capable we are of coping with present and future events (Seligman, 1991).

Surprisingly, we did not observe differences on the optimism subscale affecting the number of positive events, cognitions, or emotions reported in their life stories. This lack of differences may be due to the increase in attention to positive facts in aging (Mather & Carstensen, 2005), which could mitigate the effect of optimism in recalling past positive events, cognitions, or emotions, thus increasing the effect of pessimism. Previous studies showed that older people tend to attribute more positive features to past choices and more negative features to the options they refused (Mather & Johnson, 2000). In this regard, older people indicate more positive feelings than young people, as well as less complexity associated with negative memories, suggesting that older people more frequently use positive reappraisal (Folkman et al. 1987), which affects their recall in a positive way. Thus, older people increase their positive point of view about

their health behaviors and routines with age (Kennedy et al., 2004). In fact, in line with the *socioemotional selectivity theory* (Carstensen et al. 1999), in the present study we observed more positive events, cognitions, and emotions reported, regardless of the dispositional optimism or the optimism and pessimism groups.

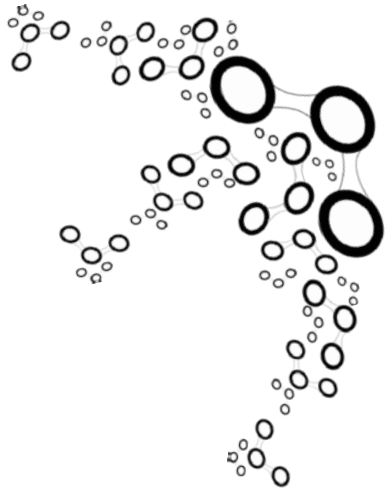
Finally, our results showed a positive relationship between the optimism subscale and the CAR, and this relationship was also significant in the additional analyses with dispositional optimism. However, when we replicated the analyses only with participants who showed a positive CAR on both sampling days, the relationship disappeared. This result agrees with Ebrecht et al. (2004), who found no relationship between dispositional optimism and CAR in young people; and partially with Jobin et al. (2013), who observed significant relationships between dispositional optimism and CAR only on days with high stress perception in older people. By contrast, Lai et al. (2005) reported that high dispositional optimism is related to reduced CAR in young men. A similar study performed with 422 older people reported that dispositional optimism is related to a blunted CAR response (Endrighi et al., 2011). It is possible that the sample size and characteristics, as well as differences in methodological cortisol assessment, could contribute to the discrepancies between our results and those reported by previous studies. First, the age-related changes in the independence of the optimism and pessimism subscales have to be taken into account, especially in people over 50 years old. These changes recommend studying the effect of optimism and pessimism separately in older people, and not as part of a continuum as in young people (Puig-Perez et al., 2015; Rasmussen et al., 2009; Herzberg et al., 2006). Thus, in our opinion, these age-related changes may contribute to differences in the effects of this trait on physiological systems, and they could help to explain the discrepancies between our study and Lai et al. (2005). On the other hand, in the Endrighi et al. (2011) study, the sample size was larger than in our study, but the CAR was assessed on only one day. The need for more than one sampling day has been suggested to ensure a reliable CAR

measurement (Stadler et al., 2016; Hellhammer et al., 2007), which led us to register the CAR on two consecutive weekdays. In any case, further research is needed to clarify the relationship between dispositional optimism and its subscales and morning cortisol release.

The results reported in the present study should be viewed in light of the following limitations. First, we tried to reduce social desirability by avoiding the pressure of performing this personal task while being observed by the experimenter. However, the fact that the participants did not perform the life story review in our presence leads to less control over the way they performed the task, and we could not know whether they did it while relaxing, although we encouraged them to do so. Second, although we tried to enhance the CAR registering accuracy by following most of the recommendations in the literature (see Stalder et al., 2016), we did not use a mechanism to objectively test whether the first sample was taken exactly when participants awakened, such as actigraphy or polisomnography. In any case, we made an effort to increase participant adherence (e.g. flexibility in choosing the saliva collection days). Additionally, we collected saliva samples on two consecutive days (Clow et al. 2010b, Hellhammer et al. 2007), and we controlled for participants' possible non-adherence to the salivary sampling protocol by analyzing the cortisol secretion profile on both days (Almela et al. 2012; Thorn, et al. 2006). Finally, characteristics of our sample (healthy older people, with medium-high SES, good educational level, and low chronic stress) make it difficult to generalize our results to the general population. However, strict control over confounding factors by using a homogenous sample could provide a clearer picture of the relationships studied.

In conclusion, low dispositional optimism, but more specifically, pessimism, seems to increase the focus on negative aspects of the past, which may lead to a worse perception of life in aging. On the other hand, optimism was related to a higher CAR, but this relationship was not significant without suspected non-adherent participants. Although the CAR's function remains unclear (Clow et al., 2010b), previous research

highlighted the negative effect of a blunted CAR on health (Clow et al., 2010b; Fries et al., 2009). Therefore, these results may contribute to increasing the knowledge about how optimism might play a protective role, whereas pessimism may have a harmful effect on health (Carver et al., 2010), with optimism being more related to physiological factors and pessimism to psychological processes (Puig-Perez et al., 2015).



Chapter 7

Optimism, the stress response and diurnal cortisol release in older people with type II diabetes.

Main results of the present chapter are under review: Puig-Perez, S., Hackett, R., Salvador, A. & Steptoe, A. (2016). Optimism moderates psychophysiological responses to stress in older people with type 2 diabetes. *Psychophysiology*.

1. INTRODUCTION

There is increasing interest in psychological characteristics that could have a protective role in preventing the development of disease (Rasmussen et al., 2009; Boehm & Kubzansky, 2012). Optimism is a psychological trait characterised by positive expectations about future outcomes that has been associated with better psychological and physical wellbeing, particularly during times of stress (Scheier & Carver, 1992; Smith & MacKenzie, 2006). Optimism is thought to play a protective role in stress-related conditions such as the metabolic syndrome (Cohen et al., 2010), reduced immune functioning (Brydon et al., 2009; Roy et al., 2010) and cardiovascular diseases (Giltay et al., 2006; Nabi et al., 2010; Tindle et al., 2010).

The pathways underlying the potential protective effect of optimism on health remain unclear. Optimism may affect health indirectly through health behaviours, as well as directly through psychophysiological processes. Both of these pathways can influence the development of cardiovascular and other chronic diseases (Tinker et al., 2007; Matthews et al., 2004). There is considerable evidence that the protective role of optimism may involve in part greater engagement in health protective behaviours (Carver et al., 2010). In line with behavioural self-regulation theory (Carver & Scheier, 2000), the way in which people face challenges or difficulties influences how they cope with stress (Carver et al., 2010). Having an optimistic point of view increases confidence, motivating individuals to achieve goals as well as increasing positive affect and wellbeing (Solberg Nes et al., 2005) Optimism is linked with adaptive coping styles and health protective behaviours (e.g. such as better treatment adherence, lower consumption of saturated fat, increased vitamin intake, as well as increased physical activity (Giltay et al., 2006; Nabi et al., 2010; Shepperd et al., 1996; Leedham et al., 1995). However, associations between optimism and health outcomes in many studies persist after

controlling for these factors, suggesting that other pathways may be involved.

The influence of optimism on psychophysiological processes involved in disease can be investigated using acute mental stress testing. Acute stress studies assess dynamic psychophysiological responses to stress under controlled conditions, which reduce the impact of possible confounding factors (Steptoe & Poole, 2010). Previous studies of healthy individuals have found that greater optimism is associated with increased HR stress responses (Solberg Nes et al., 2005), as well as faster cortisol recovery after stress (Brydon et al., 2009). However, in older people, associations have not been consistently detected. Endrighi et al. (2011) failed to detect a relationship between optimism and cortisol responses to stress, while Puig-Perez et al. (2015) found that older men and women with higher optimism had lower HR responses to acute psychosocial stress as well as a faster return to baseline levels post-task in cortisol and HR. It is possible that optimistic people demonstrate greater task engagement and this induces greater psychophysiological activation, resulting in heightened cardiovascular and endocrine responses (Solberg Nes et al., 2005). But at the same time, an optimistic perspective could protect the individual from exaggerated stress reactions, and thereby have a health-protective effect (Puig-Perez et al., 2015).

Given the relationship between acute stress responses and health, the study of factors that could have a protective role is important, especially in a vulnerable population such as people diagnosed with T2D. T2D is a heterogeneous metabolic disease characterized by reduced insulin sensitivity and relative insulin deficiency (International Diabetes Federation, 2015; McCrimmon et al., 2012). It is one of the most common diseases in people aged 50 and over (Scully, 2012) and is expected to continue to increase in prevalence in the coming decades (Zimmet et al., 2001; International Diabetes Federation, 2015).

There is evidence that stress may play a role in the development of T2D (Pouwer et al., 2010; Kelly & Ismail, 2015; Hackett & Steptoe, in press) Meta-analyses of prospective cohort studies suggest that people exposed to job strain (Nyberg et al., 2014) or who work long hours (Kivimäki et al., 2015) have a greater risk of developing T2D. Other longitudinal studies have also observed an increased risk of T2D in people with history of moderate and severe childhood abuse (Rich-Edwards et al., 2010) or childhood neglect (Goodwin & Stein, 2004). It has also been shown that higher perceived stress in adulthood increases the risk of T2D in a prospective 35-year follow-up study (Novak et al., 2013), although not all findings relating perceived stress with subsequent diabetes development have been consistent (Williams et al., 2013; Rod et al., 2009).

Stress-related physiology has not been studied extensively in T2D, but one useful concept in this regard is allostasis, an adaptive process by which the body responds to changes in the environment through the adjustment of multiple biological systems (McEwen, 1998). Sustained or repeated exposure to challenge can result in chronic allostatic load and a breakdown of regulatory processes. McEwen (1998; 2006) has argued that high allostatic load is associated with blunted acute responses coupled with impaired post-stress recovery. A trial carried out by our group suggests that physiological responses to stress may be dysregulated in people with T2D when compared with the responses of healthy controls, showing a pattern of responses characteristic of high allostatic load (Steptoe et al., 2014). Specifically, we observed that the participants with T2D had blunted stress reactivity and impaired recovery in blood pressure (BP), HR, cortisol, interleukin 6 and serum cholesterol in response to a laboratory stress task when compared with healthy controls (Steptoe et al., 2014).

It is worth noting that having a chronic disease such as T2D has been associated with a lower health-related quality of life (Rothrock et a., 2010) and psychological distress is common in people with T2D (Hackett & Steptoe, in press). Reduced quality of life is seen in a range of other conditions such as sarcoidosis (Wilsher, 2012) and breast cancer (Petersen

et al, 2008) and is common following trauma (Tøien et al., 2011). According to Broffenbrenner's ecological model (McLeroy et al., 1988) personality is a key influencer of quality of life. Accordingly, optimism is relevant as it may contribute to a better acceptance of living with a chronic disease such as T2D, thus translating into greater self-reported quality of life (Kepka et a., 2013; Perales-Montilla et al., 2012; Tøien et al., 2011; Petersen, et al., 2008; Steptoe et al., 2006; Schou et al., 2005; Achat et al., 2000). Therefore, studying the relationship between optimism and self-related health in people with T2D may provide valuable information about the possible protective role of optimism in people living with a chronic disease.

The daily pattern of cortisol release is also important for health (Kondratova & Kondratov, 2012) and several parameters of circadian cortisol release have been employed, such as total cortisol output, cortisol slope across the day and the cortisol awakening response (CAR). The CAR, is a sharp rise in cortisol levels in the first 30 minutes after waking (Stalder et al., 2016) which has been related to multiple psychosocial factors: for example, the CAR has been positively related to job stress and general life stress, and negatively associated with fatigue, burnout or exhaustion (Chida & Steptoe, 2009). However, the relationship between the CAR and positive psychological factors such as optimism is less consistent. Nevertheless, research by Jobin et al. (2014) found that older people with low optimism had higher daily cortisol values and a lower CAR on the days that they reported higher stress. Similarly, studies with healthy older (Endrighi et al., 2011) and middle-aged adults (Lai et al., 2005) reported that high optimism was related with lower CAR, but not with cortisol decline of the day or total cortisol output.

In the present study we expanded on our previous investigation to assess the role of optimism on psychophysiological responses to stress in people with T2D. Taking into account that optimism is a protective trait, it is plausible that individuals with T2D who have high levels of optimism could have a better pattern of physiological stress responses, that is, their pattern of response more closely resembles the profile of stress responsivity in

healthy individuals. So we hypothesized that greater optimism would be related to heightened BP reactivity and better post-stress recovery in people with T2D. No relationship was expected between optimism and acute cortisol response to stress, in line with previous research in older samples (Endrighi et al., 2011; Puig-Perez et al., 2015). However, we did hypothesise that greater optimism would be inversely associated with cortisol output over the day. Finally, in line with previous studies, we expected that optimism would be positively related to better self-related health (Kepka et a., 2013; Perales-Montilla et al., 2012; Tøien et al., 2011; Petersen, et al., 2008; Steptoe et al., 2006; Schou et al., 2005; Achat et a., 2000). Our analyses took into account covariates that might potentially moderate psychophysiological responses in this population.

2. MATERIAL & METHODS

2.1. Participants

The participants recruited for the present study were part of larger trial comparing stress responsivity in healthy individuals and people with T2D (Steptoe et al., 2014). All participants gave full informed consent to take part in the study, and the National Research Ethics Service granted ethical approval. A total of 140 participants with doctor-diagnosed T2D were recruited between March 2011 and July 2012 from diabetes outpatient and primary care clinics in London. No respondents had a history of coronary heart disease, inflammatory diseases, allergies or mood disorders. They were instructed to avoid the use of antihistamine or anti-inflammatory medication in the 7 days prior to the study, and were instructed not to consume alcohol or practice intense exercise on the evening before the test, and to avoid caffeinated beverages and smoking the 2 hours prior to the testing session.

2.2. Procedure

Sessions were held in the morning or in the afternoon, in a light- and temperature controlled laboratory. At the beginning of the session, objective measures of height and weight were obtained and body mass index (BMI) was computed. Systolic BP (SBP), Diastolic BP (DBP), and HR were continuously monitored using a Finometer device (TNO-TPD Biomedical Instrumentation, Amsterdam, Holland). After 30 minutes resting, the baseline cardiovascular values were measured in the last 5 minutes of the rest period, and respondents provided a baseline rating of subjective stress and a saliva sample for the assessment of cortisol. Participants then completed the two 5-minute mental stress tasks in a random order. SBP, DBP, and HR were measured with 5-minute recording periods during each of the tasks; and subjective stress and a second saliva sample were taken immediately after the tasks. Further cardiovascular measurements and subjective stress ratings were obtained at 45 and 75 min after the stress exposure. Additional saliva samples were collected at 20, 45 and 75 minutes after stress to assess cortisol responses.

Additionally, saliva samples were collected over a typical day in order to measure cortisol concentration over the day. Participants collected five saliva samples using Salivettes (Sarstedt), at waking, 30 min later, and then within three 30-min time windows in the morning (10:00–10:30), afternoon (16:00–16:30) and evening (20:00–20:30). They were instructed not to eat, drink tea or coffee, or smoke in the 30 minutes before sample collection. Violations of this protocol and sample timing were recorded in a log. For the majority of people cortisol sampling was carried out on the day after the laboratory visit. Saliva samples were stored at -20 degrees before analysis using a time-resolved immunoassay with fluorescence detection at the University of Dresden. Samples were analysed in duplicate, the intra-assay and inter-assay coefficients of variation were less than 8%. Overall, ten measures of cortisol were collected in the study, five from saliva samples in taken in the laboratory (baseline, immediately and 20, 45 and 75 minutes after stress) and five samples

collected over a typical day (at waking, 30 min later, 10:00-10:30, 16:00-16:30 and 20:00-20:30).

2.3. Assessment and Measures

2.3.1. Psychological Measures We measured optimism using the 10-item Life Orientation Test-Revised (LOT-R), a widely used measure of optimism trait that evaluates generalised positive or negative expectancies in life (Scheier et al., 1994). Participants were asked to indicate the extent of their agreement with each item (e.g. "I'm always optimistic about my future") from 0 (*strongly disagree*) to 4 (*strongly agree*). Six items are used to derive the optimism score, so ratings can range from 0 to 24, with higher scores indicating greater optimism. The remaining four questions on the LOT-R are filler items. The internal consistency (Cronbach α) was 0.83 in this sample. This questionnaire was completed prior to the laboratory stress testing session. Subjective stress was assessed before and after stress (immediately post-stress, 45 and 75 min after stress) using a 7-point rating scale, with higher values indicating greater perceived stress.

2.3.2. Other Measures We included household income as an indicator of socioeconomic status and the participants were categorized into low (< £ 20,000), medium (£ 20,000 - 40,000) and high (£ > 40,000) income groups. Education was categorized into less than high school, high school or equivalent, and college or higher. The participants also reported whether they were in paid work at the time of stress testing. Ethnicity, smoking status and medication were also recorded. Specifically, medication was divided into six categories: oral diabetic medication (metformin, etc.), insulin and other injected medication, aspirin, β-blockers, other hypertensive medication (e.g. angiotensin-converting enzyme inhibitors), and statins. Health status was measured using the 36-Item Short-Form Health Survey (SF-36) (Ware & Sherbourne, 1992). Eight dimensions of functioning are assessed on the SF-36, but for the purposes of this study the Physical Component Summary (PCS) and the Mental Component Study (MCS) were computed. Scores were coded and transformed to a scale

where 0 = worst possible health and 100 = best possible health. The internal consistency (Cronbach α) of the PCS was 0.91 and the MCS was 0.80 in this sample.

2.3.3. Mental Stress Tasks Two 5-minute behavioural tasks were used to induce mental stress in the laboratory. The tasks were administered in a random order. One task was the Stroop colour-word interference task, which consisted of successive presentations of target colour words printed in an incongruous colour. The other task was a mirror tracing task, in which the participant has to trace a star that could only be seen in mirror image using a mental stylus. If the respondent put the stylus outside of the star, the device emitted a loud beep (Lafayette Instruments Corp, Lafayette, IN) and a mistake was registered. Participants were told that the average person could complete five circuits of the star in the allocated 5-minute period. We selected these tasks because they have been shown previously to elicit similar appraisals of involvement and engagement from people across the social gradient and have been used in previous studies by our group (Steptoe et al., 2002).

2.4. Statistical Analysis

SBP, DBP and HR were averaged into 5-minute means at baseline, mean values of the two mental stress tasks, and at the two recovery periods (45 and 75 minutes after stress). Cortisol values were log-10 transformed before analysis because of a skewed distribution. Subjective stress, SBP, DBP and HR were analysed across four trials (baseline, task, and 45 minutes and 75 minutes after stress). Laboratory cortisol was analysed across five trials in the lab (baseline, task, and 20 minutes, 45 minutes and 75 minutes after stress). Repeated-measures analysis of variance was used to test the responses to mental stress (subjective stress, SBP, DBP, HR and laboratory cortisol), and main effects were followed up with *post hoc* tests using LSD. Repeated measures analysis of variance was also used to analyse the profile of cortisol over the day in five samples (at awakening, 30 min after awakening, 10:00-10:30h, 16:00-16:30h and 20:00-20:30h).

We computed measures of reactivity (mental stress task minus baseline values) and recovery (45 min minus baseline values) for SBP, DBP, HR and stress perception. The area under the curve with respect to the ground (AUC_G) was calculated to test the cortisol output across the laboratory session (Pruessner et al., 2003). For cortisol over the day, we computed total output (AUC_G) using the method described by Pruessner et al. (2003). Since the sample obtained 30 min after waking may distort the computation of total cortisol output, we measured the AUC_G both using all samples (Day-AUC_t) and after excluding the sample taken 30 min after waking (Day-AUC_{ds}). The CAR was calculated by subtracting the awakening cortisol concentrations from the 30 min post-awakening sample.

Associations with optimism were analysed using multivariable linear regression on cardiovascular baseline levels, reactivity and recovery measures, stress perceptions, and the measures of cortisol output in the laboratory and over the day. Optimism was entered into the regression models along with age, sex, smoking status and baseline values (laboratory cortisol, HR, SBP and DBP) to test associations with physiological stress responses. Stress responses differ by sex in some studies (Steptoe et al., 2002; Kudielka et al., 2004) and also sex differences in optimism have been reported (Helliwell et al., 2012). Sex was therefore included as a covariate in all analyses along with age. Smoking is known to impact physiological stress responses (Evans et al., 2012; Phillips et al., 2009) so smoking was controlled for in all analyses. Additionally, for laboratory cortisol, time of testing was entered as covariate in case there were differences between the morning and afternoon. Optimism was entered into the regression models along with age, sex, smoking status, time of awakening and day of sampling (week or weekend day) in the analyses of cortisol over the day. Delays between waking and taking the first saliva sample in can distort the CAR (Dockray et al., 2016), we excluded cases when the delay was more than 15 minutes after waking in the analysis of the CAR. However, we did not find a difference in the association between the CAR and optimism

after excluding such individuals, so the complete study sample was used in the final analyses. Finally, partial correlations were performed to test the relationship between optimism and physical and mental health status (SF-36 scales) taking account of age, sex, BMI and household income. Covariates for these analyses were chosen after preliminary analyses in which we checked their relationship with physiological responses assessed in the study or/and health status as measured on the SF-36, as well as research evidence of their relationship in previous studies (e.g. Cohen, 1996; Ettner, 1996; Roy et al., 1994; Yan et al., 2004; Direk et al., 2011; Stalder et al., 2016). Sensitivity analyses adding medication usage across multiple categories as an additional set of covariates did not change the pattern of results between optimism or the SF-36 and physiological responses. Therefore, medications were not included as covariates in the final models presented in this manuscript.

One participant did not complete the LOT-R, so was excluded from all the analyses. We used the Greenhouse-Geisser procedure when the requirement of sphericity in the Repeated-Measures ANOVA was violated. All *p*-values reported are two-tailed, the level of significance was set at $p < 0.05$ and 95% Confidence Interval (CI). We used SPSS 22.0 to perform the statistical analysis.

3. RESULTS

3.1. Sample Characteristics

The sample consisted on 140 participants (88 men) with doctor-diagnosed T2D. Participant characteristics are detailed in Table 1. It can be seen that the majority of respondents were white men, with an average age of 63.71 years old, and were typically overweight or obese (BMI average 30.75) with a modest household income (< £ 20,000). With regards to medication usage, 11% of the sample was using injectable anti-diabetic drugs, and a 11.8% were taking β-blockers. Optimism scores averaged 14.43

in this sample with ranging from 1 to 24. Optimism was related to age (r = 0.178, p = 0.036) and household income (r = 0.239, p = 0.006), but not with smoking status, body mass index or sex (all p > 0.228).

TABLE 1. Participant Characteristics

Characteristics		
Age, M (SD)	63.71	7.004
Sex, n (%), % men	88	62.9
Ethnicity, n (%), % white	112	80.0
Current Smoker, n (%), % smoker	20	14.3
Body Mass Index, M (SD), kg/m2	30.75	5.72
Household income, n (%)		
<£20.000	57	42.9
£20.000-40.000	38	28.6
>£40.000	38	28.6
Education, n (%)		
Less than high school	37	26.8%
High school	14	10.0%
College or higher	87	63.0%
Paid work, n (%), % yes	62	44.3%
HbA1c, M (SD)	7.25	1.42
Injectable anti-diabetic and insulin, n (%)	15	11.0
Oral anti-diabetic, n (%)	109	80.1
β-Blockers, n (%)	16	11.8
Anti hypertensive, n (%)	96	70.6
Cholesterol lowering, n (%)	106	77.9
Aspirin, n (%)	48	35.3
Other	62	45.6
Life Orientation Test-R, M (SD)	14.43	4.32

M=Mean; SD= Standard deviation; HbA1c= Glycated Haemoglobin

3.2. Psychophysiological Response to Stress

Repeated-measures ANOVAs showed a main effect of time in SBP, DBP and HR (all $p < 0.001$). The exposure to stress provoked a significant increase in SBP, DBP and HR from baseline, decreasing towards baseline in the post-stress period (all $p < 0.001$). Cortisol concentrations were highest at baseline, decreasing across to session until 45 min after the stress tasks (all $p < 0.001$) (see Table 2).

Stress perceptions peaked during tasks ($p < 0.001$), then returned to baseline in the post-stress period.

Table 2	Baseline	Stress Task	20 min	45 min	75 min
SBP mm Hg	M = 126.08 SD = 13.55	M = 149.35 SD = 20.58		M = 134.24 SD = 20.32	M = 137.05 SD = 17.02
DBP mm Hg	M = 71.74 SD = 10.15	M = 84.25 SD = 12.51		M = 78.04 SD = 14.89	M = 79.51 SD = 13.80
HR beats/min	M = 71.78 SD = 12.36	M = 76.34 SD =12.23		M = 70.18 SD = 12.23	M = 70.15 SD = 11.94
Stress Perception	M = 1.50 SD = 0.90	M = 4.50 SD = 1.52		M = 1.53 SD = 0.93	M = 1.43 SD = 0.93
Cortisol nmol/mL	M = 10.03 SD = 5.34	M = 8.74 SD = 4.35	M = 7.74 SD = 3.92	M = 6.89 SD = 4.02	M = 7.17 SD = 5.48

HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure. Values are presented in rows as means (standard deviation).

3.3. Cortisol Output Over the Day

As expected, there was a main effect of time in the analysis of cortisol sampled over the day ($p < 0.001$). Cortisol concentration averaged 19.98 ± 11.98 on waking, increasing to 26.60 ± 14.81 30 minutes later ($p < 0.001$). After that, the cortisol concentration decreased over the course of the day to its lowest level in the evening (mean 5.48 ± 5.9, $p < 0.001$).

3.4. Optimism and Responses to Mental Stress

Regression analyses showed no relationship between optimism and baseline SBP ($p > 0.805$) or DBP ($p > 0.757$) with and without controlling for covariates.

Regression analyses showed that optimism was associated with higher SBP (β = 0.185, CI = 0.041 to 1.325, $p = 0.037$) and DBP (β = 0.176, CI = 0.003 to 0.568, $p = 0.047$) responses to stress (Table 3). So more optimistic people with diabetes were more reactive in terms of BP after controlling for age, sex, smoking status and baseline levels. There was no association between optimism and SBP and DBP recovery, HR reactivity or recovery, or with laboratory cortisol AUC_G (all $p > 0.175$).

Regression analyses showed no relationship between optimism and increases in perceived stress in response to tasks (β = -0.083, CI = -0.090 to 0.027, $p = 0.287$) or with the decrease in stress ratings 45 min post-stress (β = -0.021, CI = -0.042 to 0.032, $p = 0.781$).

Table 3 **Summary of regression analyses with optimism and physiological stress response**

	SBP				**DBP**				**HR**				**Cortisol**	
	Reactivity		**Recovery**		**Reactivity**		**Recovery**		**Reactivity**		**Recovery**		**AUCg**	
	R^2=0.041		R^2=0.062		R^2=0.042		R^2=0.024		R^2=0.074		R^2=0.060		R^2=0.580	
	p=0.356		*p*=0.135		*p*=0.345		*p*=0.674		*p*=0.071		*p*=0.145		*p*<0.001	
	β	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***	**β**	***p***
Age	0.050	0.575	-0.006	0.945	0.057	0.534	-0.037	0.687	-0.056	0.527	0.004	0.968	-0.001	0.986
Sex	-0.029	0.744	0.151	0.087	0.014	0.881	0.047	0.608	-0.089	0.301	0.091	0.293	-0.079	0.204
Smoking	0.015	0.866	0.088	0.321	-0.051	0.559	0.042	0.638	-0.116	0.175	-0.147	0.089	0.090	0.156
Baseline	-0.016	0.857	-0.158	0.077	0.036	0.701	0.074	0.437	-0.216	0.014	-0.151	0.086	0.188	0.002
Time of testing	-	-	-	-	-	-	-	-	-	-	-	-	0.694	<0.001
Optimism	**0.185**	**0.037**	0.118	0.175	**0.176**	**0.047**	0.109	0.220	0.115	0.180	0.038	0.664	0.075	0.225

3.5. Optimism and Cortisol Over the Day

There was a significant negative association between optimism and cortisol output over the day, with (Day-AUCt: β = -0.261, CI = -7.339 to -1.387, p = 0.004) and without including the 30 min after waking sample in the model (Day-AUCds: β = -0.283, CI = -6.072 to -1.521, p = 0.001) after controlling for age, sex, educational level, BMI, smoking status, time of awakening and day of sampling (Table 4). Regression analyses showed no significant relationship between optimism and the CAR after controlling for covariates (p = 0.531).

Table 4 **Summary of regression analyses with optimism and daily cortisol**

	Day-AUC$_t$		**Day-AUC$_{ds}$**		**CAR**	
	R^2=0.144		R^2=0.194		R^2=0.047	
	p=0.014		p=0.001		p=0.560	
	β	***P***	**β**	***p***	**β**	***p***
Age	0.015	0.878	0.023	0.803	0.031	0.755
Sex	-0.102	0.266	-0.100	0.254	0.046	0.623
Smoking	-0.029	0.748	-0.056	0.524	0.039	0.675
BMI	0.123	0.206	0.192	0.039	-0.003	0.976
Day of testing	-0.171	0.059	-0.182	0.036	-0.093	0.310
Time of awakening	-0.119	0.189	-0.133	0.126	-0.163	0.078
Optimism	**-0.261**	**0.004**	**-0.283**	**0.001**	0.058	0.531

3.6. Optimism and Self-Reported Health Status

Partial correlations with age, sex, BMI and household income as covariates showed significant relationships between optimism and SF-36 total scores of physical and mental health. Optimism was positively related to better outcomes in physical (PCS) (r = 0.303, p < 0.001) and mental health (MCS) total scores (r = 0.355, p < 0.001).

4. DISCUSSION

This study investigated the relationship between optimism and cardiovascular, neuroendocrine and psychological response to acute stress and cortisol secretion over the day in middle aged and older people with T2D. The behavioural tasks elicited marked increases in cardiovascular activity along with subjective distress, indicating that they were effective in stimulating acute stress responses. Our main finding was that people with T2D with low optimism showed blunted SBP and DBP responses to stress. No associations between optimism and cortisol response to acute stress and CAR were found, but the low optimism group showed higher diurnal cortisol concentrations, and poorer health status as measured with the SF-36.

Agreeing with previous studies (Endrighi et al., 2011; Puig-Perez et al., 2015), optimism was not related with the cortisol response to stress, but was related to cardiovascular responses to stress (SBP and DBP) in people with T2D. In contrast with Solberg Nes et al., (2005), we observed a relationship with SBP and DBP, but not with HR. It should be taken into account that participants of the present study had diagnosed T2D and these individuals have previously been shown to have blunted SBP and DBP responses to stress when compared with healthy matched controls (Steptoe et al., 2014). Therefore, the results of the present study suggest that optimism in people with T2D is associated with heightened BP responses to stress, which is in line with stress responsivity observed in healthy individuals. Considering that people with T2D show blunted cardiovascular response to stress (Steptoe et al., 2014), our results contribute to the evidence for the protective effect of optimism in T2D, supporting the protective role of an optimistic perspective (Carver & Scheier, 1990; Carver et al., 2010; Scheier & Carver, 1993). That is, our results support the assumption that optimism helps people with T2D to preserve a better physiological adjustment to stress. It is plausible that this could make optimistic people with T2D less likely to develop common comorbid diseases. The stress response is an adaptive response to the challenges of the environment through the adjustment of multiple physiological systems, but repeated or sustained stimulation of these

systems can disrupt dynamic responses to acute challenges or stress resulting in impaired stress reactivity and recovery (McEwen, 1998). For this reason, factors such as optimism that facilitate similar responsivity to stressful circumstances in people with T2D, as in healthy individuals could help to reduce the consequences of impaired cardiovascular function, which is of importance as CVD is one of the most common comorbid problems in T2D (Grundy et al., 1999).

In keeping with our hypotheses and previous literature (Jobin et al., 2014), low optimism was related to higher diurnal cortisol pattern in people with T2D. Considering that T2D is characterized by heightened daily cortisol (Steptoe et al., 2014), the lack of optimism might accentuate disruption of daily cortisol release in this population. However, contrary to our hypothesis and several studies (Endrighi et al., 2011; Jobin et al., 2014; Lai et al., 2005), we did not find any relationship between the CAR and optimism. But, it should be taken into account that the above mentioned studies (Endrighi et al., 2011; Jobin et al., 2014; Lai et al., 2005) were conducted with healthy participants and not people with T2D, thus different results could be expected between optimism and daily cortisol in a diseased versus healthy population.

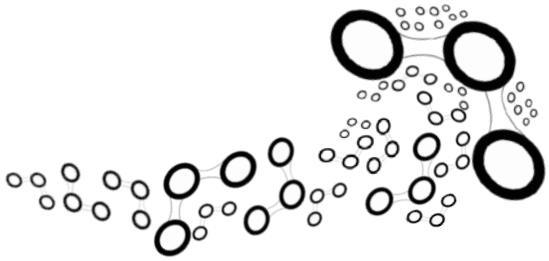
Finally, and agreeing with previous studies (Kepka et a., 2013; Perales-Montilla, García-León, Reyes-del-Paso, 2012; Tøien et al., 2011; Petersen, et al., 2008; Steptoe, Wright, Kunz-Ebrecht, & Iliffe, 2006; Schou, Ekeberg & Ruland, 2005; Achat, Kawachi, Spiro, DeMolles & Sparrow, 2000), optimism was related to better physical and mental subjective well-being. Therefore, our results support that an optimistic point of view in T2D is associated with better ratings of subjective physical and mental health, as well as a pattern of stress responsivity closer to that of healthy individuals. Taking into account the damaging impact of chronic diseases on health-related quality of life (Rothrock et al., 2010) and psychological well-being (Wilsher, 2012), our results provide valuable information about the possible protective role of optimism in a diseased population.

The present study should be interpreted in the light of various limitations. Firstly, the sample recruited were T2D patients without history of coronary heart disease from the London area and the majority of participants were of white European origin. Thus, it is possible that these results may not apply to other groups. Furthermore, cortisol was only assessed over one day which might have limited our ability to assess physiological functioning. This is a cross sectional study and further longitudinal research is needed to understand more how optimism is associated with cardiovascular and cortisol functioning in people with T2D over time. Finally, it is important note that, there some studies investigate optimism and pessimism poles as distinct constructs (Puig-Perez et al., 2015; Herzberg et al., 2006; Lai et al., 2005; Lai, 1994, 1997; Mroczek et al. 1993; Plomin et al., 1992; Robinson-Whelen, 1997) whereas in the present study we assessed optimism as a continuous measure. The exploration of this issue was beyond the scope of the present study. We did not have information on diabetes duration or age of diabetes onset and the protective effect of optimism could differ depending on disease duration and severity. Optimism was not correlated with HbA1c or medication usage in this study (data not shown) which makes it unlikely that diabetes severity impacted the relationships presented in this paper. Nevertheless, this possibility cannot be completely excluded based on the information available.

Despite these considerations, the results suggest that SBP and DBP responses to stress are blunted in people with T2D with low optimism. Moreover, these people had higher cortisol concentrations over the day. Both blunted cardiovascular response to stress and heightened daily cortisol concentrations could result in future health problems such as CVD. However, further studies are required to confirm these pathways.

Chapter 8

General Discussion, limitations and future directions.



1. General discussion

The studies that comprise this doctoral dissertation mainly explored the damaging or protective impact of neuroticism and optimism on ANS and HPA functioning in older people. The first and second studies investigated the relationship between some psychological factors considered harmful for health, such as neuroticism and depressive mood, and the functioning of ANS and HPA in healthy older people under stress and in basal situations. The third, fourth and fifth studies were focused on studying the relationships among optimism, the functioning of the ANS and HPA under stress, and daily HPA functioning.

The main results of each study are discussed in each chapter separately. This chapter presents a general discussion of the main results of this dissertation thesis, general limitations, and future directions, based on the results from the different studies.

1.1. Key factors involved in worse ANS and HPA functioning with and without stress

Previous studies investigated the relationship between neuroticism and the psychophysiological stress response, showing mixed results: heightened autonomic activation and decreased cortisol response to stress in young people higher in neuroticism (Oswald et al., 2006; Philips et al., 2005; Kennedy & Hughes, 2004; Schwebel & Suls, 1999; Kaiser, Beauvale & Bener, 1997; Kirschbaum et al., 1993; Stemmler & Meinhardt, 1990), or no significant relationships between neuroticism and physiological stress response in young people (Verschoor & Markus, 2011; Wirtz et al., 2007; Knyazev, Slobodskaya & Wilson, 2002; Schommer et al., 1999; Kirschbaum et al., 1992, 1995; Kirkcaldy, 1984; Hinton & Craske, 1977). Even more important was the fact that, in spite of the interest in this relationship in recent decades and the impact of age on the main systems involved in the

stress response (Nicolini et al., 2011; Otte et al., 2005), only one study investigated this relationship in middle-aged people (55 to 59 years old), showing a negative relationship between neuroticism and autonomic and cortisol stress response (Bibbey et al., 2013).

Apart from the neuroticism trait, depressive mood is another important factor to take into account in the stress response. However, as in the studies focused on neuroticism and the stress response, the results from studies on the relationship between depression and the stress response are mixed. In a clinical population, both higher and lower cortisol release have been found (Burke et al., 2005; Heim et al., 2000; 2002; Morris et al., 2012; Rooij et al., 2010). In addition, it has been suggested that the relationship between depression and stress-related cortisol release may depend on sex, with women showing increased cortisol release (Chopra et al., 2009; Trestman et al., 1991) and men showing decreased release (Brooks & Robles, 2009). Regarding the autonomic nervous system, most of the studies reported negative associations between depression and HR or blood pressure reactivity to stress (Carroll et al., 2007; Ehrenthal et al., 2010; Schwerdtfeger & Rosenkainer, 2011; Rooij et al., 2010; Salomon et al., 2009; Burke et al., 2005). However, as occurred in the neuroticism and stress response research, previous studies on depression have been performed in young people or large mixed-age samples. Only one study in older people with coronary artery disease showed lower cardiovascular reactivity to stress in those who reported high depressive mood (York et al., 2007).

In order to enhance and complete previous knowledge about the relationships among neuroticism, depression, and the stress response in older people, the first study of this thesis was carried out. The results showed no significant relationship in stressful situations between neuroticism and the psychophysiological stress response, but depressive mood was related to a disrupted ANS and HPA coordination: enhanced HPA functioning versus decreased cardiovascular response to stress (Puig-Perez et al., 2016a).

Another way to explore the underlying mechanisms that relate neuroticism and health is to investigate the relationship between neuroticism and morning cortisol release. Interestingly, this relationship has commonly been explored along with the extraversion trait, which was considered *the protective trait* compared to neuroticism. Studies that investigated these relationships in samples with a large age range showed mixed results: Hill et al. (2013), and van Santen et al. (2011) reported higher AUC_G in people high in extraversion, but they did not find a significant relationship between neuroticism and morning cortisol release. Portella et al. (2005) observed that neuroticism was positively related to CAR and morning cortisol AUC_G. In adolescents, low extraversion and high neuroticism were shown to be related to lower CAR (Hauner et al., 2008). Finally, in adults, different studies found no significant relationships between neuroticism or extraversion and CAR or morning cortisol AUC_G (Chang et al., 2007; Munafo et al., 2006; Lacuelle et al., 2015), except for Mangold et al. (2012), who showed a positive relationship mediated by acculturation. The most interesting aspect to take into account is that no previous studies investigated this relationship in older people, regardless of (i) the impact of age on the CAR (Fries et al., 2009; Clow et al., 2010a; 2010b) and personality (Eysenck, 1988) and (ii) the age-related impact of neuroticism and extraversion on key brain structures involved in CAR regulation (Jackson et al., 2011).

Taking previous studies into account, the second study in this thesis was conducted, which showed that neuroticism (but not extraversion) is related to a reduced morning cortisol release in healthy older men and women, as well as increased CAR only in healthy older women (Puig-Perez et al., 2016b).

Both the first and second studies contributed to extending previous results to older people, an unexplored age period in this regard. In sum, it can be inferred that neuroticism can be considered an important factor to take into account in diurnal HPA functioning, although not in stress conditions. Therefore, neuroticism may exert an important age-related

effect on brain structures related to CAR regulation (Jackson et al., 2011), which, in turn, would lead to poorer HPA regulation, thus increasing the risk of disease over the years. Previous studies related enhanced CAR to physical and mental health risks, such as depression (Dedovic & Ngiam, 2015) and coronary artery disease (Bhattacharyya et al., 2008). Interestingly, neuroticism has also been highly related to greater risk of depression and cardiovascular disorders (for a review, see Ormel et al., 2013). These coincidences may suggest an underlying physiological mechanism involving neuroticism, mood, ANS and HPA axis functioning. In fact, neuroticism could interact indirectly with the ANS and HPA axis functioning in stress conditions through depressive mood. Therefore, further research is needed in order to better understand these relationships.

1.2. Optimism as the protective trait: the promotion of psychological and physiological well-being in older people

In the last part of the thesis, from the third to the fifth studies, the focus was on investigating the protective role of optimism in healthy and older people diagnosed with T2D.

Previous studies investigated the role of optimism in the psychophysiological stress response. In young people, optimism was related to higher cortisol and HR reactivity to a stressful mental task (Solberg Nes et al., 2005), as well as faster cortisol recovery (Brydon et al., 2009). However, the only study performed with older people did not find significant relationships between optimism and stress-related cortisol release (Endrighi et al., 2011). These studies reported a lower stress perception and higher engagement on the laboratory task in people higher in optimism, regardless of age (Solberg Nes et al., 2005; Endrighi et al., 2011). Given (i) the supported effect of optimism on health and well-being markers (for more detail, see Carver et al., 2010) and (ii) the underexplored underlying mechanisms of this relationship, the third and fifth studies were performed.

The third study suggested that optimism works as a protective trait against exacerbated stress responses in healthy older people exposed to acute psychosocial stress, given that it was related to lower HR reactivity and faster cortisol and HR recovery after stress (Puig-Perez et al., 2015). Moreover, the pessimism subscale was related to greater difficulty and effort in coping with the acute stress task (Puig-Perez et al., 2015). In line with this study, the last study tested the hypothesis that optimism could help people diagnosed with T2D to show a cardiovascular stress response similar to healthy older counterparts (Puig-Perez, *under review*). The results of this study showed that people higher in optimism showed higher cardiovascular responses to stress. That is, taking into account that T2D people showed a blunted stress response in a previous study, optimism realigned the T2D stress response to that of healthy older counterparts. Additionally, T2D people with higher optimism showed lower cortisol throughout the day, making the diurnal cortisol levels of T2D people closer to those of healthy older people. It is worth noting that T2D people had a lower stress perception during the session, and higher self-reported physical and mental wellbeing was reported in those who showed higher optimism (Puig-Perez, *under review)*.

Finally, this research focused on the relationships among optimism, the CAR, and re-evaluation of past life. Two main aspects were taken into account in the design of this study:

First, given the close relationship between the CAR and health (for more details, see Clow et al., 2010), the possibility of studying the relationship between diurnal cortisol-release and optimism was considered, in order to clarify the ways this trait might affect health. Previous studies reported mixed results in young people. Lai et al. (2005) found that optimism is related to lower CAR, whereas Ebrecht et al. (2004) showed no significant relationships. Regarding older adults, Endrighi et al. (2011) reported that optimism was related to lower CAR. However, a recent study specified that this relationship was significant only in people who reported high stress perception on the sampling day, but not in those with a normal stress perception (Jobin et al., 2013).

Second, the generation of positive or negative future expectations (i.e. be optimist or pessimist) is not detached from other cognitive functions such as autobiographical memory, even showing an overlapping in certain brain structures (Spreng et al., 2009, 2010; Addis et al., 2007). The relevance of the autobiographical memory process and the re-evaluation of negative past experiences stand out as key factors in death acceptance (Cappeliez et al., 2007; Webster, 1993). According to Seligman's assumption (Seligman, 1991), the way we perceive our past experiences can affect our perceptions of how capable we are of coping with present and future events. Therefore, optimistic people may evaluate their past by focusing on positive events from the past (avoiding negative ones), which could contribute to (i) more positively perceiving their ability to cope with present and future life events, and (ii) to partly explaining why optimistic people cope and adjust better to life events (for a review, see Carver et al., 2010). Therefore, we consider that the past perception may be another important aspect to take into account in the way optimism affects our lives.

The results of this study showed that optimism was not related to morning cortisol release, but it was related to a higher bias toward positive re-evaluation of past life-events, given that people with higher optimism reported more positive events, cognitions, and emotions. Thus, optimism contributed to a more positive re-evaluation of past-life events and to avoiding negative memories, which have been related to depression and anxiety (Ricarte et al., 2011; Raes et al., 2003). Along these lines, the possibility could be considered that optimism may lead to positive re-evaluation, which, in turn, promotes psychological wellbeing in the last period in life, coinciding with the *socioemotional selectivity theory* (Löckhenhoff & Carstensen, 2004).

In sum, the results of the third, fourth and fifth studies support the consideration of optimism as a protective trait. We observed a direct relationship between this trait and its subscales with the functioning of the ANS and HPA axis under stress conditions, as well as with the circadian HPA axis. It can be stated that optimism helps healthy older people to adjust

better to stressful situations. Moreover, in the case of older people with T2D, who show a disrupted ANS and HPA axis functioning, optimism helps them to have better functioning of these systems under stress and throughout the day. Finally, optimism has not been related to the CAR, a discrete component of the HPA axis, in people diagnosed with T2D or healthy people. However, it has been related to higher self-reported physical well-being in T2D people, and to a more positive past-life re-evaluation, understood as psychological well-being. Therefore, our studies supported a direct relationship between optimism and main psychophysiological systems that are strong determinants of physical and mental well-being in advanced ages.

2. General limitations

Limitations of each specific study have been described at the end of each study. However, in the following section, the limitations of the thesis as a whole will be reported.

First, all the studies carried out in the present thesis were cross-sectional studies. More in-depth knowledge can be obtained from longitudinal studies, especially in research on the relationship between personality and changes in ANS and HPA axis functioning throughout life. With longitudinal studies, we could follow the ANS and HPA axis functioning of people from youth to more advanced ages, and determine whether personality is involved in the evolution of the functioning of these body systems, in a damaging way in the case of negative traits, such as neuroticism, or in a good way in the case of positive traits, such as optimism.

Another important limitation is that we did not measure coping strategies in the studies. Several studies have reported the importance of coping strategies in stress and their relationship with personality (e.g. optimism and neuroticism) (for review see Connor-Smith & Flachsbart, 2007). We have also studied them in relation to psychophysiological and

behavioural responses to acute stress in young people (Villada et al. 2014) however, they were not included in the studies in this thesis. Coping strategies may mediate the relationship between these traits and the psychophysiological stress response. In the research that we are currently carrying out, we have re-introduced the measurement of coping strategies in order to improve the study of this relationship with older people investigated in this thesis.

3. Future directions

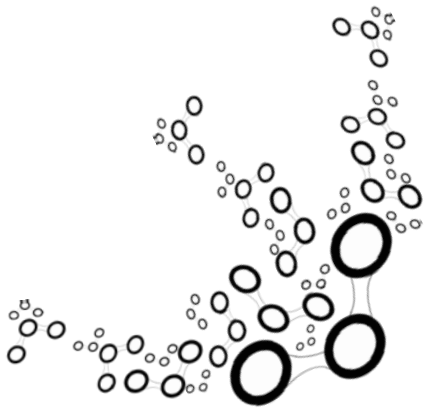
Based on the findings of the different studies presented in this doctoral dissertation, new interesting questions have been raised. First, it would be interesting to extend the research not only to physiological markers of stress but also to behavioural makers and performance on the stress task. A large amount of evidence has been found about the effects of stress on cognitive performance. Thus, considering that an optimistic perspective increases task engagement and adaptive coping strategies, it would be interesting to investigate whether this kind of approach leads to better results in the end, as part of a healthier stress response. If people have a better psychophysiological approach to a stressful situation, people higher in optimism might tend to show better behaviour and better performance in stress conditions, or even afterwards, given that they show faster recovery. It is even possible that optimism can reduce the damaging effects of stress on cognitive aspects.

In line with what was described above, it would be interesting to investigate possible mediator variables in the relationship between personality factors and the psychophysiological stress response. One of the proposed aspects to study would be the coping strategies. In future studies, it would be important to test a model in which personality variables were set as predictors of the multiple psychophysiological markers of ANS and HPA functioning, but adding coping strategies as mediator. Apart from coping strategies, it would be interesting to explore more in depth the

phenomenon of stimulus appraisal and whether it interacts in the way personality traits are related to better or worse psychophysiological adjustment to stress.

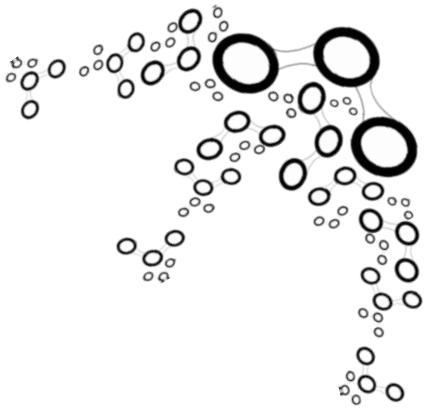
Taking into account that dysregulation of HPA axis functioning is related to alterations in memory and cognitive performance, it is possible that personality traits that affect the HPA axis play a role in cognitive processes. Previous studies supported the hypothesis that changes in the neuroticism trait in older people predict dementia development. Thus, in future studies it would be interesting to explore the relationship between personality traits and cognitive performance more in depth.

Although we investigated the effect of personality dimensions on some markers of diurnal HPA function, it would be interesting to expand our analyses to diurnal ANS functioning, as well as to other cardiovascular indexes related to health, such as Heart Rate Turbulence. At the same time, it would be interesting to test the possible effect of personality traits (e.g. optimism or neuroticism) in people diagnosed with any kind of CVD, such as hypertension. In the present dissertation thesis, optimism helped T2D people to show a physiological stress response similar to that of healthy counterparts. Therefore, it would be interesting to assess whether optimism could help people with CVD (e.g. hypertension) to have better ANS and HPA axis functioning, which, in turn, would promote psychophysiological well-being in the long run. Apart from this disease, it would be interesting to investigate the role of personality traits in people with other stress-related diseases, such as mood disorders (anxiety or depression), or people exposed to chronic stress (e.g. chronic pain such as fibromyalgia), given the evidence of disruptions in ANS and HPA axis functioning in these populations.



Chapter 9

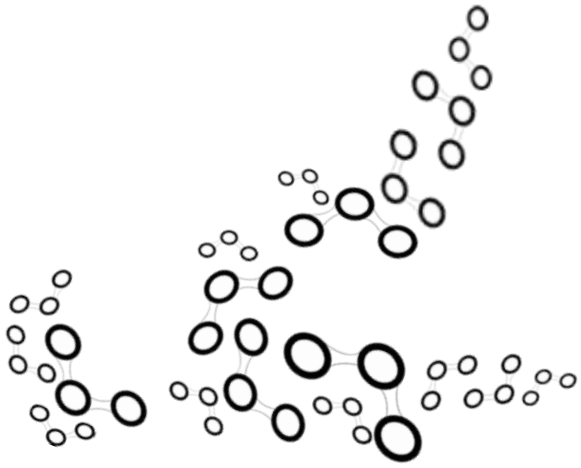
Main Conclusions



Main Conclusions

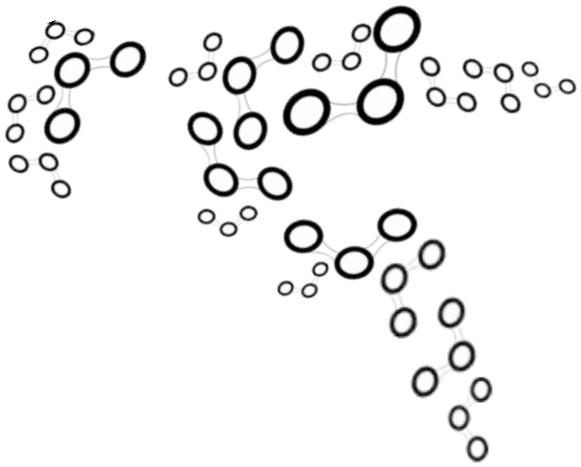
The following main conclusions can be drawn from the studies included in this thesis:

1. Neuroticism is not directly related to the ANS and HPA axis functioning under stress conditions in healthy older people.
2. Depressive mood has been related to an uncoordinated response to stress of the ANS and HPA axis: higher cortisol release and lower heart rate in response to stress in healthy older people.
3. Neuroticism was related to overall morning cortisol release in healthy older people, regardless of sex.
4. A positive relationship was found between neuroticism and CAR in women, but not in men. Sex hormones could be responsible for the sex moderation of the relationship between neuroticism and CAR.
5. Optimism was related to faster cortisol recovery after stress, but not to reactivity, in healthy older people.
6. Pessimism was only related to a more negative perception of the stressful stimuli in healthy older people, but not to the physiological stress response.
7. Optimism increased the cardiovascular response to stress and reduced the cortisol release throughout the day in people with T2D, making their stress response more similar to healthy counterparts.
8. T2D diagnosed people high in optimism reported higher subjective physical and mental well-being.
9. Optimism was not related to the CAR, a discrete component of the HPA axis, in healthy or T2D diagnosed people.
10. Higher positive bias was found in healthy older people with high optimism when they narrated their own lives, reflecting higher psychological well-being.



Chapter 10

Resumen General



1. Mecanismos psicofisiológicos implicados en la homeostasis

El organismo se adapta a los cambios del entorno a través del ajuste de múltiples sistemas fisiológicos (el sistema neuroendocrino, el metabólico, el inmune o el sistema nervioso autónomo) (McEwen, 2006). Este proceso de ajuste, conocido como *alostasis*, es necesario para preservar la adaptación a las demandas. No obstante, la estimulación repetida y mantenida de dichos sistemas puede conllevar lo que se conoce como *carga alostática*, lo cual incrementa el riesgo de desarrollar una enfermedad (Mattei et al., 2010).

Hace dos décadas McEwen propuso que la *carga alostática* altera la respuesta dinámica a retos o estresores agudos, lo cual puede generar un menoscabo en la capacidad de reacción y recuperación (McEwen, 1998). A pesar del papel adaptativo que tiene el incremento de actividad del sistema nervioso autónomo (SNA) y el eje hipotálamo-hipófiso-adrenal (HHA) ante un estrés agudo, una desregulación crónica de ambos sistemas conduce a un incremento en el riesgo de problemas de salud tanto físicos como mentales (Chida & Steptoe, 2010; McEwen, 2006; McEwen & Gianaros, 2011). Por lo tanto, el correcto funcionamiento del SNA y del eje HHA es necesario para el mantenimiento de la salud.

1.1. El Sistema Nervioso Autónomo

El SNA es uno de los sistemas más importantes para la homeostasis. Inerva la musculatura lisa y todos los órganos corazón y glándulas de nuestro organismo. Sus funciones se ejecutan, principalmente, bajo un control indirecto no voluntario, y se centran en: (i) mantener el medio interno del organismo constante, esto es, la homeostasis; (ii) ajustar la función del cuerpo a las circunstancias cambiantes internas o externas (p.ej. alimentación, deprivación de agua, etc.); y (iii) controlar órganos y sistemas implicados en la homeostasis (Jänig, 1989). Este complejo sistema se compone de tres ramas: el Sistema Nervioso Simpático (SNS), el Sistema Nervioso Para simpático (SNP) y el Sistema Nervioso Enteral (SNE).

El SNE es un sistema centrado en el tracto gastrointestinal, con una función independiente de los impulsos de la médula espinal y del tronco del encéfalo. Pero el SNS y SNP trabajan en un estado de equilibrio, siendo el SNS el involucrado en la movilización energética y el SNP el que se centra en la conservación de recursos. El músculo del corazón se encuentra inervado por ambos, SNS y SNP, ofreciendo así un claro ejemplo de las acciones antagonistas de estas dos ramas: el SNS incrementa la frecuencia cardíaca (FC), la conducción atrioventricular, la contracción del músculo cardíaco y dilata las arterias coronarias, mientras que el SNP reduce la FC y la contracción del músculo cardíaco (Gabella, 2001).

Los centros neurales del SNA están localizados en núcleos específicos del hipotálamo, el tronco del encéfalo y la médula espinal. El hipotálamo es la estructura mayor implicada en el funcionamiento del SNA, ejerciendo el principal control directo sobre el SNA y el sistema endocrino (Gabella, 2001). Aparte de las proyecciones desde el hipotálamo al tálamo, la glándula pituitaria y el prosencéfalo, un considerable número de neuronas del hipotálamo se conectan a los centros autonómicos que controlarán la actividad cardiaca (núcleo parabraquial, núcleo ambiguo, núcleo del tracto solitario y núcleo dorsal motor del nervio vago) y a neuronas preganglionares del SNS y SNP en la médula espinal (Gabella, 2001).

1.2. El eje Hipotálamo-Hipófiso-Adrenal

El eje HHA es un sistema fisiológico complejo que puede ser activado por inputs físicos y psicológicos. Inicialmente, las neuronas hipofisotrópicas del núcleo paraventricular (PVN) del hipotálamo son activadas por estímulos internos o externos, de naturaleza fisiológica o psicológica. Las neuronas CRH del PVN sintetizan hormona liberadora de corticotropina (CRH), mientras que las neuronas magnocelulares y CRH del PVN, junto con las neuronas del núcleo supraóptico (SON), sintetizan arginina-vasopresina (AVP). Ambas hormonas, CRH y AVP, son liberadas al sistema circulatorio portal de la eminencia media. Estas hormonas activan la hipófisis anterior induciendo la liberación al sistema circulatorio de

hormona adenocorticortropa (ACTH) por las células corticortropas. La ACTH viaja a través del torrente sanguíneo, estimulando el córtex de las glándulas adrenales. Esta estimulación induce la síntesis y liberación de glucocorticoides al torrente sanguíneo, siendo el cortisol el glucocorticoide más importante en humanos (Jacobson, 2005).

El control de la ACTH circulando en el organismo es un factor clave para la liberación de glucocorticoides por la glándula adrenal. Teniendo en cuenta que los glucocorticoides juegan un papel central en la regulación del eje HHA, la inhibición debida a la retroalimentación negativa que los glucocorticoides inducen sobre la liberación de ACTH hace posible regular sus concentraciones. En el caso de altos niveles de glucocorticoides, las neuronas del PVN inhiben la liberación de CRH y AVP (Whithall, 1993). Además, otras estructuras, como el hipocampo y el córtex prefrontal, median la regulación del eje HHA.

El eje HHA sigue un ritmo circadiano. Además, la liberación de CRH y AVP por las neuronas del PVN siguen un ciclo pulsátil con una frecuencia de secreción de 2-3 pulsos por hora (Liu et al., 1994). Las concentraciones de cortisol cambian a lo largo del día, mostrando mayores niveles de concentración por la mañana, con un incremento del 50% a 160% entre los 30 y 45 minutos después del despertar. Este fenómeno es conocido como la respuesta matutina de cortisol (CAR de las siglas en inglés, cortisol awakening response), y es observable aproximadamente en un 70-80% de los adultos sanos (Clow et al., 2010a; 2010b; Fries et al., 2009; Elder et al., 2014; Stalder et al., 2016). Tras dicho incremento, las concentraciones de cortisol muestran un descenso progresivo, alcanzando sus niveles más bajos durante la primera mitad del período de sueño (Pruessner et al., 1997). Pero en la última parte del periodo de sueño, los niveles de cortisol empiezan a aumentar de nuevo hasta el momento del despertar (Elder et al., 2014).

La primera descripción del CAR sugirió que este fenómeno puede ser un buen indicador de la actividad adrenocortical (Pruessner et al., 1997). Posteriormente, un amplio rango de estudios destacó la importancia

de estudiar el efecto de su desregulación en la salud (Chida & Steptoe, 2009; Stalder et al., 2016). Desafortunadamente, actualmente no se conoce el propósito del CAR, aunque se ha observado que el CAR es independiente de los niveles de cortisol durante el resto del día (Edwards et al., 2001a; Maina et al., 2009) y de la reactividad de cortisol inducida por estresores psicosociales (Bouma et al., 2009).

2. La respuesta psicofisiológica de estrés

A lo largo de la vida, las personas se ven expuestas a un amplio rango de circunstancias estresantes que implican la activación del SNA y del eje HHA. En dichas situaciones, ambos sistemas son activados debido a la percepción de estímulos que han sido considerados como un estresor, siendo algunos de ellos estímulos psicosociales.

2.1. ¿Cómo y qué puede incitar una respuesta de estrés?

Cuando se percibe un estímulo potencialmente estresante, las primeras áreas que se activan son el tálamo y el córtex prefrontal con el objetivo de integrar la información sobre el estímulo y el contexto. A continuación, se evalúa cómo de importante es el estímulo y su significado. Dependiendo de todo ello, el estímulo puede ser percibido como estresante elicitando una respuesta emocional. Aquí interviene el córtex prefrontal y otras estructuras que son parte del sistema límbico (p.ej. amígdala, hipocampo) y que están conectadas al hipotálamo, una de las principales estructuras implicadas en el control del SNA y el eje HHA, que finalmente induce o no la respuesta fisiológica de estrés (Gabella, 2001).

Según la *teoria social de la auto-preservación*, la motivación de preservar nuestro *ser social* induce una respuesta fisiológica de estrés (Dickerson & Kemeny, 2004). El *ser social* refleja la estima social, valor y estatus principalmente basado en la percepción de la sociedad sobre el valor de uno mismo (Gilbert, 1997; de Waal, 1989). Aquellas situaciones que producen una amenaza al *ser social* inducen una respuesta psicológica, fisiológica y conductual para afrontar dicha amenaza. La magnitud de esta respuesta depende principalmente de: la intensidad de la amenaza,

el contexto y la disponibilidad de factores protectores en el propio individuo o en la sociedad que lo rodea. En base a lo expuesto anteriormente, se han desarrollado múltiples estrategias para generar estresores psicosociales en laboratorio.

Selye (1956) consideró que todos los tipos de estresores provocan una respuesta fisiológica similar. No obstante, se ha observado una amplia variabilidad en la respuesta fisiológica evocada por estímulos psicológicos, lo cual pone de manifiesto la importancia de las respuestas emocionales específicas (Mason, 1968a; 1968b; 1975). Mientras que el SNA es más fácilmente activado, el eje HHA se activa en condiciones concretas. Estudios previos apoyan la sensibilidad del eje HHA a factores físicos y psicológicos (Dickerson & Kemeny, 2004), centrándose en la descripción de qué características deben tener los estímulos para inducir una respuesta del eje HHA. Los principales hallazgos sugieren que son aquellos estímulos percibidos como incontrolables (Henry & Grim, 1990; Sapolsky, 1993), impredecibles (Mason, 1968a; 1968b) o amenazantes (Blascovich & Tomaka, 1996) los capaces de inducir una respuesta del eje HHA. En un esfuerzo por delimitar qué características son necesarias en los estímulos psicológicos para elicitar una respuesta del eje HHA, Dickerson y Kemeny (2004) realizaron un meta-análisis con el que concluyeron que el hecho de afrontar una tarea motivante puede generar sentimientos de incontrolabilidad, resultados negativos inevitables o éxito inalcanzable a pesar del esfuerzo invertido. De hecho, afrontar el estrés que implica una situación de amenaza debido a la de evaluación social es la mejor estrategia para generar una respuesta del eje HHA. En conclusión, aquellas tareas que incluyan sensación de incontrolabilidad y amenaza por evaluación social son las más apropiadas para inducir una mayor secreción de cortisol. Es por ello que el Trier Social Stress Test (TSST, Kirschbaum et al., 1993) es uno de los mejores ejemplos y uno de los mejores protocolos de laboratorio para generar una respuesta de estrés.

2.2. La respuesta fisiológica a estrés

Afrontar situaciones estresantes implica la movilización de recursos para superar satisfactoriamente este tipo de dificultades. En esta línea, Cannon (1932) afirmó que las emociones negativas eran factores clave para iniciar la movilización de recursos fisiológicos necesarios para afrontar el estrés. De hecho, el primer paso para la respuesta psicofisiológica es la apreciación de la situación, esto es, evaluarla como estresante.

Existen diferentes teorías para explicar cómo la percepción del estrés induce cambios en los estados psicológicos y fisiológicos del individuo. Una de las más importantes es el *modelo biopsicosocial* (BPS) (Blacovich & Tomaka, 1996). Esta teoría destaca la importancia de la apreciación de estrés para la respuesta, ya que la forma en que es percibida y evaluada la situación tiene un enorme impacto en cómo se responde física y psicológicamente a la situación, así como en la ejecución de la respuesta (cognitiva y conductual) para afrontar la situación con éxito. La BPS establece dos formas como se aprecia la situación estresante: como una amenaza o como un reto. El hecho de considerar la situación de una forma u otra depende de la percepción de que la situación sobrepasa o no los recursos de los que se dispone para superarla. Aunque en ambos casos se produce una respuesta fisiológica, la percepción de la situación como un reto supone la generación de una respuesta más eficiente, acompañada de una mejor ejecución.

2.3. La respuesta del SNA

Cuando un estímulo es percibido como estresante, el primer sistema fisiológico activado es el SNA para inducir la respuesta de *fight or flight*, esto es, lucha o huida (Selye, 1936). El estímulo estresante es integrado y evaluado por el PVN, el cual interactúa con el SON, el nervio vago, la sección toracolumbar de la médula espinal y el locus coeruleus (Kyrou & Tsigos, 2009). Como resultado, el SNS se activa, induciendo la liberación de grandes concentraciones de catecolaminas: adrenalina desde la médula

adrenal y noradrenalina desde los nervios simpáticos (Granger et al., 2007; Iversen et al., 2000).

Además, como resultado de la activación del SNS, se produce un incremento de la frecuencia cardíaca, del flujo sanguíneo y de la presión arterial, así como una reducción en otras funciones vegetativas, lo cual refleja la predominancia del SNS sobre la actividad del SNP (Chrousos, 2009; Chrousos & Gold, 1992). El incremento de la influencia de la rama simpática puede, por tanto, ser observada a través de la frecuencia cardíaca y su variabilidad (Stein & Kleiger, 1999; Steptoe et al., 2009). No obstante, en los últimos años, se ha sugerido que el alfa-amilasa en saliva (sAA) puede ser considerado un indicador indirecto de la actividad del SNA en situaciones de estrés (Granger et al., 2007; Nater & Rohleder, 2009; Rohleder & Nater, 2009). Como resultado de la activación de la noradrenalina liberada por neuronas del SNS en los receptores beta-adrenérgicos de las glándulas salivares, se produce la liberación de sAA (principalmente por las glándulas parótidas).

2.4. La respuesta del eje HHA

Secundariamente, el eje HHA se activa, induciendo un rápido incremento en los niveles de cortisol (Kudielka & Wüst, 2010; Dickerson & Kemeny, 2004). Esta activación es producida por la acción integrada de tres estructuras: el hipotálamo, la glándula pituitaria y la glándula suprarrenal. Tras la evaluación del estímulo como estresante, el hipotálamo induce la producción y liberación de grandes cantidades de CRH y AVP desde el PVN. Estas hormonas son liberadas al sistema portal y estimulan la glándula pituitaria, la cual estimula la liberación de ACTH al torrente sanguíneo. La ACTH es transportada por la sangre, activando así las glándulas suprarrenales, las cuales son responsables de la liberación de glucocorticoides al torrente sanguíneo (Ulrich-Lai & Herman, 2009). En humanos, el producto final de la activación del eje HHA es el cortisol, considerado uno de los más importantes marcadores de la respuesta de estrés. La concentración de cortisol suele incrementarse y alcanzar su pico máximo entre 10-30 minutos después del estrés, siendo necesarios

alrededor de 60 minutos para recuperar los niveles basales (Ulrich-Lai & Herman, 2009; Dickerson & Kemeny, 2004).

2.5. La respuesta de estrés como una función integrada

Cabe destacar que la respuesta de estrés es un fenómeno complejo que debe ser entendido como una unidad (ver Figure 1). Como una parte no formal del eje HHA, el PVN tiene proyecciones descendentes al tronco del encéfalo y la médula espinal que expresan CRH, vasopresina y oxitocina (Palkovits, 1999). Estas proyecciones descendentes terminan monosinápticamente en neuronas preganglionares simpáticas y parasimpáticas, dando lugar a una conexión indirecta con la inervación simpática de la médula adrenal (Jacobson, 2005). En consecuencia, existe una conexión entre la función del eje HHA y el sistema nervioso central (ver Figure 1). Por esta razón, numerosos estudios se han focalizado en los efectos de la desregulación de la respuesta de estrés, es decir, en la disociación entre el SNA y el eje HHA ante el estrés. Se considera que la respuesta coordinada de ambos sistemas es más adaptativa que la función descoordinada (Bauer et al., 2002), de forma que la desregulación de la respuesta de estrés se ha visto asociada a un mayor número de problemas de salud (Ali & Pruessner, 2012).

En la última década, Gordis et al. (2006; 2008) y El-Sheik et al. (2008) analizaron la desregulación del SNA y el eje HHA a través de métodos de regresión. Recientemente, Ali y Pruessner (2012) desarrollaron un método que analiza la desregulación del SNA y el eje HHA y destacaron la relevancia de estudiar la interacción entre ambos biomarcadores. No obstante, es necesaria más investigación para establecer un método estándar y general para el estudio de la coordinación funcional entre el SNA y el eje HHA.

3. Factores moduladores del SNA y el eje HHA

Estudios previos han mostrado amplias diferencias entre los individuos en la respuesta autonómica y de cortisol ante estrés agudo. Por tanto, un conocimiento más profundo de los mecanismos implicados en la

regulación del eje HHA y del SNA podrían ayudar a entender dicha variabilidad. A su vez, el estudio de los factores moduladores de la actividad autonómica y del eje HHA permitiría una mejor aproximación en el conocimiento del funcionamiento de ambos sistemas fisiológicos, así como en su papel en los procesos de salud y enfermedad.

3.1. Edad

El envejecimiento ha sido definido como la pérdida gradual de la habilidad del organismo (i) para mantener la homeostasis, y (ii) para ajustarse a condiciones cambiantes (p.ej. estresores) (Masoro, 2005; Pardon, 2007). Esta pérdida progresiva de habilidad de adaptación a situaciones cambiantes puede ser explicada por el cambio asociado a la edad en el buen funcionamiento del SNA y del eje HHA.

Durante el proceso de envejecimiento, se ha observado una atenuación de la percepción de situaciones estresantes (Aldwin et al., 1996). Las personas mayores tienden a expresar menos emociones negativas que los jóvenes (Almela et al., 2011b; Labouvie-Vief et al., 1987; Lawtin et al., 1993). Además, las personas mayores tienden a percibir menor frustración en situaciones de estrés agudo comparado con jóvenes (Hidalgo et al., 2015).

Se ha observado, además, un cambio significativo de la capacidad de regulación en la actividad autonómica, observándose una mayor pérdida de regulación conforme envejecemos (Laitinen et al. 2004; Nicolini et al., 2012). No obstante, el efecto de la edad sobre el SNA en la respuesta de estrés sigue siendo controvertido debido a los resultados contradictorios encontrados en la literatura: algunos estudios sugieren que no hay cambios con la edad (Esler et al., 1995; Wood et al., 2002; Almela et al., 2011b), otros que la respuesta a estrés se encuentra atenuada (Kudielka et al., 2004b, 2004c; Strahler et al., 2010) y otros acentuada (Pascualya et al., 1999; Uchino et al., 1999).

Tal y como observamos en los parámetros cardiovasculares, la edad también afecta a la sAA. En condiciones basales no se ha observado

ningún cambio con la edad (Aguirre et al., 1987; Pajukoski et al., 1997; Salvolini et al., 1999). En contraste, un estudio reciente centrado en observar el ritmo circadiano del sAA en 185 participantes de entre 21 y 81 años mostró mayores niveles de sAA y una curva atenuada de sAA con la edad (Nater et al., 2013). En situaciones de estrés se ha observado una respuesta atenuada de sAA en personas mayores en comparación con jóvenes (Strahler et al., 2010). Aunque, por otra parte, también se ha observado una mayor respuesta de sAA en personas mayores en comparación a jóvenes (Almela et al., 2011b).

Respecto al eje HHA, la edad es uno de los factores más importantes en la regulación de los sistemas hormonales del organismo (Otte et al., 2005). Los cambios asociados a la edad en el eje HHA han sido explicados por la hipótesis de la cascada de glucocorticoides (Sapolsky et al., 1986). Según esta hipótesis, la exposición acumulada a glucocorticoides a lo largo de la vida provoca la degeneración de las estructuras cerebrales responsables de la regulación del eje HHA. Es decir, la secreción aumentada de cortisol contribuye a cambios degenerativos de las neuronas hipocampales, las cuales se vuelven incapaces de inhibir adecuadamente la liberación de glucocorticoides. En resumen, la cascada de retroalimentación negativa cambia con la edad, incrementando el riesgo de consecuencias fisiopatológicas en personas mayores (ver Figure 2). Estos hechos pueden observarse ante situaciones de estrés agudo, en las que diferentes estudios han observado mayor secreción de cortisol en personas mayores en comparación con jóvenes (Almela et al., 2011a; Kudielka et al., 2004 a; 2004c). Si bien es cierto que otros estudios sólo observaron una diferencia marginal con la edad en hombres (Kudielka et al., 2000; Rohleder et al., 2002) e incluso ninguna diferencia con la edad (Nicolson et al., 1997).

3.2. Sexo

Tal y como sucede con la edad, el sexo es uno de los factores más relevantes a tener en cuenta en el estudio de los mecanismos psicofisiológicos involucrados en la respuesta de estrés. De hecho, el sexo

es uno de los factores moduladores de la salud, incrementado el riesgo de desarrollar ciertos tipos de enfermedades dependiendo de si se es hombre o mujer (Verbrugge, 1989; Macintyre et al., 1996).

Estudios previos han analizado las diferencias sexuales en la respuesta psicológica de estrés mostrando resultados inconsistentes. Mientras que algunos estudios han observado un mayor incremento de ansiedad estado y estado negativo tras la exposición a un estresor en mujeres comparadas con hombres (Carrillo et al., 2001; Schmaus et al., 2008; Kelly et al., 2008; Childs et al., 2010), otros estudios no han encontrado diferencias (Kudielka et al., 2004a; Preuss & Wolf, 2009).

Respecto a la activación autonómica, estudios previos han observado mayor respuesta en mujeres jóvenes en comparación con hombres (Kudielka et al., 2004b; Fichera & Andreasi, 2000). No obstante, otros estudios no encontraron diferencias sexuales en la respuesta de estrés a diferentes tipos de estresores (Earle et al., 1999; Carrillo et al., 2001; Sgoifo et al., 2003; Kelly et al., 2008).

En el caso de la sAA, las diferencias han sido estudiadas tanto en las concentraciones diurnas como en la respuesta de estrés, observando que no hay diferencias a lo largo día en adultos (Nater et al., 2007), ni en situaciones de estrés en niños, adultos y personas mayores (Hidalgo et al., 2014, 2012; Thoma et al., 2012; Almela et al. 2011b; Bagley et al., 2011; Davis & Granger, 2009; Rohleder & Nater, 2009).

Finalmente, las diferencias sexuales en el funcionamiento del eje HHA han sido establecidas claramente. Existe amplia evidencia de mayor liberación de cortisol en respuesta al estrés en hombres en comparación con mujeres (Goel et al., 2014; Otte et al., 2005). No obstante, las características del estresor psicológico son altamente relevantes para explicar dichas diferencias entre sexos. En situaciones de interacción social, las mujeres muestran mayor liberación de cortisol que los hombres (Stroud et al., 2002). Pero si los participantes son expuestos a una situación

de evaluación psicosocial (p.ej. TSST), los hombres son más respondientes que las mujeres (Goel et al., 2014).

3.3. Factores psicológicos

Estudios previos han mostrado gran interés en estudiar la relación entre diferentes factores psicológicos y la respuesta del SNA y del eje HHA a estrés. Específicamente, se ha producido un incremento en los estudios centrados en estudiar la relación existente entre la personalidad y la salud a lo largo de las últimas décadas (Rasmussen et al., 2009). Se ha sugerido que las diferencias de personalidad afectan a la percepción del estrés (Connor-Smith & Flachsbart, 2007), que, a su vez, afecta a los sistemas biológicos implicados en la respuesta de estrés (Carver & Connor-Smith, 2010; Dickerson & Kemeny, 2004). Por tanto, la personalidad puede predisponer a un mejor o peor funcionamiento del SNA y del eje HHA, lo cual reduciría o aumentaría, respectivamente, el riesgo de desarrollar una patología. En esta línea, Chida y Hamer (2008) concluyeron tras un metanálisis que los estados psicológicos negativos, como la ansiedad o la hostilidad, están relacionados con una mayor respuesta cardiovascular a estrés, así como con una peor recuperación tras el estrés. Mientras que los estados o rasgos positivos, como la felicidad, parecen ejercer un efecto protector reduciendo la reactividad del eje HHA ante el estrés.

4. ¿Por qué los factores psicológicos importan?

Durante las últimas décadas, diferentes estudios han investigado el efecto perjudicial de los factores psicológicos negativos y el efecto protector de las actitudes positivas en la salud (Tindle et al., 2010; Chida & Hamer, 2008; Steptoe et al., 2009).

4.1. Factores perjudiciales

Actualmente, el rasgo de neuroticismo es la dimensión de personalidad más estudiada y relacionada con múltiples problemas conductuales y psicofisiológicos de salud (Kotov et al. 2010; Lahey, 2009). Existe amplia evidencia que establece el rasgo de neuroticismo como el

rasgo de personalidad más robusto predictor de psicopatología (Ormel et al., 2013; Lahey et al., 2009).

Los primeros intentos de establecer las bases biológicas del rasgo de neuroticismo fueron impulsados por las teorías de Eyseck y Gray, en las que el neuroticismo fue caracterizado como una respuesta exacerbada de estructuras cerebrales específicas que promovían la patología en personas con alto neuroticismo (Eysenck, 1967; Eysenck & Eysenk, 1985; Gray & McNaughton, 2000). No obstante, los avances en neuroimagen permiten una mejor comprensión de los circuitos cerebrales relacionados con este rasgo (ver Figure 3, de Ormel et al., 2013).

La respuesta exacerbada y generalizada asociada al neuroticismo no sólo se refiere a la actividad cerebral, especialmente de las estructuras límbicas, sino también a una mayor activación periférica (SNA y eje HHA). El rasgo de neuroticismo se ha relacionado con una mayor reactividad emocional a diferentes tipos de estresores, así como a una mayor percepción de los eventos estresantes (Bolger & Schiling, 1991; Suls et al., 1998; Vollrath, 2000; Connor-Smith & Flachbart, 2007). Estudios previos han relacionado el neuroticismo con un mayor descenso en afecto positivo, así como a un mayor aumento de afecto negativo tras la exposición a estrés en jóvenes adultos (Gomez et al., 2000; Rusting & Larsen, 1997; Larsen & Ketelaar, 1991; 1989). Sorprendentemente, esta relación no ha sido testada en edades avanzadas.

Otros estudios han investigado la relación entre este rasgo de personalidad y la respuesta de los principales sistemas fisiológicos con el objetivo de clarificar los posibles mecanismos subyacentes que relacionan el neuroticismo con el desarrollo de patologías vinculadas al estrés (Ormel et al., 2013). Estudios previos observaron que el neuroticismo se relacionaba con una mayor activación autonómica, pero menor liberación de cortisol, en jóvenes expuestos a estrés (Oswald et al., 2006; Phillips et al., 2005; Kennedy & Hughes, 2011; Schwebel & Suls, 1999; Kaiser et al., 1997; Kirschbaum et al., 1993; Stemmler & Meinhardt, 1990). Contrariamente, otros muchos estudios no encontraron dicha relación

(Verschoor & Markus, 2011; Wirtz et al., 2007; Knyazev et al., 2002; Schommer et al., 1999; Kirschbaum et al., 1992, 1995; Kirkcaldy, 1984; Hinton & Craske, 1977). Sólo conocemos un estudio que haya analizado la relación entre neuroticismo y la respuesta de estrés en personas de edad avanzada (entre 55 y 59 años), mostrando que el neuroticismo se encuentra asociado a una respuesta autonómica y de cortisol aplanada en situaciones estresantes (Bibbey et al., 2013).

Algunos estudios han investigado la relación entre la actividad basal del SNA y el neuroticismo, encontrando una relación negativa (Bleil et al., 2008; Riese et al., 2007) o ninguna relación significativa (Knyazev et al., 2002; Vassend & Knardahl, 2005). Respecto al eje HHA, diferentes estudios han investigado la relación entre neuroticismo (junto con el rasgo de extraversión), el CAR y las concentraciones totales de cortisol matutino a través del área bajo la curva con respecto a la base (AUC_G). En adolescentes, Hauner et al. (2008) mostraron que un mayor neuroticismo e intraversión estaba relacionado con una baja AUC_G. Van Santen et al. (2011) encontraron resultados similares con extraversion, pero no obtuvieron resultados significativos con neuroticismo. Contrariamente, Hill et al. (2013) encontraron una relación positiva entre los niveles de extraversión y AUC_G, mientras que no encontraron ninguna relación significativa con neuroticismo en una muestra con un amplio rango de edad (17 to 78 años). Portella et al. (2005) investigaron la relación entre neuroticismo, CAR y AUC_G en personas de entre 21 y 57 años, mostrando una relación positiva. En línea con estos resultados, Mandold et al. (2012) mostraron una relación positiva entre neuroticismo y AUC_G mediada por el nivel de aculturación en adultos. No obstante, algunos estudios no han observado relaciones significativas entre neuroticismo, extraversión, CAR y AUC_G (e.g., Chan et al. 2007; Munafo et al. 2006; Laceulle et al., 2015).

4.2. Factores protectores

En las últimas décadas, una serie de estudios se han centrado en investigar aquellos factores que pueden tener un efecto protector en el desarrollo de patologías. De hecho, factores protectores, como la

resiliencia, se consideran hoy en día temas de gran interés científico (Crump et al., 2016; Steptoe, 2016).

Entre los rasgos protectores, el optimismo es uno de los más importantes, siendo el cuestionario "Life Orientation Test" (LOT) (Scheier and Carver, 1985), que fue posteriormente revisado (LOT-R) (Scheier et al., 1994) el más usado para medir dicho rasgo. El LOT-R mide el optimismo disposicional a través de dos subescalas: optimismo y pesimismo. Aquellas personas más optimistas son las que tienden a mostrar expectativas más positivas respecto a su futuro, mientras que las personas con mayor pesimismo generan expectativas negativas (Carver et al., 2010; Scheier & Carver, 1992; Scheier et al., 1994).

Una de las razones más importantes para estudiar el optimismo es la falta de estudios centrados en investigar los mecanismos que subyacen a la relación existente entre el optimismo y el estado de salud. Debido al contrastado impacto del optimismo sobre la salud, ha habido un incremento en explicar cómo este rasgo podría ejercer un impacto protector en la salud (Rasmussen et al., 2009).

Es altamente probable que el efecto protector del optimismo ocurra debido a una mayor propensión de las personas optimistas a realizar conductas saludables (Carver et al., 2010). De acuerdo con la teoría de la auto-regulación (Carver & Scheier, 2000), la forma en que las personas afrontan los retos o dificultades podrían afectar a cómo afrontan el estrés (Carver et al., 2010). En general, las personas con mayor optimismo tienden a usar un estilo de afrontamiento activo cuando se enfrentan a un reto, y son menos propensas a adoptar un afrontamiento evitativo, el cual ha sido asociado a peores resultados a largo plazo (Carver & Connor-Smith, 2010; Taylor & Stanton, 2007; Solberg Nes & Segerstrom, 2006).

A pesar de la reconocida contribución de las conductas saludables y los estilos de afrontamiento al efecto protector del optimismo, los resultados de la mayoría de estudios persisten a pesar de controlar estos factores (e.g. Giltay et al., 2006; Nabi et al., 2010). Este hecho sugiere que otras vías pueden estar involucradas, lo que genera la necesidad de estudiar la relación entre el optimismo y los mecanismos psicofisiológicos

asociados al desarrollo de patologías vinculadas al estrés (SNA y eje HHA). La investigación centrada en estudiar dicha relación no ha mostrado resultados consistentes. Por una parte, se ha observado una respuesta de cortisol y frecuencia cardíaca mayor ante estrés en jóvenes con mayor optimismo (Solberg Nes et al., 2005), así como una rápida recuperación del cortisol (Brydon et al., 2009). En contraste, en personas mayores no se ha observado una relación significativa (Endrighi et al., 2011).

Teniendo en cuenta que las personas con alto optimismo tienden a mostrar un afrontamiento proactivo y una mayor implicación en las tareas estresantes de laboratorio (Carver & Connor-Smith, 2010; Solberg Nes & Segerstrom, 2006), es posible un alto optimismo genere un arousal mayor cuando la persona sienta que se enfrenta a un reto, lo cual conllevaría una respuesta cardiovascular y endocrina mayor (Solberg Nes et al., 2005). No obstante, al mismo tiempo, se espera que una perspectiva optimista proteja a la persona de respuestas exacerbadas de estrés.

Algunos estudios se han centrado en investigar la relación entre optimismo, cortisol circadiano y CAR. Jobin et al. (2014) mostraron en personas mayores que un bajo optimismo se relaciona con niveles de cortisol más altos a lo largo del día y bajo CAR, pero sólo en aquellos días en que las personas expresaron una mayor estrés subjetivo. No obstante, otros estudios con personas mayores (Endrighi et al., 2011) y con adultos mayores (Lai et al., 2005) mostraron una relación negativa entre el optimismo y el CAR, pero no encontraron relación alguna con el declive de cortisol a lo largo del día, el perfil de cortisol o el total de cortisol liberado a lo largo del día.

Hoy en día, los mecanismos subyacentes al efecto protector o perjudicial que tienen diferentes factores psicológicos sobre la salud permanecen desconocidas. Por esta razón, en las últimas décadas ha habido un incremento en el interés por clarificar los posibles mecanismos psicofisiológicos implicados en la relación entre los factores psicológicos y la salud (Rasmussen et al., 2009).

Respecto a los mecanismos psicológicos, estudios previos han sugerido que la reevaluación positiva de los eventos de nuestra vida tiene un papel importante en nuestro bienestar psicológico en edades avanzadas (Löckenhoff & Carstensen, 2004; Carstensen et al., 1999) y en la aceptación de la muerte (Cappeliez et al., 2007; Webster, 1993). La teoría de selección socio-emocional (Carstensen et al., 1999) sugiere la idea de que las personas muestran un sesgo hacia la positividad a medida que envejecen. De hecho, las personas mayores tienden a mostrar más atención hacia hechos positivos y a recordar más palabras o imágenes positivas (Mather & Carstensen, 2005). Es por ello que resulta de interés investigar cuándo las personas que muestran expectativas positivas de futuro reevalúan su pasado de una forma más positiva, contribuyendo de esta forma a un mayor bienestar psicológico. E Existen evidencias de un solapamiento en las estructuras cerebrales involucradas en la generación de expectativas futuras y el recuerdo de eventos autobiográficos (Addis et al., 2007; Buchner & Carroll, 2007; Spreng et al., 2009, 2010). Además, de acuerdo con la propuesta de Seligman (1991), la generación de expectativas de futuro deriva de la percepción de nuestro pasado. Por tanto, sería razonable pensar que el hecho de generar expectativas de futuro positivas deriva de una percepción positiva de nuestro pasado. Y lo contrario sería esperado de aquellas personas que tienden a mostrar expectativas negativas de su pasado. En esta línea, por ejemplo, las personas con mayor neuroticismo tienden a relatar más emociones negativas cuando narran eventos personales (Webster, 1994). Por otra parte, un estado de ánimo negativo incrementa el uso de palabras con valencia negativa como ocurre en el caso de personas con depresión (Fromholt et al., 1995; Mathews & MacLeod, 1994; Mat et al., 1992).

Respecto a los mecanismos fisiológicos que se encuentran implicados en la relación entre factores psicológicos y la salud, Tindle et al. (2010) sugirieron un modelo conceptual que trata de explicar cómo las actitudes positivas o negativas pueden reducir o incrementar, respectivamente, el riesgo de desarrollo de patologías cardiovasculares. En este modelo se sugiere que el optimismo y el pesimismo pueden afectar

a la salud indirectamente a través de la ejecución o no de conductas saludables, y directamente a través de procesos psicofisiológicos (incluidos la activación de los sistemas de respuesta de estrés). Estas dos vías pueden, por tanto, incrementar o reducir el riesgo de desarrollo de patologías vinculadas al estrés, como las patologías cardiovasculares o la diabetes tipo II (T2D) (e.g. Tinker et al., 2007; Matthews et al., 2004). La T2D es una de las enfermedades más prevalentes en personas mayores de 50 años (8-10%) (Scully, 2012). De hecho, la T2D comprende alrededor del 90% de los diagnósticos de diabetes en población adulta del mundo (Zimmet et al., 2001). La T2D es considerada una enfermedad metabólica heterogénea que se caracteriza por una reducida sensibilidad a la insulina, acompañada de una relativa deficiencia de insulina (McCrimmon et al., 2012). Desafortunadamente, el estilo de vida (por ejemplo, un estilo de vida sedentario, el consumo de alcohol o el sobrepeso) y los factores socioeconómicos relacionados con el estilo de vida actuales están incrementando la prevalencia de T2D esperada para las próximas décadas (Zimmet et al., 2001). Existen evidencias que muestran la relación entre la T2D y el desarrollo de otras patologías, como retinopatías (Samiec et al., 1998), disfunción cognitiva (Kloppenborg et al., 2008; van Harten et al., 2007) y enfermedad de Alzheimer y riesgo de demencia vascular (Biessels et al., 2008). No obstante, el riesgo de desarrollar patologías cardiovasculares en la T2D es el más preocupante debido a que el 80% de las muertes de pacientes con T2D son causadas por patologías cardiovasculares (Sowers et al., 2001). Además, es importante destacar que la T2D a menudo suele ser una manifestación del síndrome metabólico, una constelación de factores de riesgo de patología cardiovascular y diabetes como la hiperglicemia, la hipertensión, la dislipidemia y la obesidad central (Zimmet et al., 2001). En esta línea, niveles elevados de interleuquina-6 (IL-6) se han visto relacionados con un mayor riesgo de patologías cardiovasculares (Danesh et al., 2008) y T2D (Steptoe et al., 2014). Estos hallazgos sugieren que los pacientes con T2D se caracterizan por alteraciones en la función autonómica, neuroendocrina

y del sistema inmune, aumentando así su vulnerabilidad hacia la enfermedad.

En conclusión, el estudio de los factores que contribuyen al detrimento de la salud, o que, contrariamente, pueden ayudar a reducir el riesgo de desarrollar una enfermedad son de gran relevancia, especialmente en personas mayores debido a su mayor vulnerabilidad. Es posible que los factores que afectan a la actividad del SNA y al eje HHA sean factores clave en el desarrollo de patologías vinculadas al estrés, como es el caso de la T2D. A su vez, es posible que las personas con diagnóstico de aquellas patologías que se han visto relacionadas con una desregulación del SNA y del eje HHA puedan preservar una mejor salud gracias al efecto protector de factores psicológicos. Por tanto, la investigación en estos campos de conocimiento podría ayudar a entender cómo y por qué motivos algunas personas muestran un envejecimiento satisfactorio, así como a reducir el impacto de ciertas enfermedades extendidas en población mayor, como la T2D.

5. Objetivos e hipótesis

Como hemos comentado anteriormente, sólo unos pocos estudios han investigado el papel protector o perjudicial de factores psicológicos sobre los principales sistemas fisiológicos involucrados en la respuesta de estrés en personas mayores. Considerando la importancia que éstos sistemas fisiológicos en la salud, el objetivo principal de esta tesis doctoral es investigar los posibles factores psicológicos que protegen o contribuyen a un mal funcionamiento del SNA y del eje HHA.

Objetivo 1. El objetivo principal fue investigar la relación entre el rasgo de neuroticismo, el estado de ánimo depresivo, y la respuesta psicofisiológica a estrés en personas mayores sanas. Esperamos una desregulación entre el SNA y el eje HHA en situaciones de estrés en aquellas personas con mayor neuroticismo y estado de ánimo depresivo, así como una mayor ansiedad y afecto negativo tras la exposición a estrés.

Objetivo 2. El segundo objetivo fue investigar el efecto moderador de los rasgos de neuroticismo y extraversión sobre el CAR y la liberación total de cortisol matutino (AUC_G) en personas mayores sanas. En línea con estudios previos, esperamos encontrar una relación positiva entre CAR, AUC_G y extraversión. Pero debido a los resultados contradictorios hayados en estudios previos en neuroticismo, no establecimos ninguna dirección específica de la relación con este rasgo. Además, exploramos el efecto moderador del sexo en las relaciones estudiadas.

Objetivo 3. En este objetivo nos focalizamos en aquellos factores protectores de la función del SNA y del eje HHA, por lo que nos centramos en estudiar la relación entre el optimismo disposicional y sus sub-escalas y la respuesta psicofisiológica de estrés en personas mayores sanas. Esperamos una relación positia entre el optimismo y la reactividad del SNA y el eje HHA, así como una recuperación más rápida. Respecto al pesimismo, esperamos relaciones opuestas. Además, exploramos el efecto del optimismo y el pesimismo en la apreciación del estresor.

Objetivo 4. Este objetivo se centra en el papel del optimismo disposicional y sus sub-escalas en el CAR y la reevaluación de la vida en personas mayores sanas, entendiendo que una reevaluación positiva se relaciona con un mayor bienestar. Se esperó un menor CAR en personas con mayor optimismo comparado con personas con algo optimismo. Respecto a la forma en que las personas optimistas reevaluaban eventos pasados de sus vidas, esperamos un mayor foco en eventos, cogniciones y emociones positivas en aquellas personas que mostraron un mayor optimismo.

Objetivo 5. El último objetivo de esta tesis fue analizar el efecto del optimismo disposicional en la respuesta psicofisiológica a estrés, así como la actividad diurna del eje HHA, en personas con diagnóstico de T2D. Debido a que se espera una respuesta atenuada a estrés en personas con T2D, esperamos que el optimismo ayude a estas personas a mostrar una respuesta más parecida a personas mayores sanas. Por tanto, esperamos

una mayor respuesta fisiológica de estrés en personas con diagnóstico de T2D con alto optimismo, así como un menor nivel de cortisol diurno.

6. Estudios desarrollados

Estudio 1

En el presente estudio nos hemos centrado en analizar el posible efecto negativo del rasgo de personalidad neuroticismo y del estado de ánimo depresivo en la respuesta psicofisiológica de estrés en personas mayores sanas. Para ello, 36 voluntarios sanos mayores fueron expuestos a un estresor psicosocial estandarizado (TSST) mientras que 35 voluntarios fueron sometidos a una tarea no estresante. A lo largo de la sesión experimental, fueron registrados los niveles de cortisol, alfa-amilasa en saliva y frecuencia cardíaca antes, durante y después de la exposición a la tarea estresante y no estresante. Además, se registró el nivel de ansiedad estado, afecto positivo y afecto negativo antes y después de la exposición a dichas tareas. Los participantes también completaron el Eysenck Personality Questionnaire (EQP) y el Beck Depression Inventory (BDI) con el objetivo de medir el rasgo de neuroticismo y el estado de ánimo depresivo, respectivamente. Los resultados mostraron que el neuroticismo no afectaba a la respuesta psicofisiológica de estrés. En cambio, un mayor estado de ánimo depresivo se vio relacionado con una mayor respuesta de cortisol, así como una respuesta aplanada de la frecuencia cardíaca ante estrés. Los principales resultados del presente estudio han sido publicados en: Puig-Perez, S., Villada, C., Pulopulos, M.M., Hidalgo, V., Salvador, A. (2016). How are neuroticism and depression related to the psychophysiological stress response to acute stress in healthy older people? Physiology & Behavior, 156, 128-136.

Estudio 2

El objetivo del segundo estudio se centró en el papel de los rasgos de personalidad de neuroticismo y extraversión sobre la respuesta matutina de cortisol (CAR) y los niveles de cortisol matutinos (AUC_G), estudiando además la existencia de diferencias sexuales en dicha relación. Para ello, un total de 160 de personas mayores de 55 años y sanas recogieron 3 muestras de saliva en el momento del despertar y 30 y 45 minutos después de despertar durante dos días consecutivos entre semana. Además, completaron el Eysenck Personality Questionnaire (EQP). Los resultados mostraron que, sin tomar en consideración el sexo, el rasgo de neuroticismo no se vio relacionado con el CAR, pero sí con una menor concentración de cortisol matutino (AUC_G). Tras analizar el efecto del sexo/género, observamos que éste afectó a la relación entre neuroticismo y CAR, pero no entre neuroticismo y AUC_G. Observamos que sólo en las mujeres, el neuroticismo se relacionó con mayor CAR. Finalmente, el rasgo de extraversión no mostró relación alguna con el CAR ni con el AUC_G, incluso después de explorar el efecto del sexo/género en dichas relaciones. Los principales resultados del presente estudio han dado lugar a un manuscrito que ha sido publicado: Puig-Perez, S., Pulopulos, M. M., Hidalgo, V., & Salvador, A. (2016). Are Neuroticism or extraversion related to morning cortisol release in healthy older peole? *International Journal of Psychophysiology* (in press).

Estudio 3

El objetivo del presente estudio fue analizar el efecto del optimismo disposicional y sus sub-escalas sobre la respuesta fisiológica al estrés y la apreciación del estresor en un total de 72 personas mayores sanas de entre 55 y 76 años de edad. En dicho estudio, los participantes fueron asignados aleatoriamente a dos grupos: grupo de estrés y grupo control. El grupo estrés fue sometido a un estresor psicosocial estandarizado (Trier Social Stress Test, TSST), mientras que el grupo control realizó una tarea control ad

hoc similar a la realizada por el grupo experimental, pero sin el componente estresante. A lo largo de ambas sesiones se registraron los niveles de cortisol y frecuencia cardíaca a nivel basal, durante la exposición a la tarea estresante o a la tarea control y después. Además, los participantes completaron el cuestionario Live Orientation Test – Revised (LOT-R) y establecieron qué nivel de estrés, frustración, esfuerzo y dificultad tenía la tarea a la que habían sido expuestos, así como la importancia que había tenido para ellos hacerla correctamente. Nuestros resultados mostraron que la sub-escala de optimismo estaba relacionada con una recuperación más rápida de los niveles de cortisol y de frecuencia cardíaca en aquellas personas expuestas a estrés. Mientras que la sub-escala de pesimismo no se relacionó con la respuesta fisiológica, sí que facilitó una percepción más negativa del estresor incrementando la sensación de dificultad y esfuerzo requerido para afrontarlo. Los principales resultados del presente estudio han sido publicados en: Puig-Perez, S., Villada, C., Pulopulos, M.M., Almela, M., Hidalgo, V., Salvador, A. (2015). Optimism and pessimism are related to different components of the stress response in healthy older people. International Journal of Psychophysiology, 98 (2-Part 1), 213-221.

Estudio 4

Este estudio se ha focalizado en analizar el efecto del optimismo disposicional y sus sub-escalas (optimismo y pesimismo) sobre la respuesta matutina de cortisol (CAR) y el recuerdo de eventos autobiográficos positivos y negativos. Un total de 77 participantes completaron el LOT-R, recogieron 3 muestras de saliva al despertar y a los 30 y 45 minutos después de despertar en dos días consecutivos entre semana, y resumieron en una hoja los eventos, cogniciones y emociones más importantes de su vida. Tres jueces externos valoraron las narraciones de las personas que participaron de acuerdo a 4 categorías, realizando un conteo del número de eventos positivos o negativos que la persona relató en su evento, así como de pensamientos o emociones positivas o negativos plasmadas en su

narración. En general, las mujeres mostraron un mayor número de cogniciones y emociones que los hombres. Aquellas personas con mayor optimismo disposicional mostraron un mayor porcentaje de recuerdo de eventos, emociones y cogniciones positivas. Del mismo modo, las personas con mayor pesimismo se focalizaron en aquellos eventos, emociones y cogniciones negativas. Finalmente, el optimismo disposicional y la sub-escala de optimismo se relacionó con un mayor CAR, no obstante, este resultado no se replicó cuando repetimos los resultados sólo con aquellas personas que mostraron respuesta del CAR en los dos días medidos. Los resultados de este estudio han sido sometidos para su publicación en: Puig-Perez, S., Pulopulos, M. Hidalgo, V., & Salvador, A. Being an Optimist or a Pessimist and its Relationship with Morning Cortisol Release and Past Life Review in Healthy Older People. *Psychology & Health.*

Estudio 5

El último estudio se focalizó en el efecto del optimismo disposicional sobre la respuesta psicofisiológica ante estrés, así como en la función circadiana del eje hipotálamo-hipofiso-adrenal (respuesta matutina de cortisol y secreción diurna de cortisol), en personas con diabetes tipo II. Un total de 140 personas con diagnóstico de diabetes tipo II del área metropolitana de Londres fueron expuestas a un estresor estandarizado de laboratorio (Stroop Test y Mirror Strar Tracing Test). Antes, durante y después del estresor, se registraron los niveles de presión arterial, frecuencia cardíaca, cortisol y estrés percibido. Además, de forma ambulatoria las personas recogieron 5 muestras de saliva a lo largo de un día (en el momento del despertar, 30 minutos después de despertar, entre las 10 y las 10:30, entre las 16:00 y 16:30, y entre las 20:00 y 20:30) y completaron el LOT-R. Las personas con diabetes tipo II participantes en este estudio mostraron una respuesta fisiológica aplanada a estrés comparada con personas sanas (Steptoe et al., 2014). Nuestros resultados muestran que aquellas personas con mayor optimismo disposicional presentaron una respuesta cardiovascular al estrés menos aplanada que las personas con bajo

optimismo disposicional, percibiendo además la sesión experimental como menos estresante. Además, aunque el optimismo disposicional no se relacionó con el CAR, sí que observamos que las personas con mayor optimismo mostraron menos niveles de cortisol a lo largo del día. Los resultados de este estudio han dado lugar a un manuscrito que está actualmente en revisión: Puig-Perez, S., Hackett, R., Salvador, A. & Steptoe, A. (2016). Optimism moderates psychophysiological responses to stress in older people with type 2 diabetes. *Psychophysiology*.

7. Conclusiones principales

Las principales conclusiones derivadas de los estudios que contiene la presente tesis doctoral son:

1. El rasgo de neuroticismo no se ha encontrado relacionado con la actividad del SNA ni del eje HHA en situaciones de estrés en personas mayores sanas.
2. El estado de ánimo depresivo se ha visto relacionado con una respuesta descoordinada ante estrés del SNA y del eje HHA: alta liberación de cortisol y baja respuesta cardiovascular en situaciones de estrés agudo en personas mayores sanas.
3. El rasgo de neuroticismo ha sido relacionado con las concentraciones totales de cortisol durante los primeros 45 minutos después del despertar en personas mayores sanas, independientemente del sexo.
4. Las hormonas sexuales pueden ser responsables del efecto moderador del sexo en la relación entre el rasgo de neuroticismo y el CAR: existe una relación positiva en mujeres, pero no en hombres.
5. El optimismo ha sido relacionado con una recuperación más rápida de los niveles de cortisol en personas mayores sanas.
6. El pesimismo se relaciona con una percepción más negativa de los estímulos estresantes en personas mayores sanas.

7. El optimismo se ha visto relacionado con una mayor respuesta cardiovascular a estrés en personas mayores con diabetes mellitus, lo cual hace más similar su respuesta a las personas mayores sanas.
8. Las personas con diabetes mellitus con alto optimismo mostraron mayor bienestar subjetivo a nivel físico y psicológico.
9. El optimismo no se relacionó con el CAR, componente del eje HHA, en personas mayores sanas o con diagnóstico de diabetes mellitus.
10. Hay un sesgo positivo mayor en personas mayores sanas con alto optimismo cuando narran sus propias vidas, reflejando un mayor bienestar psicológico.

Financiación

La autora de esta tesis doctoral recibió una beca de doctorado por parte del Ministerio de Educación Español dentro del programa para la Formación de Personal Universitario (beca nº FPU12/04597).

Los estudios presentados en esta tesis doctoral han sido financiados por el Ministerio de Ciencia y Tecnología Español (proyecto nº PSI2010/21343), la Generalitat Valenciana (proyectos nº ACOMP/2013/0200, ACOMP/2012/0240, ACOMP/2011/0133, ISIC/2013/01, PROMETEO 2011/048, PROMETEOII 2015/020) y la British Heart Foundation.

Funding source

The author of this doctoral dissertation has been supported by the Spanish Ministry of Education (grant no FPU12/04597).

The studies reported in this doctoral dissertation have been supported by the Spanish Ministry of Science and Technology (project no. PSI2010/21343), Generalitat Valenciana (projects no. ACOMP/2013/0200, ACOMP/2012/0240, ACOMP/2011/0133, ISIC/2013/01, PROMETEO 2011/048, PROMETEOII 2015/020) and the British Heart Foundation.

REFERENCES

Achat, H., Kawachi, I., Spiro, A., DeMolles, D. A., & Sparrow, D. (2000). Optimism and depression as predictors of physical and mental health functioning: the Normative Aging Study. *Annals of Behavioral Medicine*, *22*(2), 127-130.

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*, *34*(10), 1423–1436.

Addis, D. R., Wong, A. T., & Schacter, D. L. (2007). Remembering the past and imagining the future: Common and distinct neural substrates during event construction and elaboration. *Neuropsychologia*, *45*(7), 1363-1377.

Adler, N. E., Epel, E. S., Castellazzo, G., & Ickovics, J. R. (2000). Relationship of subjective and objective social status with psychological and physiological functioning: Preliminary data in healthy, White women. *Health Psychology*, *19*, 586–592.

Aguilera, G., (2011). HPA axis responsiveness to stress: implications for healthy aging. *Experimental Gerontology, 46*(2-3), 90-95.

Aguirre, A., Levine, M. J., Cohen, R. E., & Tabak, L. A. (1987). Immunochemical quantitation of α-amylase and secretory IgA in parotid saliva from people of various ages. *Archives of oral biology*, *32*(4), 297-301.

Aiken, L., & West, S.G., (1991). Multiple Regressions: Testing and Interpreting Interactions. Thousand Oaks, CA: Sage Publications, McElroy S., USA.

Aldwin, C. M., Sutton, K. J., Chiara, G., & Spiro, A. (1996). Age differences in stress, coping, and appraisal: Findings from the normative aging study. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *51*(4), P179-P188.

Ali, N., & Pruessner, J. C. (2012). The salivary alpha amylase over cortisol ratio as a marker to assess dysregulations of the stress systems. *Physiology & behavior*, *106*(1), 65-72.

Almela, M., Hidalgo, V., Villada, C., Espín, L., Gómez-Amor, J., & Salvador, A. (2011a). The impact of cortisol reactivity to acute stress on memory: sex differences in middle-aged people. *Stress*, *14*(2), 117-127.

Almela, M., Hidalgo, V., Villada, C., van der Meij, L., Espín, L., Gómez-Amor, J., & Salvador, A. (2011b). Salivary alpha-amylase response to acute psychosocial stress: The impact of age. *Biological psychology, 87*(3), 421-429.

Almela, M., Meij, L. van der, Hidalgo, V., Villada, C., & Salvador, A., (2012). The cortisol awakening response and memory performance in older men and women. *Psychoneuroendocrinology*, *37*, 1929–1940.

Appelhans, B. M., & Luecken, L. J. (2006). Heart rate variability as an index of regulated emotional responding. *Review of general psychology*, *10*(3), 229.

Aspinwall, L. G., & Richter, L. (1999). Optimism and self-mastery predict more rapid disengagement from unsolvable tasks in the presence of alternatives. *Motivation & Emotion, 23*, 221–245.

Baggett, H. L., Saab, P. G., & Carver, C. S. (1996). Appraisal, coping, task performance, and cardiovascular responses during the evaluated speaking task. *Personality and Social Psychology Bulletin*, *22*(5), 483-494.

Bagley, S. L., Weaver, T. L., & Buchanan, T. W. (2011). Sex differences in physiological and affective responses to stress in remitted depression.*Physiology & behavior*, *104*(2), 180-186.

Barry, J. A., Hardiman, P. J., Saxby, B. K., & Kuczmierczyk, A. (2011). Testosterone and mood dysfunction in women with polycystic ovarian syndrome compared to subfertile controls. *Journal of Psychosomatic Obstetrics & Gynecology*, *32*(2), 104-111.

Bauer, A. M., Quas, J. A., & Boyce, W. T. (2002). Associations between physiological reactivity and children's behavior: Advantages of a multisystem approach. *Journal of Developmental & Behavioral Pediatrics*, *23*(2), 102-113.

Baum, B. J. (1993). Principles of saliva secretion. *Annals of the New York Academy of Sciences*, *694*(1), 17-23.

Beck, A. T., Ward, C. H., Mendelson, M., Mock, J., & Erbaugh, J. K. (1961). An inventory for measuring depression. *Archives of general psychiatry*, *4*(6), 561-571.

Bhattacharyya, M. R., Molloy, G. J., & Steptoe, A. (2008). Depression is associated with flatter cortisol rhythms in patients with coronary artery disease.*Journal of psychosomatic research*, *65*(2), 107-113.

Bibbey, A., Carroll, D., Roseboom, T. J., Phillips, A. C., & de Rooij, S. R. (2013). Personality and physiological reactions to acute psychological stress. *International Journal of Psychophysiology*, *90*(1), 28-36.

Biessels, G. J., Deary, I. J., & Ryan, C. M. (2008). Cognition and diabetes: a lifespan perspective. *The Lancet Neurology*, *7*(2), 184-190.

Björntorp, P. (1991). Psychological factors and fat distribution. In: *Obesity in Europe* 1991, Ailhaud, G., Guy-Grand, B., Lafontan, M., Ricquier, D., eds. *London*: John Libbey, 1992: 377-387.

Blascovich, J., & Tomaka, J. (1996). The biopsychosocial model of arousal regulation. *Advances in experimental social psychology*, *28*, 1-52.

Bleil, M. E., Gianaros, P. J., Jennings, J. R., Flory, J. D., & Manuck, S. B. (2008). Trait negative affect: toward an integrated model of understanding psychological risk for impairment in cardiac autonomic function.*Psychosomatic Medicine*, *70*(3), 328-337.

Boehm, J. K., & Kubzansky, L. D. (2012). The heart's content: the association between positive psychological well-being and cardiovascular health. *Psychological bulletin*, *138*(4), 655.

Bolger, N., & Schilling, E. A. (1991). Personality and the problems of everyday life: The role of neuroticism in exposure and reactivity to daily stressors. *Journal of personality*, *59*(3), 355-386.

Bouma, E. M., Riese, H., Ormel, J., Verhulst, F. C., & Oldehinkel, A. J. (2009). Adolescents' cortisol responses to awakening and social stress; effects of gender, menstrual phase and oral contraceptives. The TRAILS study. *Psychoneuroendocrinology*, *34*(6), 884-893.

Brenes, G. A., Rapp, S. R., & Rejeski, W. J. (2002). Do optimism and pessimism predict physical functioning? *Journal of Behavioral Medicine, 25*, 219–231.

Brindle, R. C., Ginty, A. T., & Conklin, S. M. (2013). Is the association between depression and blunted cardiovascular stress reactions mediated by perceptions of stress? *International Journal of Psychophysiology*, *90*(1), 66-72.

Broderick, J.E., Arnold, D., Kudielka, B.M., & Kirschbaum, C., (2004). Salivary cortisol compliance: comparison of patients and healthy volunteers. *Psychoneuroendocrinology, 29*, 636-650.

Brooks, K. P., & Robles, T. F. (2009). Recent depressive and anxious symptoms predict cortisol responses to stress in men. *Psychoneuroendocrinology*, *34*(7), 1041-1049.

Brydon, L., Walker, C., Wawrzyniak, A., Chart, H., & Steptoe, A. (2009). Dispositional optimism and stress-induced changes in immunity and negative mood. *Brain, Behavior, and Immunity*, *23*(6).

Buchanan, T. W., Kern, S., Allen, J. S., Tranel, D., & Kirschbaum, C. (2004). Circadian regulation of cortisol after hippocampal damage in humans. *Biological psychiatry*, *56*(9), 651-656.

Buckner, R.L. & Carroll, D.C. (2007). Self-projection and the brain. *Trends in Cognitive Sciences*, 11(2): 49-57.

Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: a meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846-856.

Cannon, W. B. (1932). Homeostasis. *The wisdom of the body. Norton, New York*.

Cappeliez, P., Rivard, V., & Guindon, S. (2007). Functions of reminiscence in later life: proposition of a model and applications. *Revue Européenne de Psychologie Appliquée/European Review of Applied Psychology*, *57*(3), 151-156.

Carrillo, E., Moya-Albiol, L., González-Bono, E., Salvador, A., Ricarte, J., & Gómez-Amor, J. (2001). Gender differences in cardiovascular and electrodermal responses to public speaking task: the role of anxiety and mood states. *International Journal of Psychophysiology*, *42*(3), 253-264.

Carroll, D., Phillips, A. C., Hunt, K., & Der, G. (2007). Symptoms of depression and cardiovascular reactions to acute psychological stress: evidence from a population study. *Biological psychology*, *75*(1), 68-74.

Carstensen, L. L., Isaacowitz, D. M., & Charles, S. T. (1999). Taking time seriously: A theory of socioemotional selectivity. *American psychologist*, *54*(3), 165.

Carver, C. S., & Connor-Smith, J. (2010). Personality and coping. *Annual review of psychology*, *61*, 679-704.

Carver, C. S., & Scheier, M. F. (1990). Origins and functions of positive and negative affect: a control-process view. *Psychological review*, *97*(1), 19.

Carver, C. S., Scheier, M. F., (2000). On the structure of behavioral self-regulation. In M. Boekaerts, P. Pintrich, M. Zeidner (Eds.), Handbook of self-regulation (pp. 42–85). San Diego, CA: Academic Press.

Carver, C., Scheier, M., F. & Segerstrom, S. (2010). Optimism. *Clinical psychology review*, *30*(7), 879–89.

Castro-Diehl, C., Roux, A. V. D., Seeman, T., Shea, S., Shrager, S., & Tadros, S. (2014). Associations of socioeconomic and psychosocial factors with urinary measures of cortisol and catecholamines in the Multi-

Ethnic Study of Atherosclerosis (MESA). *Psychoneuroendocrinology*, *41*, 132-141.

Chan, S. W., Goodwin, G. M., & Harmer, C. J. (2007). Highly neurotic never-depressed students have negative biases in information processing. *Psychological Medicine*, *37*(09), 1281-1291.

Chico, E., (2002). Optimismo disposicional como predictor de estrategias de afrontamiento. [Dispositional Optimism as a predictor of coping strategies]. *Psicothema, 14*(3), 544-550.

Chida, Y., & Hamer, M. (2008). Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. *Psychological bulletin*, *134*(6), 829.

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. *Biological psychology*, *80*(3), 265-278.

Chida, Y., & Steptoe, A. (2010). Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status a meta-analysis of prospective evidence. *Hypertension*, *55*(4), 1026-1032.

Chida, Y., Hamer, M., (2008). Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. *Psychological bulletin, 134*(6), 829-885.

Childs, E., Dlugos, A., & De Wit, H. (2010). Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. *Psychophysiology*, *47*(3), 550-559.

Chopra, K. K., Ravindran, A., Kennedy, S. H., Mackenzie, B., Matthews, S., Anisman, H., ... & Levitan, R. D. (2009). Sex differences in hormonal responses to a social stressor in chronic major depression. *Psychoneuroendocrinology*, *34*(8), 1235-1241.

Christenfeld, N. J., Sloan, R. P., Carroll, D., & Greenland, S. (2004). Risk factors, confounding, and the illusion of statistical control. *Psychosomatic Medicine*, *66*(6), 868-875.

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews Endocrinology*, *5*(7), 374-381.

Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. *Jama*, *267*(9), 1244-1252.

Clow, A., Hucklebridge, F., & Thorn, L. (2010b). The cortisol awakening response in context. *International review of neurobiology*, *93*, 153-175.

Clow, A., Hucklebridge, F., Stalder, T., Evans, P., & Thorn, L. (2010a). The cortisol awakening response: more than a measure of HPA axis function. *Neuroscience & Biobehavioral Reviews*, *35*(1), 97-103.

Cohen, B. E., Panguluri, P., Na, B., & Whooley, M. A. (2010). Psychological risk factors and the metabolic syndrome in patients with coronary heart disease: findings from the Heart and Soul Study. *Psychiatry research*, *175*(1), 133-137.

Cohen, G. (1996). Age and health status in a patient satisfaction survey. *Social science & medicine*, *42*(7), 1085-1093.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of health and social behavior*, 385-396.

Conde, V., & Useros, E. (1975). Adaptación castellana de la escala de evaluación conductual para la depresión de Beck. *Revista de Psiquiatría y Psicología Médica de Europa y América Latina*, *12*(4), 217-236.

Connor-Smith, J. K., & Flachsbart, C. (2007). Relations between personality and coping: a meta-analysis. *Journal of personality and social psychology*, *93*(6), 1080.

Conrad, R., Schilling, G., Haidl, G., Geiser, F., Imbierowicz, K., & Liedtke, R. (2002). Relationships between personality traits, seminal parameters and hormones in male infertility. *Andrologia*, *34*(5), 317-324.

Costa, R. & Salvador, A., (2012). Associations between success and failure in a face-to-face competition and psychobiological parameters in young women. *Psychoneuroendocrinology, 37*(11), 1780-1790.

Crump, C., Sundquist, J., Winkleby, M. A., & Sundquist, K. (2016). Stress resilience and subsequent risk of type 2 diabetes in 1.5 million young men. *Diabetologia*, 1-6.

Cullinan, W. E., Herman, J. P., & Watson, S. J. (1993). Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. *Journal of Comparative Neurology*, *332*(1), 1-20.

Dallman, M. F., Akana, S. F., Levin, N., Walker, C. D., Bradbury, M. J., Suemaru, S., & Scribner, K. S. (1994). Corticosteroids and the Control of Function in the Hypothalamo-Pituitary-Adrenal (HPA) Axisa. *Annals of the New York Academy of Sciences*, *746*(1), 22-31.

Danesh, J., Kaptoge, S., Mann, A. G., Sarwar, N., Wood, A., Angleman, S. B., ... & Rumley, A. (2008). Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med*, *5*(4), e78.

Dar-Nimrod, I., Chapman, B. P., Franks, P., Robbins, J., Porsteinsson, A., Mapstone, M. & Duberstein, P. R., (2012). Personality factors moderate the associations between apolipoprotein genotype and cognitive function as well as late onset Alzheimer disease. *American Journal of Geriatric Psychiatry, 20*, 1026-1035.

Davis, E. P., & Granger, D. A. (2009). Developmental differences in infant salivary alpha-amylase and cortisol responses to stress.*Psychoneuroendocrinology*, *34*(6), 795-804.

de Kloet, E. R., Oitzl, M. S., & Joëls, M. (1999). Stress and cognition: are corticosteroids good or bad guys? *Trends in neurosciences*, *22*(10), 422-426.

de Waal, F. (1989). *Chimpanzee politics: Power and sex among apes.* Baltimore: Johns Hopkins University Press.

Dedovic, K., & Ngiam, J., (2015). The cortisol awakening response and major depression: examining the evidence. *Neuropsychiatric Disease and Treatement, 11*, 1181-1189.

DeSoto, M. C., & Salinas, M., (2015). Neuroticism and cortisol: The importance of checking for sex differences. *Psychoneuroendocrinology, 62*, 174-179.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological bulletin*, *130*(3), 355.

Diener, E., Suh, E., Lucas, R.E., & Smith, H.L., (1999). Subjective well being: Three decades of progress. *Psychological bulletin, 125*, 276-302.

Direk, N., Newson, R. S., Hofman, A., Kirschbaum, C., & Tiemeier, H. (2011). Short and long-term effects of smoking on cortisol in older adults. *International Journal of Psychophysiology*, *80*(2), 157-160.

Earle, T. L., Linden, W., & Weinberg, J. (1999). Differential effects of harassment on cardiovascular and salivary cortisol stress reactivity and recovery in women and men. *Journal of psychosomatic research, 46*(2), 125-141.

Ebrecht, M., Hextall, J., Kirtley, L.-G., Taylor, A., Dyson, M., & Weinman, J., (2004). Perceived stress and cortisol levels predict speed of wound healing in healthy male adults. *Psychoneuroendocrinology, 29*, 798–809.

Edwards, S., Clow, A., Evans, P., & Hucklebridge, F. (2001). Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life sciences*, *68*(18), 2093-2103.

Ehrenthal, J. C, Herrmann-Lingen, C., Fey, M. & Schauenburg, H. (2010). Altered cardiovascular adaptability in depressed patients without heart disease. *The World Journal of Biological Psychiatry,* 11 (3), 586-593

Ekholm, U. B., Turkmen, S., Hammarbäck, S., & Bäckström, T. (2014). Sexuality and androgens in women with cyclical mood changes and pre-menstrual syndrome. *Acta obstetricia et gynecologica Scandinavica*, *93*(3), 248-255.

Elder, G. J., Wetherell, M. A., Barclay, N. L., & Ellis, J. G. (2014). The cortisol awakening response–applications and implications for sleep medicine. *Sleep medicine reviews*, *18*(3), 215-224.

Eller, N.H., Netterstrøm, B., & Allerup, P., (2005). Progression in intima media thick- ness—the significance of hormonal biomarkers of chronic stress. *Psychoneuroendocrinology, 30*, 715–723.

El-Sheikh, M., Erath, S. A., Buckhalt, J. A., Granger, D. A., & Mize, J. (2008). Cortisol and children's adjustment: The moderating role of sympathetic nervous system activity. *Journal of Abnormal Child Psychology*, *36*(4), 601-611.

Endrighi, R., Hamer, M., & Steptoe, A. (2011). Associations of Trait Optimism With Diurnal Neuroendocrine Activity, Cortisol Responses to Mental Stress, and Subjective Stress Measures in Healthy Men and Women. *Psychosomatic Medicine*, *73*(8), 672-678.

Engert, V., Vogel, S., Efanov, S. I., Duchesne, A., Corbo, V., Ali, N., & Pruessner, J. C. (2011). Investigation into the cross-correlation of salivary cortisol and alpha-amylase responses to psychological stress. *Psychoneuroendocrinology*, *36*(9), 1294-1302.

Esch, T., Stefano, G. B., Fricchione, G. L., & Benson, H. (2002). Stress in cardiovascular diseases. *Medical Science Monitor*, *8*(5), RA93-RA101.

Esler, M. D., Thompson, J. M., Kaye, D. M., Turner, A. G., Jennings, G. L., Cox, H. S., ... & Seals, D. R. (1995). Effects of aging on the responsiveness of the human cardiac sympathetic nerves to stressors. *Circulation*, *91*(2), 351-358.

Ettner, S. L. (1996). New evidence on the relationship between income and health. *Journal of health economics*, *15*(1), 67-85.

Evans, B. E., Greaves-Lord, K., Euser, A. S., Tulen, J. H. M., Franken, I. H. A., & Huizink, A. C. (2012). Alcohol and tobacco use and heart rate reactivity to a psychosocial stressor in an adolescent population. *Drug and Alcohol Dependence*, *126*(3), 296–303.

Eysenck H. J., Eysenck S. B. G. (1997). *Cuestionario de Personalidad de Eysenck revisado (EPQ-R).* Manual. Madrid: TEA Ediciones SA.

Eysenck, H. J. (1987). Personality and ageing: An exploratory analysis. *Journal of Social Behavior and Personality*, *3*(1), 11.

Eysenck, H. J., & Eysenck, S. B. G. (1975). *Manual of the Eysenck Personality Questionnaire (junior and adult)*. Hodder and Stoughton.

Eysenck, H. J., Eysenck, M. W., (1985). *Personality and Individual Differences. A Natural Science Approach*. Plenum Press, New York.

Eysenck, H.J., (1967). *The Biological Basis of Personality*. Charles C. Thomas, Springfield, IL.

Fendler, K., Karmos, G., & Telegdy, G. (1961). The effect of hippocampal lesion on pituitary-adrenal function. *Acta Physiologica Academiae Scientiarum Hungaricae*, *20*, 293.

Ferrando, P.J., Chico, E., & Tous, J.M., (2002). Psychometric properties of the optimism test Live Orientation Test [Propiedades psicométricas del test de optimismo Live Orientation Test]. *Psicothema, 14* (3), 673-680.

Fichera, L. V., & Andreassi, J. L. (2000). Cardiovascular reactivity during public speaking as a function of personality variables. *International Journal of Psychophysiology*, *37*(3), 267-273.

Firk, C., & Markus, C. R. (2009). Mood and cortisol responses following tryptophan-rich hydrolyzed protein and acute stress in healthy subjects with high and low cognitive reactivity to depression. *Clinical Nutrition*, *28*(3), 266-271.

Fischette, C. T., Komisaruk, B. R., Edinger, H. M., Feder, H. H., & Siegel, A. (1980). Differential fornix ablations and the circadian rhythmicity of adrenal corticosteroid secretion. *Brain research*, *195*(2), 373-387.

Folkman, S. et al. (1987). Age differences in stress and coping processes. *Psychology and Ageing*, 2, 171–184.

Friedman, L. C., Nelson, D. V., Baer, P. E., Lane, M., Smith, F. E., & Dworkin, R. J. (1992). The relationship of dispositional optimism, daily life stress, and domestic environment to coping methods used by cancer patients. *Journal of Behavioral Medicine*, *15*(2), 127-141.

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): facts and future directions. *International Journal of Psychophysiology*, *72*(1), 67-73.

Fromholt, P., Larsen, P., & Larsen, S. F. (1995). Effects of late-onset depression and recovery on autobiographical memory. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *50*(2), P74-P81.

Gabella, G. (2001). Autonomic nervous system. *eLS*.

Gale, C. R., Booth, T., Mõttus, R., Kuh, D., & Deary, I. J. (2013). Neuroticism and Extraversion in youth predict mental wellbeing and life satisfaction 40 years later. *Journal of Research in Personality*, 47, 687-697.

Garcia-Banda, G., Chellew, K., Fornes, J., Perez, G., Servera, M., & Evans, P. (2014). Neuroticism and cortisol: pinning down an expected effect. *International Journal of Psychophysiology*, *91*(2), 132-138.

Gilbert, P. (1997). The evolution of social attractiveness and its role in shame, humiliation, guilt and therapy. *British Journal of Medical Psychology*, *70*(2), 113-147.

Giltay, E. J., Kamphuis, M. H., Kalmijn, S., Zitman, F. G., & Kromhout, D. (2006). Dispositional optimism and the risk of cardiovascular death: the Zutphen Elderly Study. *Archives of internal medicine*, *166*(4), 431.

Goel, N., Workman, J. L., Lee, T. T., Innala, L., & Viau, V. (2014). Sex differences in the HPA axis. *Comprehensive Physiology*, *4*, 1121-1155.

Gomez, R., Cooper, A., & Gomez, A. (2000). Susceptibility to positive and negative mood states: Test of Eysenck's, Gray's and Newman's theories. *Personality and Individual Differences*, *29*(2), 351-365.

Gonzalez-Bono, E., Moya-Albiol, L., Salvador, A., Carrillo, E., Ricarte, J., & Gomez-Amor, J. (2002). Anticipatory autonomic response to a public speaking task in women: the role of trait anxiety. *Biological Psychology*, *60*(1), 37-49.

Goodwin, R. D., & Stein, M. B. (2004). Association between childhood trauma and physical disorders among adults in the United States. *Psychological medicine*, *34*(03), 509-520.

Gordis, E. B., Granger, D. A., Susman, E. J., & Trickett, P. K. (2008). Salivary alpha amylase–cortisol asymmetry in maltreated youth. *Hormones and behavior*, *53*(1), 96-103.

Gordis, E. B., Granger, D. A., Susman, E. J., Trickett, P.K. (2006). Asymmetry between salivary cortisol and alpha-amylase reactivity to stress: relation to aggressive behavior in adolescents. *Psychoneuroendocrinology*, *31,* 976–87.

Gordon, T., Kannel, W. B., Hjortland, M. C., & McNamara, P. M. (1978). Menopause and coronary heart disease: the Framingham Study. *Annals of Internal Medicine*, *89*(2), 157-161.

Gragnoli, C. (2012). Depression and type 2 diabetes: cortisol pathway implication and investigational needs. *Journal of cellular physiology*, *227*(6), 2318-2322.

Granger, D. A., Kivlighan, K. T., El-Sheikh, M. O. N. A., Gordis, E. B., & Stroud, L. R. (2007). Salivary α-amylase in biobehavioral research. *Annals of the New York Academy of Sciences*, *1098*(1), 122-144.

Gray, J. A., & McNaughton, N. (2000). Neural anxiety systems: relevant fault-lines to trace and treat disorders. In *European Journal of Neuroscience* (Vol. 12, pp. 311-311).

Grundy, S. M., Benjamin, I. J., Burke, G. L., Chait, A., Eckel, R. H., Howard, B. V., ... & Sowers, J. R. (1999). Diabetes and cardiovascular disease a

statement for healthcare professionals from the American Heart Association. *Circulation*, *100*(10), 1134-1146.

Hackett, R. A., Kivimäki, M., Kumari, M., & Steptoe, A. (2016). Diurnal cortisol patterns, future diabetes and impaired glucose metabolism in the Whitehall II cohort study. *The Journal of Clinical Endocrinology & Metabolism*, jc-2015.

Hackett, R.A. & Steptoe, A. (in press) Psychosocial Factors in Diabetes and Cardiovascular Risk. *Current Cardiology Reports, 18* (95). doi:10.1007/s11886-016-0771-4

Hamburg, S., Hendler, R., & Sherwin, R. S. (1980). Influence of small increments of epinephrine on glucose tolerance in normal humans. *Annals of internal medicine*, *93*(4), 566-568.

Hamer, M., Tanaka, G., Okamura, H., Tsuda, A., & Steptoe, A. (2007). The effects of depressive symptoms on cardiovascular and catecholamine responses to the induction of depressive mood. *Biological psychology*, 74(1), 20-25.

Hauner, K. K., Adam, E. K., Mineka, S., Doane, L. D., DeSantis, A. S., Zinbarg, R., ... & Griffith, J. W. (2008). Neuroticism and introversion are associated with salivary cortisol patterns in adolescents. *Psychoneuroendocrinology*, *33*(10), 1344-1356.

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., ... & Nemeroff, C. B. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *Jama*, *284*(5), 592-597.

Heim, C., Newport, D. J., Wagner, D., Wilcox, M. M., Miller, A. H., & Nemeroff, C. B. (2002). The role of early adverse experience and adulthood stress in the prediction of neuroendocrine stress reactivity in women: a multiple regression analysis. *Depression and anxiety*, *15*(3), 117-125.

Hellhammer, J., Fries, E., Schweisthal, O. W., Schlotz, W., Stone, A. A., & Hagemann, D. (2007). Several daily measurements are necessary to reliably assess the cortisol rise after awakening: state-and trait components. *Psychoneuroendocrinology*, *32*(1), 80-86.

Helliwell, J., Layard, R. & Sachs, J. 2012.World Happiness Report. The Earth Institute, Columbia University. Commissioned for the April 2nd 2012 United Nations Conference on HappinessInternational Diabetes Federation. (2015). *IDF Diabetes Atlas* (7th ed.). Brussels, Belgium: International Diabetes Federation.

Henry, J. P., & Grim, C. E. (1990). Psychosocial mechanisms of primary hypertension. *Journal of hypertension*, *8*(9), 783-793.

Heponiemi, T., Elovainio, M., Pulkki, L., Puttonen, S., Raitakari, O., & Keltikangas-Järvinen, L. (2007). Cardiac autonomic reactivity and recovery in predicting carotid atherosclerosis: the cardiovascular risk in young Finns study. *Health Psychology*, *26*(1), 13.

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo–pituitary–adrenocortical responsiveness. *Frontiers in neuroendocrinology*, *24*(3), 151-180.

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *29*(8), 1201-1213.

Herzberg, P. Y., Glaesmer, H., & Hoyer, J. (2006). Separating optimism and pessimism: a robust psychometric analysis of the revised Life Orientation Test (LOT-R). *Psychological assessment*, *18*(4), 433.

Hidalgo, V., Almela, M., Villada, C., & Salvador, A. (2014). Acute stress impairs recall after interference in older people, but not in young people.*Hormones and behavior*, *65*(3), 264-272.

Hidalgo, V., Pulopulos, M. M., Puig-Perez, S., Espin, L., Gomez-Amor, J., & Salvador, A. (2015). Acute stress affects free recall and recognition of pictures differently depending on age and sex. *Behavioural brain research*, *292*, 393-402.

Hidalgo, V., Pulopulos, M. M., Puig-Perez, S., Espin, L., Gomez-Amor, J., & Salvador, A. (2015). Acute stress affects free recall and recognition

of pictures differently depending on age and sex. *Behavioural brain research*, *292*, 393-402.

Hidalgo, V., Villada, C., Almela, M., Espín, L., Gómez-Amor, J., & Salvador, A. (2012). Enhancing effects of acute psychosocial stress on priming of non-declarative memory in healthy young adults. *Stress*, *15*(3), 329-338.

Hill, E. M., Billington, R., & Krägeloh, C. (2013). The cortisol awakening response and the big five personality dimensions. *Personality and Individual Differences*, *55*(5), 600-605.

Hinton, J. W., & Craske, B. (1977). Differential effects of test stress on the heart rates of extraverts and introverts. *Biological Psychology*, *5*(1), 23-28.

Holsboer, F. (2001). Stress, hypercortisolism and corticosteroid receptors in depression: implicatons for therapy. *Journal of affective disorders*, *62*(1), 77-91.

Hutchinson, J. G., & Ruiz, J. M. (2011). Neuroticism and cardiovascular response in women: evidence of effects on blood pressure recovery. *Journal of personality*, *79*(2), 277-302.

Iversen, S., Iversen, L., & Saper, C. B. (2000). The autonomic nervous system and the hypothalamus. *Principles of neural science*, *4*, 960-981.

Jackson, J., Balota, D. A., & Head, D. (2011). Exploring the relationship between personality and regional brain volume in healthy aging. *Neurobiology of aging*, *32*(12), 2162-2171.

Jacobson, L. (2005). Hypothalamic–pituitary–adrenocortical axis regulation. *Endocrinology and metabolism clinics of North America*, *34*(2), 271-292.

Jänig, W. (1989). Autonomic nervous system. *Human physiology*. Springer Berlin Heidelberg, 333-370.

Jobin, J., Wrosch, C., & Scheier, M. (2014). Associations between dispositional optimism and diurnal cortisol in a community sample:

When stress is perceived as higher than normal. *Health Psychology*, *33*(4), 382.

Kagan, J., Snidman, N., Arcus, D., & Reznick, J. S. (1994). *Galen's prophecy: Temperament in human nature*. Basic Books.

Kaiser, J., Beauvale, A., & Bener, J. (1997). The evoked cardiac response as a function of cognitive load differs between subjects separated on the main personality dimensions. *Personality and individual differences*, *22*(2), 241-248.

Kajantie, E., & Phillips, D. I. (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress.*Psychoneuroendocrinology*, *31*(2), 151-178.

Karatsoreos, I. N., & McEwen, B. S. (2011). Psychobiological allostasis: resistance, resilience and vulnerability. *Trends in cognitive sciences*, *15*(12), 576-584.

Keller-Wood, M. E., & Dallman, M. F. (1984). Corticosteroid Inhibition of ACTH Secretion. *Endocrine reviews*, *5*(1), 1-24.

Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., & Carpenter, L. L. (2008). Sex differences in emotional and physiological responses to the Trier Social Stress Test. *Journal of behavior therapy and experimental psychiatry*, *39*(1), 87-98.

Kelly, S. J., & Ismail, M. (2015). Stress and type 2 diabetes: a review of how stress contributes to the development of type 2 diabetes. *Annual review of public health*, *36*, 441-462.

Kennedy, D. K., & Hughes, B. M. (2011). The optimism-neuroticism question: An evaluation based on cardiovascular reactivity in female college students. *The Psychological Record*, *54*(3), 4.

Kennedy, Q., Mather, M., & Carstensen, L. L. (2004). The role of motivation in the age-related positivity effect in autobiographical memory. *Psychological Science*, *15*(3), 208-214.

Kepka, S., Baumann, C., Anota, A., Buron, G., Spitz, E., Auquier, P., ... & Mercier, M. (2013). The relationship between traits optimism and anxiety and health-related quality of life in patients hospitalized for chronic diseases: data from the SATISQOL study. *Health and quality of life outcomes*, *11*(1), 1.

Kirkcaldy, B. D. (1984). Individual differences in tonic activity and reactivity of somatic functioning. *Personality and individual differences*, *5*(4), 461-466.

Kirschbaum, C., Bartussek, D., & Strasburger, C. J. (1992). Cortisol responses to psychological stress and correlations with personality traits. *Personality and Individual Differences*, *13*(12), 1353-1357.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The "Trier Social Stress Test"—A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28,* 76–81.

Kirschbaum, C., Prussner, J. C., Stone, A. A., Federenko, I., Gaab, J., Lintz, D., ... & Hellhammer, D. H. (1995). Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. *Psychosomatic medicine*, *57*(5), 468-474.

Kirschbaum, C., Wüst, S., & Hellhammer, D. (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic medicine*, *54*(6), 648-657.

Kivimäki, M., Virtanen, M., Kawachi, I., Nyberg, S. T., Alfredsson, L., Batty, G. D., ... & Dragano, N. (2015). Long working hours, socioeconomic status, and the risk of incident type 2 diabetes: a meta-analysis of published and unpublished data from 222 120 individuals. *The Lancet Diabetes & Endocrinology*, *3*(1), 27-34.

Kleiger, R. E., Stein, P. K., & Bigger, J. T. (2005). Heart rate variability: measurement and clinical utility. *Annals of Noninvasive Electrocardiology*, *10*(1), 88-101.

Kloppenborg, R. P., van den Berg, E., Kappelle, L. J., & Biessels, G. J. (2008). Diabetes and other vascular risk factors for dementia: which factor matters most? A systematic review. *European journal of pharmacology*, *585*(1), 97-108.

Knyazev, G. G., Slobodskaya, H. R., & Wilson, G. D. (2002). Psychophysiological correlates of behavioural inhibition and activation. *Personality and Individual Differences*, *33*(4), 647-660.

Kondratova, A. A., & Kondratov, R. V. (2012). The circadian clock and pathology of the ageing brain. *Nature Reviews Neuroscience*, *13*(5), 325-335. doi: 10.1038/nrn3208

Kotov, R., Gamez, W., Schmidt, F., & Watson, D. (2010). Linking "big" personality traits to anxiety, depressive, and substance use disorders: a meta-analysis. *Psychological bulletin*, *136*(5), 768.

Kudielka, B. M., & Wüst, S. (2010). Human models in acute and chronic stress: assessing determinants of individual hypothalamus–pituitary–adrenal axis activity and reactivity. *Stress*, *13*(1), 1-14.

Kudielka, B. M., Broderick, J. E., & Kirschbaum, C. (2003). Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosomatic medicine*, *65*(2), 313-319.

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004a). HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology*, *29*(1), 83-98.

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004b). Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: the impact of age and gender. *International journal of behavioral medicine*, *11*(2), 116-121.

Kudielka, B. M., Hellhammer, D. H., Kirschbaum, C. (2007). Ten years of research with the trier social stress test revisited. In: Harmon-Jones E, Winkielman P, editors. *Social neuroscience: integrating biological and psychological explanations of social behavior*. New York: Guilford Press (Chapter 4), pp. 56–83.

Kudielka, B. M., Schmidt-Reinwald, A. K., Hellhammer, D. H., & Kirschbaum, C. (1999). Psychological and endocrine responses to psychosocial stress and dexamethasone/ corticotropin-releasing hormone in

healthy postmenopausal women and young controls: the impact of age and a two - week estradiol treatment. *Neuroendocrinology*, *70*(6), 422-430.

Kudielka, B. M., Schmidt-Reinwald, A. K., Hellhammer, D. H., Schürmeyer, T., & Kirschbaum, C. (2000). Psychosocial stress and HPA functioning: no evidence for a reduced resilience in healthy elderly men. *Stress*, *3*(3), 229-240.

Kudielka, B. M., Schommer, N. C., Hellhammer, D. H., & Kirschbaum, C. (2004c). Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. *Psychoneuroendocrinology*, *29*(8), 983-992.

Kuzma, E., Sattler, C., Toro, P., Schönknecht, P., & Schröder, J. (2011). Premorbid personality traits and their course in mild cognitive impairment: results from a prospective population-based study in Germany. *Dementia and geriatric cognitive disorders*, *32*(3), 171-177.

Kyrou, I., & Tsigos, C. (2009). Stress hormones: physiological stress and regulation of metabolism. *Current opinion in pharmacology*, *9*(6), 787-793.

Laakso, M. (1999). Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes*, *48*(5), 937-942.

Labouvie-Vief, G., Hakim-Larson, J., & Hobart, C. J. (1987). Age, ego level, and the life-span development of coping and defense processes. *Psychology and aging*, *2*(3), 286.

Laceulle, O. M., Nederhof, E., Aken, M. A., & Ormel, J. (2015). Adolescent personality: Associations with basal, awakening, and stress-induced cortisol responses. *Journal of personality*, *83*(3), 262-273.

Lahey, B. B. (2009). Public health significance of neuroticism. *American Psychologist*, *64*(4), 241.

Lai, J. C. (1994). Differential predictive power of the positively versus the negatively worded items of the Life Orientation Test. *Psychological Reports*,*75*(3 suppl), 1507-1515.

Lai, J. C. (1997). Relative predictive power of the optimism versus the pessimism index of a Chinese version of the Life Orientation Test. *The Psychological Record*, *47*(3), 399-410.

Lai, J. C., Evans, P. D., Ng, S. H., Chong, A. M., Siu, O. T., Chan, C. L., ... Chan, C. C. (2005). Optimism, positive affectivity, and salivary cortisol. *British journal of health psychology*, *10*(Pt 4), 467–84.

Laitinen, T., Niskanen, L., Geelen, G., Länsimies, E., & Hartikainen, J. (2004). Age dependency of cardiovascular autonomic responses to head-up tilt in healthy subjects. *Journal of Applied Physiology*, *96*(6), 2333-2340.

Lakatta, E.G. (1993). Cardiovascular regulatory mechanisms in advanced age. *Physiological Reviews*, *73*, 413-467.

Larsen, R. J., & Ketelaar, T. (1989). Extraversion, neuroticism and susceptibility to positive and negative mood induction procedures. *Personality and individual Differences*, *10*(12), 1221-1228.

Larsen, R. J., & Ketelaar, T. (1991). Personality and susceptibility to positive and negative emotional states. *Journal of personality and social psychology*, *61*(1), 132.

Lawton, M. P., Kleban, M. H., & Dean, J. (1993). Affect and age: cross-sectional comparisons of Structure and prevalence. *Psychology and aging*,*8*(2), 165.

Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping.* New York: Springer.

Leedham, B., Meyerowitz, B. E., Muirhead, J., & Frist, W. H. (1995). Positive expectations predict health after heart transplantation. *Health Psychology*,*14*(1), 74.

Liao, D., Barnes, R. W., Chambless, L. E., Simpson Jr, R. J., Sorlie, P., Heiss, G., & ARIC investigators. (1995). Age, race, and sex differences in autonomic cardiac function measured by spectral analysis of heart rate variability—the ARIC study. *The American journal of cardiology*, *76*(12), 906-912.

Light, K. C., Kothandapani, R. V., & Allen, M. T. (1998). Enhanced cardiovascular and catecholamine responses in women with depressive symptoms. *International Journal of Psychophysiology*, *28*(2), 157-166.

Liu, J. P., Clarke, I. J., Funder, J. W., & Engler, D. (1994). Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the hypophysial-portal circulation of the conscious sheep. II. The central noradrenergic and neuropeptide Y pathways cause immediate and prolonged hypothalamic-pituitary-adrenal activation. Potential involvement in the pseudo-Cushing's syndrome of endogenous depression and anorexia nervosa. *Journal of Clinical Investigation, 93(4)*, 1439.

Löckenhoff, C. E., & Carstensen, L. L. (2004). Socioemotional selectivity theory, aging, and health: The increasingly delicate balance between regulating emotions and making tough choices. *Journal of personality*, *72*(6), 1395-1424.

Lovallo, W. R. (2011). Do low levels of stress reactivity signal poor states of health? *Biological psychology*, *86*(2), 121-128.

Lynn, R., & Martin, T. (1997). Gender differences in extraversion, neuroticism, and psychoticism in 37 nations. *The Journal of social psychology*, *137*(3), 369-373.

Macintyre, S., Hunt, K., & Sweeting, H. (1996). Gender differences in health: are things really as simple as they seem? *Social science & medicine*, *42*(4), 617-624.

Maina, G., Palmas, A., Bovenzi, M., & Filon, F. L. (2009). Salivary cortisol and psychosocial hazards at work. *American journal of industrial medicine*, *52*(3), 251-260.

Mangold, D., Mintz, J., Javors, M., & Marino, E. (2012). Neuroticism, acculturation and the cortisol awakening response in Mexican American adults. *Hormones and behavior*, *61*(1), 23-30.

Mason, J. W. (1968a). A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosomatic medicine*, *30*(5), 576-607.

Mason, J. W. (1968b). A review of psychoendocrine research on the sympathetic-adrenal medullary system. *Psychosomatic medicine*, *30*(5), 631-653.

Mason, J. W. (1975). A historical view of the stress field. *Journal of human stress*, *1*(2), 22-36.

Masoro, E. J. (2005). Overview of caloric restriction and ageing. *Mechanisms of ageing and development*, *126*(9), 913-922.

Mather, M., & Carstensen, L. L. (2005). Aging and motivated cognition: The positivity effect in attention and memory. *Trends in cognitive sciences*, *9*(10), 496-502.

Mather, M., & Johnson, M. K. (2000). Choice-supportive source monitoring: Do our decisions seem better to us as we age? *Psychology and aging*, *15*(4), 596–606.

Mathews, A., & MacLeod, C. (1994). Cognitive approaches to emotion and emotional disorders. *Annual review of psychology*, *45*, 25.

Matt, G. E., Vázquez, C., & Campbell, W. K. (1992). Mood-congruent recall of affectively toned stimuli: A meta-analytic review. *Clinical Psychology Review*,*12*(2), 227-255.

Mattei, J., Demissie, S., Falcon, L. M., Ordovas, J. M., & Tucker, K. (2010). Allostatic load is associated with chronic conditions in the Boston Puerto Rican Health Study. *Social Science & Medicine*, *70*(12), 1988-1996.

Matthews, K. A., Räikkönen, K., Sutton-Tyrrell, K., & Kuller, L. H. (2004). Optimistic attitudes protect against progression of carotid

atherosclerosis in healthy middle-aged women. *Psychosomatic medicine*, *66*(5), 640-644.

McCrimmon, R. J., Ryan, C. M., & Frier, B. M. (2012). Diabetes and cognitive dysfunction. *The Lancet*, *379*(9833), 2291-2299.

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England journal of medicine*, *338*(3), 171-179.

McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*(1), 33-44.

McEwen, B. S. (2006). Protective and damaging effects of stress mediators: central role of the brain. *Dialogues in clinical neuroscience*, *8*(4), 367.

McEwen, B. S., & Gianaros, P. J. (2011). Stress-and allostasis-induced brain plasticity. *Annual review of medicine*, *62*, 431.

McLeroy, K. R., Bibeau, D., Steckler, A., & Glanz, K. (1988). An ecological perspective on health promotion programs. *Health Education & Behavior*, *15*(4), 351-377.

Mommersteeg, P. M., & Pouwer, F. (2012). Personality as a risk factor for the metabolic syndrome: A systematic review. *Journal of psychosomatic research*,*73*(5), 326-333.

Morris, M. C., Rao, U., & Garber, J. (2012). Cortisol responses to psychosocial stress predict depression trajectories: Social-evaluative threat and prior depressive episodes as moderators. *Journal of affective disorders*, *143*(1), 223-230.

Mroczek, D. K., Spiro, A., Aldwin, C. M., Ozer, D. J., Bossé, R., (1993). Construct validation of optimism and pessimism in older men: Findings from the normative aging study. *Health Psychology, 12*(5), 406-409.

Munafo, M. R., Lee, L., Ayres, R., Flint, J., Goodwin, G., & Harmer, C. J. (2006). Early morning salivary cortisol is not associated with extraversion. *Personality and individual differences*, *40*(2), 395-400.

Nabi, H., Koskenvuo, M., Singh-Manoux, A., Korkeila, J., Suominen, S., Korkeila, K., ... & Kivimäki, M. (2010). Low pessimism protects against stroke the health and social support (HeSSup) prospective cohort study. *Stroke*,*41*(1), 187-190.

Nater, U. M., & Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*, *34*(4), 486-496.

Nater, U. M., Hoppmann, C. A., & Scott, S. B. (2013). Diurnal profiles of salivary cortisol and alpha-amylase change across the adult lifespan: evidence from repeated daily life assessments. *Psychoneuroendocrinology*, *38*(12), 3167-3171.

Nater, U. M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M. M., & Ehlert, U. (2006). Stress-induced changes in human salivary alpha-amylase activity—associations with adrenergic activity. *Psychoneuroendocrinology*, *31*(1), 49-58.

Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., & Ehlert, U. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology*, *55*(3), 333-342.

Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase.*Psychoneuroendocrinology*, *32*(4), 392-401.

Netterstrøm, B., Kristensen, T. S., Damsgaard, M. T., Olsen, O., & Sjøl, A. (1991). Job strain and cardiovascular risk factors: a cross sectional study of employed Danish men and women. *British Journal of Industrial Medicine*,*48*(10), 684. doi:10.1136/oem.48.10.684

Nicolini, P., Ciulla, M. M., Asmundis, C. D., Magrini, F., & Brugada, P. (2012). The prognostic value of heart rate variability in the elderly, changing the perspective: from sympathovagal balance to chaos theory. *Pacing and clinical electrophysiology*, *35*(5), 621-637.

Nicolson, N., Storms, C., Ponds, R., & Sulon, J. (1997). Salivary cortisol levels and stress reactivity in human aging. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *52*(2), M68-M75.

Novak, M., Björck, L., Giang, K. W., Heden-Ståhl, C., Wilhelmsen, L., & Rosengren, A. (2013). Perceived stress and incidence of Type 2 diabetes: a 35-year follow-up study of middle-aged Swedish men. *Diabetic medicine*,*30*(1), e8-e16.

Nyberg, S. T., Fransson, E. I., Heikkilä, K., Ahola, K., Alfredsson, L., Bjorner, J. B., ... & Hamer, M. (2014). Job Strain as a Risk Factor for Type 2 Diabetes: A Pooled Analysis of 124,808 Men and Women. *Diabetes Care*, *37*(8), 2268.

Obmiński, Z., Mroczkowska, H., & Tomaszewski, W. (2016). Relationships between personality traits, resting serum hormones and visuomotor ability in male judokas. *Annals of agricultural and environmental medicine: AAEM*, *23*(1), 79-83.

Ohannessian, C. M., Hesselbrock, V. M., Tennen, H., & Affleck, G. (1993). Hassles and uplifts and generalized outcome expectancies as moderators on the relation between a family history of alcoholism and drinking behaviors. *Journal of Studies on Alcohol*, *55*, 754−763.

Ormel, J., Bastiaansen, A., Riese, H., Bos, E. H., Servaas, M., Ellenbogen, M., ... & Aleman, A. (2013). The biological and psychological basis of neuroticism: current status and future directions. *Neuroscience & Biobehavioral Reviews*, *37*(1), 59-72.

Oswald, L. M., Zandi, P., Nestadt, G., Potash, J. B., Kalaydjian, A. E., & Wand, G. S. (2006). Relationship between cortisol responses to stress and personality. *Neuropsychopharmacology*, *31*(7), 1583-1591.

Otero, J.M., Luengo, A., Romero, E., Gómez, J.A., Castro, C. (1998). Psicología de personalidad. *Manual de prácticas*. Barcelona: Ariel Practicum.

Otte, C., Hart, S., Neylan, T. C., Marmar, C. R., Yaffe, K., & Mohr, D. C. (2005). A meta-analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology*, *30*(1), 80-91.

Pajukoski, H., Meurman, J. H., Snellman-Gröhn, S., Keinänen, S., & Sulkava, R. (1997). Salivary flow and composition in elderly patients referred to an acute care geriatric ward. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *84*(3), 265-271.

Palkovits, M. (1999). Interconnections between the neuroendocrine hypothalamus and the central autonomic system: Geoffrey Harris Memorial Lecture, Kitakyushu, Japan, October 1998. *Frontiers in neuroendocrinology*, *20*(4), 270-295.

Paolisso, G., Manzella, D., Barbieri, M., Rizzo, M. R., Gambardella, A., & Varricchio, M. (1999). Baseline heart rate variability in healthy centenarians: differences compared with aged subjects (> 75 years old). *Clinical science (London, England: 1979)*, *97*(5), 579.

Pardon, M. C. (2007). Stress and ageing interactions: a paradox in the context of shared etiological and physiopathological processes. *Brain research reviews*, *54*(2), 251-273.

Park, C. L., Moore, P. J., Turner, R. A., & Adler, N. E. (1997). The roles of constructive thinking and optimism in psychological and behavioral adjustment during pregnancy. *Journal of Personality and Social Psychology*, *73*(3), 584.

Pascualya, M., Petrie, E. C., Brodkin, K., Peskind, E. R., Veith, R. C., & Raskind, M. A. (1999). Effects of advanced aging on plasma catecholamine responses to the cold pressor test. *Neurobiology of aging*, *20*(6), 637-642.

Patacchioli, F. R., Simeoni, S., Monnazzi, P., Pace, M., Capri, O., & Perrone, G. (2006). Menopause, mild psychological stress and salivary cortisol: influence of long-term hormone replacement therapy (HRT). *Maturitas*, *55*(2), 150-155.

Patel, P. D., Lopez, J. F., Lyons, D. M., Burke, S., Wallace, M., & Schatzberg, A. F. (2000). Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. *Journal of psychiatric research*, *34*(6), 383-392.

Perales-Montilla, C. M., García-León, A., & Reyes-del Paso, G. A. (2012). Psychosocial predictors of the quality of life of chronic renal failure patients undergoing haemodialysis. *Nefrologia*, *32*(5), 622-30.

Petersen, L. R., Clark, M. M., Novotny, P., Kung, S., Sloan, J. A., Patten, C. A., ... & Colligan, R. C. (2008). Relationship of optimism–pessimism and

health-related quality of life in breast cancer survivors. *Journal of Psychosocial Oncology*, *26*(4), 15-32.

Phillips, A. C., Carroll, D., Burns, V. E., & Drayson, M. (2005). Neuroticism, cortisol reactivity, and antibody response to vaccination. *Psychophysiology*, *42*(2), 232-238.

Phillips, A. C., Der, G., Hunt, K., & Carroll, D. (2009). Haemodynamic reactions to acute psychological stress and smoking status in a large community sample. *International Journal of Psychophysiology*, *73*(3), 273–278.

Piccirillo, G., Bucca, C., Bauco, C., Cinti, A. M., Michele, D., Fimognari, F. L., ... & Marigliano, V. (1998). Power spectral analysis of heart rate in subjects over a hundred years old. *International journal of cardiology*, *63*(1), 53-61.

Pico-Alfonso, M. A., Mastorci, F., Ceresini, G., Ceda, G. P., Manghi, M., Pino, O., ... & Sgoifo, A. (2007). Acute psychosocial challenge and cardiac autonomic response in women: The role of estrogens, corticosteroids, and behavioral coping styles. *Psychoneuroendocrinology*, *32*(5), 451-463.

Pineles, S.L., Rasmusson, A.M., Yehuda, R., Lasko, N.B., Macklin, M.L., Pitman, R.K., Orr, S.P., 2013. Predicting emotional responses to potentially traumatic events from pre-exposure waking cortisol levels: a longitudinal study of police and firefighters. Anxiety Stress Coping 26, 241–53.

Plomin, R., Scheier, M. F., Bergeman, C. S., Pedersen, N. L., Nesselroade, J. R., & McClearn, G. E. (1992). Optimism, pessimism and mental health: A twin/adoption analysis. *Personality and individual differences*, *13*(8), 921-930.

Portella, M. J., Harmer, C. J., Flint, J., Cowen, P., & Goodwin, G. M. (2005). Enhanced early morning salivary cortisol in neuroticism. *American Journal of Psychiatry*.

Pouwer, F., Kupper, N., & Adriaanse, M. C. (2010). Does emotional stress cause type 2 diabetes mellitus? A review from the European

Depression in Diabetes (EDID) Research Consortium. *Discovery medicine*, *9*(45), 112-118.

Preuß, D., & Wolf, O. T. (2009). Post-learning psychosocial stress enhances consolidation of neutral stimuli. *Neurobiology of Learning and Memory*, *92*(3), 318-326.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, *28*(7), 916–31.

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., Von Auer, K., Jobst, S., ... & Kirschbaum, C. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life sciences*, *61*(26), 2539-2549.

Pruessner, M., Pruessner, J. C., Hellhammer, D. H., Pike, G. B., & Lupien, S. J. (2007). The associations among hippocampal volume, cortisol reactivity, and memory performance in healthy young men. *Psychiatry Research: Neuroimaging*, *155*(1), 1-10.

Puig-Perez, S., Almela, M., Pulopulos, M. M., Hidalgo, V., & Salvador, A. (2016b). Are neuroticism and extraversion related to morning cortisol release in healthy older people?. *International Journal of Psychophysiology*.

Puig-Perez, S., Villada, C., Pulopulos, M. M., Almela, M., Hidalgo, V., & Salvador, A. (2015). Optimism and pessimism are related to different components of the stress response in healthy older people. *International Journal of Psychophysiology, 98*(2), 213-221.

Puig-Perez, S., Villada, C., Pulopulos, M. M., Hidalgo, V., & Salvador, A. (2016a). How are neuroticism and depression related to the psychophysiological stress response to acute stress in healthy older people?. *Physiology & behavior*, *156*, 128-136.

Pulopulos, M. M., Almela, M., Hidalgo, V., Villada, C., Puig-Perez, S., & Salvador, A. (2013). Acute stress does not impair long-term memory retrieval in older people. *Neurobiology of learning and memory*, *104*, 16-24.

Pulopulos, M. M., Hidalgo, V., Almela, M., Puig-Perez, S., Villada, C., & Salvador, A. (2014). Hair cortisol and cognitive performance in healthy older people. *Psychoneuroendocrinology*, *44*, 100-111.

Pulopulos, M. M., Hidalgo, V., Almela, M., Puig-Perez, S., Villada, C., & Salvador, A. (2015). Acute stress and working memory in older people. *Stress*, (0), 1-10.

Raes, F., Hermans, D., de Decker, A., Eelen, P., & Williams, J. M. G. (2003). Autobiographical memory specificity and affect regulation: an experimental approach. *Emotion*, *3*(2), 201.

Rajandram, R.K., Ho, S.M., Samman N., Chan, N., McGrath, C. & Zwahlen, R.A. (2011). Interaction of hope and optimism with anxiety and depression in a specific group of cancer survivors: a preliminary study. *BMC Research Notes*, 4, 519-525.

Ramírez-Maestre, C., & Esteve, R. (2013). Disposition and adjustment to chronic pain. *Current pain and headache reports*, *17*(3), 1-11.

Rasmussen, H. N., Scheier, M. F., & Greenhouse, J. B. (2009). Optimism and physical health: A meta-analytic review. *Annals of behavioral medicine*, *37*(3), 239-256.

Rasmussen, H. N., Wrosch, C., Scheier, M. F., & Carver, C. S. (2006). Self-regulation processes and health: the importance of optimism and goal adjustment. *Journal of personality*, *74*(6), 1721-1748.

Raz, N., & Rodrigue, K. M. (2006). Differential aging of the brain: patterns, cognitive correlates and modifiers. *Neuroscience & Biobehavioral Reviews*,*30*(6), 730-748.

Remor, E. (2006). Psychometric properties of a European Spanish version of the Perceived Stress Scale (PSS). *The Spanish journal of psychology*, *9*(01), 86-93.

Ricarte, J. J., Hernández-Viadel, J. V., Latorre, J. M., & Ros, L. (2012). Effects of event-specific memory training on autobiographical memory retrieval and depressive symptoms in schizophrenic patients. *Journal of behavior therapy and experimental psychiatry*, *43*, S12-S20.

Rich-Edwards, J. W., Spiegelman, D., Hibert, E. N. L., Jun, H. J., Todd, T. J., Kawachi, I., & Wright, R. J. (2010). Abuse in childhood and adolescence as a predictor of type 2 diabetes in adult women. *American journal of preventive medicine*, *39*(6), 529-536.

Riese, H., Rosmalen, J. G., Ormel, J., Van Roon, A. M., Oldehinkel, A. J., & Rijsdijk, F. V. (2007). The genetic relationship between neuroticism and autonomic function in female twins. *Psychological medicine*, *37*(02), 257-267.

Robinson-Whelen S., Kim C., MacCallum RC. & Kiecolt-Glaser JK., (1997). Distinguishing optimism from pessimism in older adults: Is it more important be optimistic or not be pessimistic? *Journal of Personality and Social Psychology*, 73(6):1345-53.

Rod, N. H., Grønbaek, M., Schnohr, P., Prescott, E., & Kristensen, T. S. (2009). Perceived stress as a risk factor for changes in health behaviour and cardiac risk profile: a longitudinal study. *Journal of internal medicine*, *266*(5), 467-475.

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary α-amylase in humans and methodological considerations. *Psychoneuroendocrinology*, *34*(4), 469-485.

Rohleder, N., Kudielka, B. M., Hellhammer, D. H., Wolf, J. M., & Kirschbaum, C. (2002). Age and sex steroid-related changes in glucocorticoid sensitivity of pro-inflammatory cytokine production after psychosocial stress. *Journal of neuroimmunology*, *126*(1), 69-77.

Rohleder, N., Nater, U. M., Wolf, J. M., Ehlert, U., & Kirschbaum, C. (2004). Psychosocial Stress-Induced Activation of Salivary Alpha-Amylase: An Indicator of Sympathetic Activity?. *Annals of the New York Academy of Sciences*, *1032*(1), 258-263.

Rohleder, N., Wolf, J. M., Maldonado, E. F., & Kirschbaum, C. (2006). The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology*, *43*(6), 645-652.

Rooij, S. R., Schene, A. H., Phillips, D. I., & Roseboom, T. J. (2010). Depression and anxiety: Associations with biological and perceived stress

reactivity to a psychological stress protocol in a middle-aged population. *Psychoneuroendocrinology*, *35*(6), 866-877.

Rothrock, N. E., Hays, R. D., Spritzer, K., Yount, S. E., Riley, W., & Cella, D. (2010). Relative to the general US population, chronic diseases are associated with poorer health-related quality of life as measured by the Patient-Reported Outcomes Measurement Information System (PROMIS). *Journal of clinical epidemiology*, *63*(11), 1195-1204.

Roy, B., Diez-Roux, A., Seeman, T., Ranjit, N., Shea, S., & Cushman, M. (2010). Association of Optimism and Pessimism With Inflammation and Hemostasis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Psychosomatic Medicine*, *72*(2), 134.

Roy, M. P., Steptoe, A., & Kirschbaum, C. (1994). Association between smoking status and cardiovascular and cortisol stress responsivity in healthy young men. *International journal of behavioral medicine*, *1*(3), 264–283.

Rusting, C. L., & Larsen, R. J. (1997). Extraversion, neuroticism, and susceptibility to positive and negative affect: A test of two theoretical models. *Personality and Individual Differences*, *22*(5), 607-612.

Ryff, C. D., & Singer, B. (1998). The contours of positive human health.*Psychological inquiry*, *9*(1), 1-28.

Salomon, K., Clift, A., Karlsdóttir, M., & Rottenberg, J. (2009). Major depressive disorder is associated with attenuated cardiovascular reactivity and impaired recovery among those free of cardiovascular disease. *Health Psychology*, *28*(2), 157.

Salvador, A., & Costa, R. (2009). Coping with competition: neuroendocrine responses and cognitive variables. *Neuroscience & Biobehavioral Reviews*,*33*(2), 160-170.

Salvolini, E., Mazzanti, L., Martarelli, D., Di Giorgio, R., Fratto, G., & Curatola, G. (1999). Changes in the composition of human unstimulated whole saliva with age. *Aging (Milan, Italy)*, *11*(2), 119-122.

Samiec, P. S., Drews-Botsch, C., Flagg, E. W., Kurtz, J. C., Sternberg, P., Reed, R. L., & Jones, D. P. (1998). Glutathione in human plasma: decline in association with aging, age-related macular degeneration, and diabetes. *Free Radical Biology and Medicine*, *24*(5), 699-704.

Sandín, B., Chorot, P., Lostao, L., Joiner, T. E., Santed, M. A., & Valiente, R. M. (1999). Escalas PANAS de afecto positivo y negativo: validación factorial y convergencia transcultural. *Psicothema*, *11*(1), 37-51.

Sapolsky, R. M. (1993). Endocrinology alfresco: psychoendocrine studies of wild baboons. *Recent progress in hormone research*, *48*, 437.

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis*. *Endocrine reviews*, *7*(3), 284-301.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions 1. *Endocrine reviews*, *21*(1), 55-89.

Scannapieco, F. A., Torres, G., & Levine, M. J. (1993). Salivary α-amylase: role in dental plaque and caries formation. *Critical Reviews in Oral Biology & Medicine*, *4*(3), 301-307.

Scheier, M. F., & Carver, C. S. (1993). On the power of positive thinking: The benefits of being optimistic. *Current Directions in Psychological Science*. Retrieved from http://www.jstor.org/stable/20182190

Scheier, M. F., & Carver, C. S. (1985). Optimism, coping, and health: assessment and implications of generalized outcome expectancies. *Health psychology: official journal of the Division of Health Psychology, American Psychological Association*, *4*(3), 219–47.

Scheier, M. F., & Carver, C. S. (1992). Effects of optimism on psychological and physical well-being: Theoretical overview and empirical update. *Cognitive therapy and research*, *16*(2), 201-228.

Scheier, M. F., & Carver, C. S. (1993). On the power of positive thinking: The benefits of being optimistic. *Current Directions in Psychological Science*, *2*(1), 26-30.

Scheier, M. F., Carver, C. S., & Bridges, M. W. (1994). Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. *Journal of personality and social psychology*, *67*(6), 1063–78.

Schmaus, B. J., Laubmeier, K. K., Boquiren, V. M., Herzer, M., & Zakowski, S. G. (2008). Gender and stress: Differential psychophysiological reactivity to stress reexposure in the laboratory. *International Journal of Psychophysiology*,*69*(2), 101-106.

Schommer, N. C., Kudielka, B. M., Hellhammer, D. H., & Kirschbaum, C. (1999). No evidence for a close relationship between personality traits and circadian cortisol rhythm or a single cortisol stress response. *Psychological reports*, *84*(3), 840-842.

Schou, I., Ekeberg, Ø., & Ruland, C. M. (2005). The mediating role of appraisal and coping in the relationship between optimism-pessimism and quality of life. *Psycho-Oncology*, *14*(9), 718-727.

Schwebel, D. C., & Suls, J. (1999). Cardiovascular reactivity and neuroticism: Results from a laboratory and controlled ambulatory stress protocol. *Journal of Personality*, *67*(1), 67-92.

Schwebel, D. C., & Suls, J. (1999). Cardiovascular reactivity and neuroticism: Results from a laboratory and controlled ambulatory stress protocol. *Journal of Personality*, *67*(1), 67-92.

Schwerdtfeger, A., & Rosenkaimer, A. K. (2011). Depressive symptoms and attenuated physiological reactivity to laboratory stressors. *Biological Psychology*, *87*(3), 430-438.

Scully, T. (2012). Diabetes in numbers. *Nature*, *485*(7398), S2-S3.

Segerstrom, S. C. (2001). Optimism and attentional bias for negative and positive stimuli. *Personality and Social Psychology Bulletin*, *27*(10), 1334-1343.

Seligman, M. E. P. (1991). Learned optimism. Knopf: New York.

Sellers, J. G., Mehl, M. R., & Josephs, R. A. (2007). Hormones and personality: testosterone as a marker of individual differences. *Journal of Research in Personality*, *41*(1), 126-138.

Selye, H. (1936). A syndrome produced by diverse nocuous agents. *Nature*, *138*(3479), 32.

Selye, H. (1956). The Stress of Life. New York: McGraw Hill.

Sgoifo, A., Braglia, F., Costoli, T., Musso, E., Meerlo, P., Ceresini, G., & Troisi, A. (2003). Cardiac autonomic reactivity and salivary cortisol in men and women exposed to social stressors: relationship with individual ethological profile. *Neuroscience & Biobehavioral Reviews*, *27*(1), 179-188.

Shepperd, J. A., Maroto, J. J., & Pbert, L. A. (1996). Dispositional optimism as a predictor of health changes among cardiac patients. *Journal of Research in Personality*, *30*, 517−534.

Shimizu, K., Arai, Y., Hirose, N., Yonemoto, T., & Wakida, Y. (2002). Prognostic significance of heart rate variability in centenarians. *Clinical and experimental hypertension*, *24*(1-2), 91-97.

Sisk, C. L., & Zehr, J. L. (2005). Pubertal hormones organize the adolescent brain and behavior. *Frontiers in neuroendocrinology*, *26*(3), 163-174.

Solberg Nes, L. S., & Segerstrom, S. C. (2006). Dispositional optimism and coping: A meta-analytic review. *Personality and social psychology review*, *10*(3), 235-251.

Solberg Nes, L., Segerstrom, S., & Sephton, S. (2005). Engagement and Arousal: Optimism's Effects During a Brief Stressor. *Personality and Social Psychology Bulletin*, *31*(1), 111–120.

Sowers, J. R., Epstein, M., & Frohlich, E. D. (2001). Diabetes, hypertension, and cardiovascular disease an update. *Hypertension*, *37*(4), 1053-1059.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). *State-trait anxiety inventory (self-evaluation questionnaire)*. Palo Alto, CA: Consulting Psychologists Press.

Spielberger, C. D., Seisdedos, N., Gorsuch, R. L., Lushene, R. E. (1982) State-trait anxiety inventory. TEA Ediciones, Madrid.

Spreng, R. N., & Grady, C. L. (2010). Patterns of brain activity supporting autobiographical memory, prospection, and theory of mind, and their relationship to the default mode network. *Journal of cognitive neuroscience*,*22*(6), 1112-1123.

Spreng, R.N., Mar, R.A. & Kim, A.S. (2009). The common neural basis of autobiographical memory, prospection, navigation, theory of mind, and the default mode: A quantitative meta-analysis. *Journal of Cognitive Neuroscience*, 21(3): 489e510.

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., ... & Miller, R. (2016). Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology*, *63*, 414-432.

Stein, P. K., & Kleiger, R. E. (1999). Insights from the study of heart rate variability. *Annual review of medicine*, *50*, 249.

Stemmler, G., & Meinhardt, E. (1990). Personality, situation and physiological arousability. *Personality and individual differences*, *11*(3), 293-308.

Steptoe, A. (2016). Diabetes: Stress resilience and risk of type 2 diabetes mellitus. *Nature Reviews Endocrinology*.

Steptoe, A., & Poole, L. (2010). Use of biological measures in behavioral medicine. In *Handbook of Behavioral Medicine* (pp. 619-632). Springer New York.

Steptoe, A., Dockray, S., & Wardle, J. (2009). Positive affect and psychobiological processes relevant to health. *Journal of personality*, *77*(6), 1747-1776.

Steptoe, A., Feldman, P. J., Kunz, S., Owen, N., Willemsen, G., & Marmot, M. (2002). Stress responsivity and socioeconomic status. A mechanism for increased cardiovascular disease risk?. *European Heart Journal*, *23*(22), 1757-1763.

Steptoe, A., Hackett, R. A., Lazzarino, A. I., Bostock, S., La Marca, R., Carvalho, L. A., & Hamer, M. (2014). Disruption of multisystem responses to stress in type 2 diabetes: investigating the dynamics of allostatic load. *Proceedings of the National Academy of Sciences*, *111*(44), 15693-15698.

Steptoe, A., Wright, C., Kunz-Ebrecht, S. R., & Iliffe, S. (2006). Dispositional optimism and health behaviour in community-dwelling older people: Associations with healthy ageing. *British journal of health psychology*, *11*(1), 71-84.

Strahler, J., Mueller, A., Rosenloecher, F., Kirschbaum, C., & Rohleder, N. (2010). Salivary α-amylase stress reactivity across different age groups. *Psychophysiology*, *47*(3), 587-595.

Stroud, L. R., Salovey, P., & Epel, E. S. (2002). Sex differences in stress responses: social rejection versus achievement stress. *Biological psychiatry*, *52*(4), 318-327.

Sugaya, N., Izawa, S., Kimura, K., Ogawa, N., Yamada, K. C., Shirotsuki, K., ... & Shimada, H. (2012). Adrenal hormone response and psychophysiological correlates under psychosocial stress in individuals with irritable bowel syndrome. *International Journal of Psychophysiology*, *84*(1), 39-44.

Suls, J., & Martin, R. (2005). The daily life of the garden-variety neurotic: Reactivity, stressor exposure, mood spillover, and maladaptive coping. *Journal of personality*, *73*(6), 1485-1510.

Suls, J., Green, P., & Hillis, S. (1998). Emotional reactivity to everyday problems, affective inertia, and neuroticism. *Personality and Social Psychology Bulletin*.

Taylor, S. E., & Stanton, A. L. (2007). Coping resources, coping processes, and mental health. *Annu. Rev. Clin. Psychol.*, *3*, 377-401.

Thoma, M. V., Kirschbaum, C., Wolf, J. M., & Rohleder, N. (2012). Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biological psychology*, *91*(3), 342-348.

Thorn, L., Hucklebridge, F., Evans, P., & Clow, A. (2006). Suspected non-adherence and weekend versus week day differences in the awakening cortisol response. *Psychoneuroendocrinology*, *31*(8), 1009-1018.

Tilbrook, A. J., & Clarke, I. J. (2006). Neuroendocrine mechanisms of innate states of attenuated responsiveness of the hypothalamo-pituitary adrenal axis to stress. *Frontiers in neuroendocrinology*, *27*(3), 285-307.

Tindle, H., Davis, E. & Kuller, L. (2010). Attitudes and cardiovascular disease. *Maturitas*, 67(2), 108-113.

Tinker, L. F., Rosal, M. C., Young, A. F., Perri, M. G., Patterson, R. E., Van Horn, L., ... & Wu, L. (2007). Predictors of dietary change and maintenance in the Women's Health Initiative Dietary Modification Trial. *Journal of the American Dietetic Association*, *107*(7), 1155-1165.

Tøien, K., Bredal, I. S., Skogstad, L., Myhren, H., & Ekeberg, Ø. (2011). Health related quality of life in trauma patients. Data from a one-year follow up study compared with the general population. *Scandinavian journal of trauma, resuscitation and emergency medicine*, *19*(1), 1.

Trestman, R. L., Coccaro, E. F., Bernstein, D., Lawrence, T., Gabriel, S. M., Horvath, T. B., & Siever, L. J. (1991). Cortisol responses to mental arithmetic in acute and remitted depression. *Biological psychiatry*, *29*(10), 1051-1054.

Uchino, B. N., Uno, D., Holt-Lunstad, J., & Flinders, J. B. (1999). Age-related differences in cardiovascular reactivity during acute psychological stress in men and women. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *54*(6), P339-P346.

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*, *10*(6), 397-409.

van der Meij, L., Buunk, A. P., Almela, M., & Salvador, A. (2010). Testosterone responses to competition: the opponent's psychological state makes it challenging. *Biological psychology*, *84*(2), 330-335.

van Harten, B., Oosterman, J., Muslimovic, D., Van Loon, B. J. P., Scheltens, P., & Weinstein, H. C. (2007). Cognitive impairment and MRI correlates in the elderly patients with type 2 diabetes mellitus. *Age and ageing*, *36*(2), 164-170.

van Santen, A., Vreeburg, S. A., Van der Does, A. W., Spinhoven, P., Zitman, F. G., & Penninx, B. W. (2011). Psychological traits and the cortisol awakening response: results from the Netherlands Study of Depression and Anxiety.*Psychoneuroendocrinology*, *36*(2), 240-248.

van Stegeren, A. H., Wolf, O. T., & Kindt, M. (2008). Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. *International Journal of Psychophysiology*, *69*(1), 33-40.

Vassend, O., & Knardahl, S. (2005). Personality, affective response, and facial blood flow during brief cognitive tasks. *International journal of psychophysiology*, *55*(3), 265-278.

Veldhuis, J. D. (2013). Changes in pituitary function with ageing and implications for patient care. *Nature Reviews Endocrinology*, *9*(4), 205-215.

Verbrugge, L. M. (1989). The twain meet: empirical explanations of sex differences in health and mortality. *Journal of health and social behavior*, 282-304.

Verschoor, E., & Markus, C. R. (2011). Affective and neuroendocrine stress reactivity to an academic examination: influence of the 5-HTTLPR genotype and trait neuroticism. *Biological psychology*, *87*(3), 439-449.

Villada, C., Hidalgo, V., Almela, M., & Salvador, A. (2014b). Individual differences in the psychobiological response to psychosocial stress (Trier Social Stress Test): the relevance of trait anxiety and coping styles. *Stress and Health*, *32* (2), 90-99.

Villada, C., Hidalgo, V., Almela, M., Mastorci, F., Sgoifo, A., & Salvador, A. (2014a). Coping with an acute psychosocial challenge: behavioral and physiological responses in young women. *PLoS One, 9*(12), e114640.

Vollrath, M. (2000). Personality and hassles among university students: A three-year longitudinal study. *European Journal of Personality*, *14*(3), 199-215.

Von Dawans, B., Kirschbaum, C., & Heinrichs, M. (2011). The Trier Social Stress Test for Groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group format. *Psychoneuroendocrinology*, *36*(4), 514-522.

Ware Jr, J. E., & Sherbourne, C. D. (1992). The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Medical care*, 473-483.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of personality and social psychology*, *54*(6), 1063.

Watson, S., & Mackin, P. (2006). HPA axis function in mood disorders. *Psychiatry*, *5*(5), 166-170.

Webster, J. D. (1994). Predictors of reminiscence: A lifespan perspective. *Canadian Journal on Aging/La Revue canadienne du vieillissement*, *13*(01), 66-78.

Webster, J. D., & Cappeliez, P. (1993). Reminiscence and autobiographical memory: Complementary contexts for cognitive aging research. *Developmental review*, *13*(1), 54-91.

Webster, J. D., & McCall, M. E. (1999). Reminiscence functions across adulthood: A replication and extension. *Journal of Adult Development*, *6*(1), 73-85.

Weiss, A., & Costa Jr, P. T. (2005). Domain and facet personality predictors of all-cause mortality among Medicare patients aged 65 to 100. *Psychosomatic medicine*, *67*(5), 724-733.

Whitnall, M. H. (1993). Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. *Progress in neurobiology*, *40*(5), 573-629.

Williams, E. D., Magliano, D. J., Tapp, R. J., Oldenburg, B. F., & Shaw, J. E. (2013). Psychosocial stress predicts abnormal glucose metabolism: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. *Annals of Behavioral Medicine*, *46*(1), 62-72.

Wilsher, M. L. (2012). Psychological stress in sarcoidosis. *Current opinion in pulmonary medicine*, *18*(5), 524-527.

Wing, R. R., Epstein, L. H., Blair, E., & Nowalk, M. P. (1985). Psychologic stress and blood glucose levels in nondiabetic subjects. *Psychosomatic Medicine*, *47*(6), 558-564.

Wirtz, P. H., Elsenbruch, S., Emini, L., Rüdisüli, K., Groessbauer, S., & Ehlert, U. (2007). Perfectionism and the cortisol response to psychosocial stress in men. *Psychosomatic Medicine, 69*(3), 249-255.

Wolf, O. T., Fujiwara, E., Luwinski, G., Kirschbaum, C., & Markowitsch, H. J. (2005). No morning cortisol response in patients with severe global amnesia. *Psychoneuroendocrinology*, *30*(1), 101-105.

Wood, R., Maraj, B., Lee, C. M., & Reyes, R. (2002). Short-term heart rate variability during a cognitive challenge in young and older adults. *Age and ageing*, *31*(2), 131-135.

Xhyheri, B., Manfrini, O., Mazzolini, M., Pizzi, C., & Bugiardini, R. (2012). Heart rate variability today. Progress in cardiovascular diseases, 55(3), 321-331.

Yan, L. L., Daviglus, M. L., Liu, K., Pirzada, A., Garside, D. B., Schiffer, L., ... & Greenland, P. (2004). BMI and Health-related quality of life in adults 65 years and older. *Obesity research*, *12*(1), 69-76.

Yim, I. S., Quas, J. A., Cahill, L., & Hayakawa, C. M. (2010). Children's and adults' salivary cortisol responses to an identical psychosocial laboratory stressor. *Psychoneuroendocrinology*, *35*(2), 241-248.

York, K. M., Hassan, M., Li, Q., Li, H., Fillingim, R. B., & Sheps, D. S. (2007). Coronary artery disease and depression: patients with more depressive symptoms have lower cardiovascular reactivity during laboratory-induced mental stress. *Psychosomatic Medicine*, *69*(6), 521-528.

Young, E. A., Lopez, J. F., Murphy-Weinberg, V., Watson, S. J., & Akil, H. (2000). Hormonal evidence for altered responsiveness to social stress in major depression. *Neuropsychopharmacology*, *23*(4), 411-418.

Zakowski, J. J., & Bruns, D. E. (1985). Biochemistry of human alpha amylase isoenzymes. *CRC Critical reviews in clinical laboratory sciences*, *21*(4), 283-322.

Zimmet, P., Alberti, K. G. M. M., & Shaw, J. (2001). Global and societal implications of the diabetes epidemic. *Nature*, *414*(6865), 782-787.