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Stress and memory performance in older men and women:

The role of the Hypothalamus-Pituitary-Adrenal axis.

Dissertation

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Esta tesis doctoral es el fruto de un largo camino de aprendizaje y superación personal. Un camino que tuvo sus cuestas, difíciles de subir y donde la vista no alcanzaba a vislumbrar el final, pero que una vez superadas, lo conseguido confirmó que había valido la pena subir y que volvería a subir la siguiente. Durante este camino, muchas son las personas que me han acompañado y apoyado, y que sin ellas, desde luego no habría conseguido llegar a la meta.

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Dissertation Outline

The Western countries are experiencing a rapid population ageing which is mainly caused by advances in research and changes in life style that have occurred in the last decades, and to a lesser extent by a decrease in birth rate in some countries. For example, in Spain, nowadays, 17 % of the population is older than 65 years, and it is expected that by 2050 this percentage will have increased up to 40 %. This projection places the Spanish population as one of the oldest populations in the world in 2050 (National Statistics Institute, 2011; Population Ageing and Development, UN, 2009). Similarly, a projection made by Eurostat indicates that 30 % of the population in the European Union will be older than 65 years by 2050 (Europop, 2004).

This scenario presses the scientific community to pursue goals that are not only directed towards increasing the life span of people, but also to pursue goals that are directed towards making people age "successfully", i.e. with well-preserved physical, mental and emotional capabilities until death occurs (Rowe and Khan, 1987). This perspective entails individual and collective benefits, because successful aging implies to avoid, or at least delay, the dependence of older people on their families, on social and health care services and, ultimately, on the community as a whole. To do so, scientific research has to focus on factors that contribute to the vulnerability of older people to diseases and functional disabilities. In this dissertation I am going to focus on the cognitive decline that accompanies the aging process and, more specifically, I will focus on the relationship between the activity and regulation of the Hypothalamus-Pituitary-Adrenal axis (HPA-axis) and the differences in memory performance observed in older people.

In fact, research has shown that there is a large heterogeneity in the age-related cognitive decline among healthy people (Rabbitt, 1993; Christensen et al., 1999). This means that some people maintain their memory relatively well as they

age, while among other people their memory deteriorates dramatically. The aim of this dissertation is to investigate whether the HPA-axis activity and regulation is related to these individual differences in memory performance.

In the first chapter of this dissertation, I am going to explain what the HPA-axis is, I will discuss its main functions, and I will explain the mainstream theories that indicate an involvement of the HPA-axis in the age-related memory decline. Additionally, I will present a short overview of studies that have previously investigated the relationship between the HPA-axis and memory performance. In the second chapter, I am going to describe the first empirical study of this dissertation, which investigated whether there is an impact of older age on the HPA-axis and the autonomic nervous system (ANS) responses to acute psychosocial stress.

In the third chapter, I am going to explain the main results of a study that investigated whether acute psychosocial stress can modulate acutely the memory performance of middle-aged people, and if sex is a moderator in this relationship. Next, in the third study of this dissertation, which is described in the fourth chapter, I tried to answer questions that emerged from the study in the previous chapter. Thus, I investigated whether the magnitude of the cortisol response to acute stress is related to the memory performance of older people when memory is tested in a non-stressful situation, i.e. to the 'basal' memory capabilities of older people.

In the fifth chapter, I will discuss the last study of this dissertation. In this study I took a different approach by investigating whether another facet of the HPA-axis activity, the cortisol awakening response (CAR), is also related to the memory performance of older people. Finally, the sixth chapter will discuss the main findings of the previous empirical chapters, it will highlight the main conclusions of this dissertation, and it will give a new direction to future studies.

Contents

Chapter 1

The link between HPA-axis activity and memory differences in older individuals

1. The Hypothalamus-Pituitary-Adrenal axis (HPA-axis)

1.1 *Activity and Regulation*

When we refer to the HPA-axis, we are referring to a complex communication system that encompasses three different regions of our body. This communication system is crucial for our survival, because it is an important part of our defense when faced with threats, and because it coordinates the re-establishment of homeostasis when the threat is over. Therefore, the activity of the HPA-axis is a part of the stress-response, and its main function is to improve our chances of survival in the presence of threats to our physical or psychological well-being.

Communication is established between the hypothalamus, the pituitary gland and the adrenal gland through the release of hormones (see figure 1). When the HPA-axis is activated due to, for example, the presence of a physical or psychological stressor, the paraventricular nucleus of the hypothalamus releases corticotropin releasing hormone (CRH) and arginine-vasopressin (AVP) into the portal circulation of the median eminence. CRH and AVP induce the pituitary gland to secrete adrenocorticotropic hormone (ACTH) into the bloodstream, which stimulates the cortex of the adrenal glands, where, finally, glucocorticoids are released into the bloodstream (Ulrich-Lai and Herman, 2009). The most notable glucocorticoid in humans is cortisol.

Under non-stressful situations, the secretion of glucocorticoids follows a circadian rhythm with the highest levels in the morning and the lowest levels at night (Ulrich-Lai and Herman, 2009). The greatest glucocorticoid secretion, under resting conditions, is produced about 30 minutes after awakening from sleep. This is a discrete and distinct component of the cortisol circadian cycle that has been called the cortisol awakening response (CAR). The function of the CAR is still not clearly understood, but it could be related to recovery from sleep and the regaining of arousal following awakening (Clow et al., 2010a).

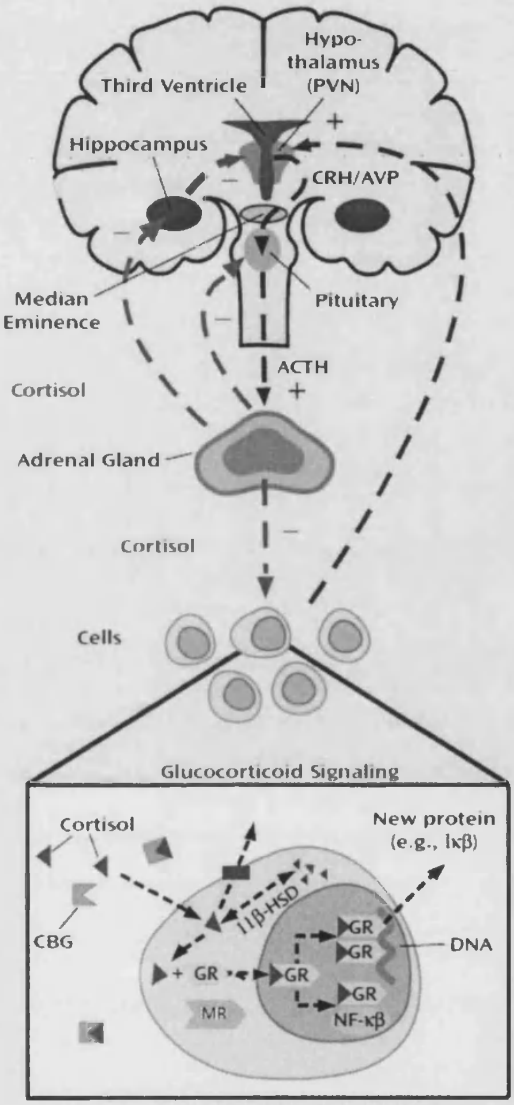

Figure 1. HPA-axis. CRH: corticotropin-releasing hormone; AVP: arginine vasopressin; PVN: paraventricular nucleus of the hypothalamus; ACTH: adrenocorticotropic hormone; CBG: corticosteroid binding globulin; 11β-HSD: 11-β-hydroxysteroid dehydrogenase; GR: glucocorticoid receptor; MR: mineralcorticoid receptor (Raison and Miller, 2003).

The secretion of glucocorticoids has to be tightly regulated because prolonged exposure to them is related to serious metabolic, immune and physiological dysfunctions (McEwen, 2008). Therefore, the HPA-axis activity is controlled by a system of

negative feedback that makes sure that glucocorticoid levels are maintained within tolerable limits (Keller-Wood and Dallman, 1984). This negative feedback is regulated by the neurons of the PVN of the hypothalamus, as the increase in cortisol levels in the bloodstream inhibits the secretion of CRH and AVP (Whitnall, 1993). Apart from this, there are also neuronal inhibitory pathways that work in parallel with the hormonal feedback. These inhibitory pathways are mediated mainly by limbic structures such as (i) the hippocampus and (ii) the prefrontal cortex (see figure 1).

(i) The hippocampus has a high degree of glucocorticoid receptivity because it has the highest levels of glucocorticoid binding of any brain structure, and it exerts an influence on the PVN that is primarily inhibitory (Patel et al., 2000; Herman et al., 2005). The hippocampus is involved in both the control of the circadian glucocorticoid rhythm and the inhibition of the HPA-axis responses to stress (Fendler et al., 1961; Fischette et al., 1980). This inhibitory role is exerted through a two-step pathway. In the first step, the glutamatergic hippocampal fibers contact the bed nucleus of the stria terminalis, the medial preoptic area, the dorsomedial hypothalamus, and other hypothalamic nuclei, which are populated with GABAergic neurons. In a second step, these GABAergic neurons project directly to the PVN, exerting the inhibitory function (Cullinan et al., 1993; Herman et al., 2003).

(ii) The medial prefrontal cortex processes stressful information, has a high density of glucocorticoids receptors, and modulates stress-induced HPA axis activity (Cullinan et al., 1995; Patel et al., 2000). In a similar way to the hippocampus, the inhibitory role of the medial prefrontal cortex is exerted through a two-step pathway. Thus, glutamatergic fibers from the medial prefrontal cortex interact with the preoptic area, the nucleus of the solitary tract, and the bed of the stria terminalis, among others, and through there, the GABAergic neurons send inhibitory inputs to the PVN. Moreover, the medial prefrontal cortex has connections with the hippocampus and the amygdala (which has an excitatory function on the HPA-axis activity); and because of this, it is positioned at the top of the response-initiation hierarchy and might

be a principal, but not the sole, limbic coordinator of physiological reactivity to stress (Ulrich-Lai and Herman, 2009).

1.2 *Functions of glucocorticoids*

The release of glucocorticoids has many complex functions in our organism that have been classified along the following dimensions: (i) permissive, (ii) stimulating, (iii) suppressive, and (iv) preparative (for an extensive review see: Sapolsky et al., 2000b). (i) Permissive and (ii) stimulating actions reinforce the first defense mechanisms through which we respond to stress. The difference between the two is that permissive effects are exerted by the basal glucocorticoids levels that were present before the onset of the stressor, and the stimulating effects are exerted by the stress-induced increase in glucocorticoid levels. As an example of a (i) permissive effect, basal glucocorticoids facilitate sympathetic interactions, and their overall physiological effects are to permissively augment cardiovascular activation during stress. When facing a stressor, one of the main functions of the first wave of the stress response is to optimize our energy resources and facilitate the use of this energy in those parts of our body where it is needed most, like, for example our muscles. The activation of the cardiovascular system, through the release of catecholamines by the sympathetic nervous system, is oriented toward this end, and it provokes an increase in arterial pressure, heart rate, and cardiac output, and diverts the blood to our muscles. In this context, the actions of glucocorticoids help the catecholamines and other vasoconstrictors to exert their full actions, by for example, inhibiting catecholamine reuptake and enhancing cardiovascular sensitivity to catecholamines by increasing the binding capacity of β-adrenergic receptors (Sakaue and Hoffman, 1991). As an example of (ii) a stimulating effect, the stress-induced release of glucocorticoids enhances the amount of energy (glucose) available by stimulating the gluconeogenesis and glycogen deposition in the liver and inhibiting the peripheral glucose transport and utiliza-

tion. All of these have the purpose of maintaining a, probably necessary, prolonged stress response (Munk and Náray-Fejes-Tóth, 1994).

Complementary, (iii) suppressive actions control the stress response and propitiate the return to homeostasis before our own stress response harms us. As an example, glucocorticoids suppress immunological and inflammatory stress responses. When facing a stressor, there is a rapid activation of the immune system which is aimed at defending us against disease, through rapidly identifying and defeating pathogens which could enter our organism as a result of possible injuries or infections. However, if this increase in immune system activity is sustained in time, there can be negative consequences for our organism, such as inflammation and autoimmune diseases. In the long-term, the glucocorticoids have the function of suppressing the stress-induced increase in immune activity and, therefore, reducing the likelihood of autoimmune overshoot.

Finally, (iv) preparative actions do not affect the immediate response to a stressor, but instead modulate our response to a subsequent stressor. As an example, glucocorticoids stimulate appetite in the long term (Dallman et al., 1993). The exposure to a stressor suppresses feeding, an effect that is probably mediated by CRH (Arase et al., 1988). Feeding is a costly process that provides energy slowly and, therefore, is expendable when we are facing a stressor. However, in the long term, glucocorticoids stimulate the appetite, which can be seen as a preparatory feature; because it helps our energy stores to get full again, in case we have to cope with a subsequent stressor.

1.3 *Pathways for glucocorticoid effects*

Glucocorticoids are capable of performing all of these complex functions through two different pathways, both of which are characterized by the time that the glucocorticoids need to exert their functions. The (i) slow alternative is via the classical or genomic pathway, through which glucocorticoids can exert their effects in a

matter of minutes to hours. This pathway is mediated by intracellular receptors which, in their inactive state, exist in the cytosol and are bound to stabilizing proteins (see figure 1). Once the glucocorticoids have passively penetrated the cellular membrane, they bind to these receptors, promoting the translocation to the cell nucleus. Once inside the nucleus, they regulate gene expression by interacting with other transcription factors or binding to specific DNA response elements (Ulrich-Lai and Herman, 2009).

Two related receptor molecules are responsible for this genomic pathway: the mineralcorticoid receptors (MRs) and the glucocorticoid receptors (GRs). MRs have a high affinity to glucocorticoids, while GRs have a ten-fold lower affinity to them (Reul and de Kloet, 1985). This difference seems to indicate that the MRs mediate the glucocorticoid effects when the glucocorticoid levels are low, such as in basal conditions. However, when glucocorticoid levels increase, such as in stressful situations, MRs saturate and the GRs begin to be occupied. Therefore, the GR receptor is probably more involved in the regulation of stress-related glucocorticoid effects, including the feedback inhibition of the HPA-axis (de Kloet et al., 2005). The highest expressivity of these two kinds of receptors has been found in brain structures strongly related to the cognitive appraisal of a stressful situation, and highly related to HPA-axis feedback inhibition, such as the hippocampus and the prefrontal cortex (Patel et al., 2000).

The (ii) fast alternative pathway is via the non-genomic or non-classical pathway, through which glucocorticoids can exert their effects in a matter of seconds to minutes. These fast effects include rapid negative feedback-inhibition of the HPA-axis, which occurs within minutes of the rise in circulating glucocorticoids, but also quick effects of glucocorticoids on cognitive processes such as appraisal of novel situations (Oitzl and de Kloet, 1992), extinction processes (Rodrigues et al., 2009) and retrieval processes (de Quervain et al., 2009). These effects on cognitive processes occur too fast to be mediated by classic genomic pathways (Tasker et al., 2005). The exact mechanism through which these non-genomic mediated effects occur is not

known, but some hypotheses point to membrane actions of the nuclear receptors (GRs and MRs) or to other unidentified membrane receptors (de Kloet et al., 2008; Ulrich-Lai and Herman, 2009).

Finally, it is pertinent to comment that there are proteins that interact with glucocorticoids and play an important role in the ability of the hormone to exert its effects; therefore, these proteins also can affect the regulation of the HPA-axis. Among them, I want to highlight the corticosteroid binding globulin (CBG) and the enzyme 11-β-hydroxysteroid dehydrogenase. CBG is a protein that binds cortisol with high affinity, facilitates its transport in the blood, provides a reservoir of inactive circulating hormone, and regulates the amount of free hormone available for diffusion into tissue (Mendel, 1989; Henley and Lightman, 2011). When cortisol is measured in plasma, a total value is usually obtained, which is a combination of the inactive cortisol that is bound to CBG proteins and the active portion which is free or unbound. However, when cortisol is measured in saliva, only the free or active portion of cortisol is counted because only the unbound portion can pass through the lipid membranes and diffuse into bodily fluids like saliva (Kirschbaum and Hellhammer, 2000). On the other hand, the enzyme 11-β-hydroxysteroid dehydrogenase metabolizes endogenous glucocorticoid hormones upon entry into the cell and, therefore, regulates the access of glucocorticoids to its receptors.

2. A mechanism through which HPA-axis activity can affect memory capabilities

HPA-axis activity has crucial functions that enhance our capacity to deal with threats. Therefore, it seems counter-intuitive that the same mechanism that increases our chances of survival could also produce detrimental effects in our organism and be related to the age-related memory decline. Nonetheless, research has shown that a prolonged exposure to glucocorticoids, for example during situations of chronic stress or when suffering from diseases like Cushing's, has detrimental effects on our organism and harms our body tissue. Indeed, a dysregulation of the HPA-axis activity

has been linked to several conditions, such as depression, diabetes, or even Alzheimer's disease (McEwen, 2008). The brain structures that would be more exposed to these harmful effects are those that are more involved in HPA-axis regulation, such as the hippocampus and the prefrontal cortex. These same brain structures also have a central involvement in memory processes like declarative and working memory (Scoville and Milner, 1957; Galloway et al., 2008). Therefore, if excessive exposure to glucocorticoids can cause harm to those structures, this would be translated into memory problems.

In fact, research predominantly on animals has shown that chronic stress markedly affects hippocampal and prefrontal cortex morphology. In the hippocampus, prolonged exposure to high levels of glucocorticoids suppresses neurogenesis in the dentate gyrus (Gould and Tanapat, 1999) and compromises cell survival (Sapolsky, 2000a). Additionally, it causes other structural alterations, such as dendritic atrophy (Magarinos et al., 1996), synaptic loss of excitatory glutamatergic synapses (Sandi et al., 2003), and reduction in the surface area of postsynaptic densities (Sousa et al., 2000). Similar alterations have been observed in the medial prefrontal cortex after prolonged exposure to higher glucocorticoid levels, including dendritic atrophy (Wellman, 2001) and spine loss (Cerqueira et al., 2005). All of these damaging effects have been linked to a glucocorticoid-provoked enhanced vulnerability of neurons in these brain areas to insults. Furthermore, elevated glucocorticoid levels prolonged in time also disrupt neuronal energy metabolism by exacerbating the state of energy depletion that they induce and increasing oxidative stress (Sapolsky and Pulsinelli, 1985). Not surprisingly, all of these structural changes in the hippocampus and the prefrontal cortex are accompanied by memory impairments (Sousa et al., 2000; Liston et al., 2006).

2.1 *Hypothesis of the relationship between HPA-axis activity and age-related memory decline*

Several hypotheses have been formulated that try to explain the involvement of the HPA-axis activity in the age-related memory decline. The first hypothesis formulated was the 'Glucocorticoid Cascade Hypothesis' (Sapolsky et al., 1986), re-renamed later as the 'Neurotoxicity Hypothesis' (Gilbertson et al., 2002), which is based, predominantly, on studies performed on non-human animals. According to this seminal hypothesis, memory impairments observed in older organisms are caused by the cumulative exposure to high levels of glucocorticoids across the individual's lifetime. These enhanced concentrations of glucocorticoids could have been caused by diverse factors, like episodes of chronic stress or specific diseases such as depression. Under normal conditions, periods of excessive stress and, consequently, excessive glucocorticoid secretion, produce a down-regulation of the glucocorticoid receptors at the hippocampus. Once the stressful episode has passed and, consequently, the increase in glucocorticoid secretion terminates, the down-regulation of receptors is self-corrected. However, it is possible that at a certain point the down-regulation of receptors causes a failure in the capacity of the hippocampus to inhibit the HPA-axis activation, leading to a more permanent glucocorticoid hypersecretion. This situation precipitates more down-regulation of receptors and further glucocorticoid hypersecretion in a feed-forward cycle, which ultimately will cause harm and neuronal death in the hippocampus that will be translated into cognitive problems such as memory impairments.

Support for the Glucocorticoid Cascade Hypothesis comes from studies performed predominantly in rats (Sapolsky et al., 1986). However, subsequent studies involving rats and other species failed to show hippocampal cell loss after exposure to chronic stress or enhanced glucocorticoid levels (rats: Bodnoff et al., 1995; Coburn-Litvak et al., 2004; tree shrews: Fuchs et al., 2001; non-human primates: Leverenz et al., 1999; humans: Muller et al., 2001). Moreover, the correlation between increasing

age and enhanced HPA-axis activity among humans appeared to be weak and rather heterogeneous (Lupien et al., 1996; Seeman et al., 1997), and the study of PTSD patients showed that a reduced hippocampal volume could also be accompanied by glucocorticoid hyposecretion (Meewisse et al., 2007).

All the previous evidence led to the formulation of the 'Vulnerability Hypothesis', which states that there must be some predisposition to experiencing damaging effects due to elevated glucocorticoid levels. This predisposition would be a pre-existing risk factor for suffering from stress-related disorders, and it could be represented by factors like genetics and exposure to stress in the early/later stages of the lifetime (Charney and Manji, 2004). In fact, it is possible that the two hypotheses are not exclusive but rather complementary. It is possible that the negative impact of stress on brain tissue is different depending on its developmental stage, and therefore, this negative impact is not a result of cumulative exposure to stress across the lifetime, but rather of exposure to stress during specific vulnerable windows in our development. These vulnerable windows would especially be childhood, where the hippocampus, amygdale and prefrontal cortex are still developing, and during aging, when our body has more problems maintaining homeostasis (Lupien et al., 2009).

3. Age-related changes in HPA-axis activity and their correlates in memory performance

It has been suggested that aging and stress share etiological and physiopathological processes (Pardon, 2007). In fact, several authors have defined age as the gradual loss of the ability of the body to maintain itself in a steady state (homeostasis) and of the ability to adjust itself to changing conditions (e.g. stressors) (Rowe and Kahn, 1987; Masoro, 2005; Pardon, 2007). This progressive failure to adapt to a changing environment could be related to an age-related malfunctioning of the HPA-axis because, as I mentioned above, the HPA-axis plays a crucial role in maintaining and returning to homeostasis after stress. In fact, aging provokes changes in the HPA-

axis activity, and these changes have been identified as a possible mechanism through which stress is involved in the age-related memory decline (Sapolsky et al., 1986; Lupien et al., 2005; Pardon, 2007).

In the following section, I am going to explain what changes have been shown to occur in the HPA-axis with increasing age among healthy people, and what their correlates are in memory performance. Therefore, I am going to explain the impact of age on (i) basal cortisol levels, on (ii) the cortisol reactivity to challenge, and on (iii) the CAR.

3.1 *Age-related changes in basal cortisol levels and their correlates in memory performance*

Earlier cross-sectional studies that compared basal cortisol levels between older and younger people found indications that basal cortisol levels did not change with increasing age in healthy people (West et al., 1961; Jensen and Blichert-Toft, 1971; Waltman et al., 1991). However, some studies reported small changes in circadian cortisol rhythmicity, such as a dampened amplitude and increased cortisol levels at night in older individuals (Friedman et al., 1969; Jensen and Blichert-Toft, 1971; Touitou et al., 1982). Around the same time, studies performed in animals showed a high degree of controversy, as some studies showed an increase in HPA-axis activity with older age (Tang and Phillips, 1978; Angelucci et al., 1987) and others showed no change (Lorens et al., 1990; van Eekelen et al., 1991). Interestingly, only those older animals with increased basal glucocorticoid levels exhibited memory impairments, as compared to animals who maintained their glucocorticoid levels across time (Issa et al., 1990).

Taken together, these earlier studies seemed to indicate that age did not have a huge impact on the HPA-axis, but rather the impact of age on the HPA-axis could be heterogeneous. Longitudinal studies were needed to shed more light on this issue by measuring cortisol levels of the same individuals as they aged. Thus, Lupien et al.

(1996), in a longitudinal study that ranged from 3 to 6 years, found that, in fact, there was a large heterogeneity in the changes that occurred in the HPA-axis over time. They found evidence for three subgroups of older people that showed either a progressive year to year increase in cortisol levels with high levels at the end of the study (38% of the sample), or a progressive increase in cortisol levels with moderate levels at the end of the study (47% of the sample), or a progressive decrease in cortisol levels with moderate levels at the end of the study (15%). Interestingly, the group that showed increased cortisol levels over time together with high cortisol levels at the end of the study showed both memory deficits and a 14% reduction in their hippocampal volume. Similarly, Seeman et al. (1997), in a 2.5 year follow-up longitudinal study, found an increase in cortisol levels in 20% - 26% of the sample, and a decrease in 34% - 41 %. Interestingly, only among women, the increase in cortisol secretion was associated with a decline in their memory performance. Furthermore, Li et al. (2006) found that not only was the increase in cortisol levels over a period of 3 years related to a decrease in memory performance, but higher cortisol levels at the start of the study also predicted memory declines in subsequent years.

In sum, aging has an effect on basal HPA-axis activity. However, this effect is not universal, but rather there is a huge heterogeneity between individuals, with some showing increases in their basal cortisol levels over time and others showing decreases. Interestingly, the increase in basal cortisol levels over time is associated with memory decline. In addition, although Lupien et al. (1996) and Li et al. (2006) did not test for sex effects, Seeman et al. (1997) showed that sex could be a moderator in the relationship between increasing cortisol levels over time and memory decline.

3.2 *Age-related changes in cortisol reactivity to challenge and their correlates in memory performance*

The study of the impact of aging on the HPA-axis reactivity to challenge has received much attention, because it is crucial to answer the question of whether as we age we progressively lose our capacity to adapt to a changing environment and main-

tain our homeostasis. To study this, two different approaches have been undertaken. These different approaches are the study of the impact of aging on HPA-axis reactivity (i) to pharmacological challenges and (ii) to different kinds of stressors (e.g. laboratory psychosocial and/or cognitive procedures). I will discuss these approaches in the next two sections.

3.2.1 *Age-related changes in cortisol reactivity to pharmacological challenge and its correlates in memory performance*

The use of pharmacological substances to study HPA-axis activity has led to a better understanding of the physiological changes that, for example, may be behind the age-related increase in basal cortisol levels. Several pharmacological tests have been developed, and they have made it possible to focus on the different stages of HPA-axis regulation by either stimulating or inhibiting the release of cortisol. Among the tests that focus on activating the HPA-axis, the CRH test (sometimes accompanied by vasopressin) measures the sensitivity of the pituitary gland to producing ACTH through the administration of synthetic or extracted CRH and, indirectly, the sensitivity of the adrenal gland to producing cortisol. Similarly, the ACTH test evaluates the sensitivity of the adrenal gland to producing cortisol through the administration of synthetic or extracted ACTH. The majority of studies that have used the CRH test have shown larger ACTH and cortisol responses in older people than in younger people (Pavlov et al., 1986; Heuser et al., 1994; Luisi et al., 1998; Kudielka et al., 1999), with some studies pointing to a larger effect of age in women (Greenspan et al.,1993; Heuser et al., 1994). On the contrary, the adrenal response to synthetic or extracted ACTH does not vary with increasing age (Vermeulen et al., 1982; Ohashi et al., 1986; Rasmuson et al., 1998; Martinez-Taboada et al., 2002).

It has been suggested that the elevated response to the CRH test in older people is caused by a reduction in the inhibitory feedback-sensitivity of the HPA-axis with age. To study this hypothesis, the inhibitory capacity of the HPA-axis has been com-

pared between young and older individuals. These studies involve the infusion of substances that have the potential of inhibiting the release of cortisol, such as dexamethasone or cortisol itself, and the measurement of their effects on the release of ACTH and cortisol. The results of these studies have shown that older individuals have a reduced inhibition of ACTH and cortisol release as compared to younger individuals (Wilkinson et al., 1997; Kudielka et al., 1999; Wolf et al., 2002). This failure of the HPA-axis inhibition has been attributed to a decreased feedback sensitivity of the glucocorticoid receptors (MRs and GRs) in the hippocampus of older people (Wilkinson et al., 1997; Otte et al., 2003).

To summarize, it appears that there is an enhanced cortisol response to pharmacological challenge with older age, and this increased response has been related particularly to a decreased inhibitory feedback-sensitivity in older people. Additionally, sex could be a crucial factor in this matter, to the extent that it has been suggested that the impact of age on the HPA-axis response to challenge is three times stronger in women than it is in men (Otte et al., 2005).

Besides confirming age-related changes in HPA-axis feedback sensitivity, the use of pharmacological substances to increase glucocorticoid levels has shown that acute changes in glucocorticoid levels can modulate memory performance. Thus, pharmacological doses of, for example, dexamethasone and prednisone (both are glucocorticoid agonists), acutely decrease memory performance (Wolkowitz et al., 1990; Newcomer et al., 1994). Later on, a distinction was made between the glucocorticoid effects on different phases of the memory process (e.g. consolidation and retrieval) and also on the different types of memory (e.g. declarative and working memory). Thus, it has been shown that pharmacologically increased glucocorticoid levels improve memory consolidation but impair memory retrieval (for more information on this matter see: Roozendaal et al., 2002), and have an effect on memory processes linked predominantly to hippocampal and prefrontal cortex function, such as declarative and working memory, but not to other types of memory like procedural memory (Kirschbaum et al., 1996; Lupien et al., 1999).

The majority of studies investigating the acute modulation of memory by pharmacologically increased glucocorticoid levels have been performed with young people, and only a few studies have included older people; of the latter, most of the time only men were studied (Wolf et al., 2001; Lupien et al., 2002). Despite this, results show that the memory performance of older people is also vulnerable to acute increases in glucocorticoid levels (Lupien et al., 2002), although they could be less sensitive than younger people to the acute effects of glucocorticoids on working memory (Newcomer et al., 1995; Wolf et al., 2001). This effect has been explained as a possible reduction in the sensitivity of the prefrontal cortex to elevated cortisol levels (Wolf et al., 2001).

These pharmacological studies have proved to be useful to investigate specific functions of glucocorticoids, but they also have their limitations. The most important limitation is that there are major neuroendocrine differences between pharmacologically-induced glucocorticoid elevations and stress-induced glucocorticoid elevations (Lupien and Schramek, 2006). As explained above, the activity of the HPA-axis is dynamic and implies a cascade of events that, in the end, lead to the release of glucocorticoids. However, if the increase in glucocorticoid levels is produced artificially, all the neuroendocrine events that normally occur before the release of glucocorticoids are neglected and, in some cases, can even be the opposite of what usually occurs in a natural activation of the HPA-axis. In fact, a stressful situation provokes the increase of CRH and ACTH, whereas an exogenous administration of glucocorticoids suppresses the release of CRH and ACTH and, therefore, decreases circulating levels of both. In addition, there are important differences in receptor affinities between endogenous and synthetic glucocorticoids, which limits the information derived from these studies to the activation of the specific receptor involved (Raison and Miller, 2003). For example, dexamethasone binds avidly with GRs, while endogenous cortisol has a much higher affinity for MRs and less for GRs. Finally, but not less important, stress is not equal to glucocorticoid increases because many other psychological and physiological changes occur that do not happen with artificial glucocorticoid intake, includ-

ing mood changes or autonomic activation, which can also play a role in memory modulation.

3.2.2. *Age-related changes in cortisol reactivity to stressors and their correlates to memory performance*

To overcome the previous limitations and to study stress within a more ecologically-valid context, several laboratory procedures have been developed to provoke consistent psychological and physiological stress responses. These laboratory procedures are designed to activate both the HPA-axis and the ANS, and they involve different kinds of stressful tasks ranging from public-speaking tasks, cognitive tasks (e.g. arithmetic tasks, vigilance–reaction time tasks), emotion induction procedures and noise exposure tasks to more physical stressors such as the cold pressor test. Among them, a combination of a public speaking task and a cognitive task has proved to provoke the most consistent endocrine, cardiovascular, immune and subjective stress responses (Kudielka et al., 2007), as well as the strongest cortisol response (Dickerson and Kemeny, 2004).

Studies using stressful laboratory procedures have shown marked sex differences in the salivary cortisol response to stress. Thus, stress-induced cortisol responses are up to twice as high in men as they are in women (for reviews see: Kajantie and Phillips, 2006; Kudielka et al., 2009). The same effect of sex can be observed in young and older samples (Steptoe et al., 1996; Nicolson et al., 1997; Kudielka et al., 2004b; Strahler et al., 2010b). Additionally, menstrual cycle phase also has an effect on the salivary cortisol response to stress of women. Hence, women in their luteal phase show a salivary cortisol response to stress similar to that of men, while women show lower cortisol responses when they are in their follicular phase, taking oral contraceptives, or are postmenopausal (Kirschbaum et al., 1999; Rohleder et al., 2003; Strahler et al., 2010b). The amount of circulating CBG has been pointed out as a main factor related to these sex effects (Kudielka and Kirschbaum, 2005). CBG con-

centrations are increased in women who take oral contraceptives (Wiegratz et al., 2003) as well as in postmenopausal women (Kudielka et al., 2004b). Therefore, as the cortisol concentrations measured in saliva reflect the free portion of cortisol (not bound with CBG), the blunted cortisol response observed in women can partly be explained by higher concentrations of CBG, leading to a greater percentage of the secreted cortisol bound with it.

Studies investigating the impact of age on the response to stressors are very scarce, and results are mixed (Kudielka et al., 2009). Thus, while several studies have not found differences in the cortisol response to acute stress between young and older individuals (Nicolson et al., 1997; Kudielka et al., 1999; 2000; Rohleder et al., 2002), others have found a higher cortisol response with older age (Seeman et al., 2001; Kudielka et al., 2004b; Traustadóttir et al., 2005; Strahler et al., 2010b). In addition, similar to the results using pharmacological challenges, several studies point to a modulating effect of sex on the impact of age on the cortisol response to stress. However, in the case of laboratory stressors, limited evidence suggests that it would be men and not women who show an increased cortisol response with age (Kudielka et al., 2004b; Strahler et al., 2010b).

Besides age effects on the HPA-axis response to stressors, it is also important to mention that aging can have also an impact on the ANS response to stress, which can have adverse effects on health by, for example, exacerbating cardiovascular diseases. There is some consensus that in restful conditions older people show a heightened sympathetic tone (for a review see: Seals and Dinenno, 2004). However, the influence of aging on the ANS response to stress remains controversial. While several studies have reported no changes in the ANS response to stress with older age (Esler et al., 1995; Wood et al., 2002), others have reported a decreased response (Kudielka et al., 2004a; Strahler et al., 2010b), or even an enhanced response (Pascualy et al., 1999; Uchino et al., 1999).

To summarize, the use of laboratory procedures has allowed a more complete study of the stress response. However, the results from studies investigating the im-

pact of aging on the acute stress response are far from conclusive. Therefore, the purpose of the first study of this dissertation will be to address this issue by comparing both the HPA-axis and ANS responses to stress of young and older men and women.

As I discussed above, studies using pharmacological challenges have shown that glucocorticoids can acutely modulate memory performance. A similar approach has been developed to investigate whether the stress response to laboratory procedures has an acute impact on memory performance. The majority of studies have investigated young people and focused mostly on declarative memory performance. The results of these studies are contradictory because when stress was provoked prior to learning they found on immediate recall either no effects (Elzinga et al., 2005; Hidalgo et al., 2011), impairing effects (Kirschbaum et al., 1996; Jelicic et al., 2004; Payne et al., 2006; Smeets et al., 2006) or even enhancing effects on memory performance (Smeets et al., 2007; Schwabe et al., 2008). These inconsistent findings can be explained, at least in part, by the existence of an inverted U-shaped relationship between circulating glucocorticoid levels and memory performance (for reviews see: Lupien and McEwen, 1997; de Kloet et al., 1999; Lupien et al., 2007). According to this theory, enhancing effects on memory performance would be observed under moderate concentrations of glucocorticoids, when MRs are saturated and GRs are partly occupied. However, when glucocorticoid levels are too low or too high, the memory performance would be impaired.

Nevertheless, whether the same effects can be seen in older men and women is unknown because only a few studies are available, and the results are not conclusive. Thus, two studies carried out with a sample composed only of women found that the exposure to stress prior to learning did not have any effect on declarative memory performance (Bohnen et al., 1990; Domes et al., 2002); however, Lupien et al. (1997) found that acute stress impaired declarative memory performance in both men and women. Unfortunately, the sample in the lastter study was too small (only 7 men and 7 women) to investigate sex differences. According to these results, sex

could be a moderator of the impact of acute stress on memory performance in older people, and this issue deserves further investigation. Therefore, the second study of this dissertation investigated whether the memory performance of older people is vulnerable to the acute effects of stress, and whether there are sex differences in this vulnerability.

Finally, to the best of my knowledge, no study has investigated whether the magnitude of the cortisol response to acute stress is related to the memory performance of older people when it is tested in non-stressful conditions, i.e. to the 'basal' memory performance. Some preliminary evidence comes from the study by Lupien et al. (1997), who found that high-cortisol responders to stress had poorer memory performance than non-responders, not only after the exposure to stress, but also before being exposed to the stressful task . This suggests that the magnitude of the stress-induced cortisol response could be a measure of the HPA-axis status and flexibility, which could be related to the individual differences observed in the 'basal' memory performance of older people. This issue is going to be addressed in the third study of this dissertation.

3.3 *Age-related changes in the cortisol awakening response and its correlates in memory performance*

The CAR is a short period of increased cortisol secretory activity initiated after morning awakening that typically peaks between 30 and 45 minutes post-awakening (Pruessner et al., 1997; for complete reviews see: Fries et al., 2009; Clow et al., 2010a and b). It is a complex phenomenon that is still not completely understood. The CAR seems to be independent from the cortisol release during the rest of the day because, although the total cortisol secreted after awakening is related to the total amount of cortisol secreted during the day, the dynamic of the CAR, i.e. the total increase, is not (Edwards et al., 2001). Additionally, the main function of the CAR is still not known, although it has been suggested that it could be related to the transition

from sleep to full alertness, and that it can play a role in synchronizing the body to both sleep-wake and light-dark cycles (Clow et al., 2010a). Accordingly, it appears that the CAR is controlled by different brain connections from the activation of the HPA-axis in other circumstances, because the hypothalamic suprachiasmatic nucleus (SCN) and the sympathetic nervous system seem to have a more important function in the control of the CAR (Buijs et al., 2003; Clow et al., 2010a). Furthermore, it seems that the influence of the hippocampus in the control of the CAR is actually opposite to the role that it usually plays in the HPA-axis activity during the rest of the day. Thus, it is possible that the function of the hippocampus in the CAR is permissive, instead of the inhibitory function that it exerts during the rest of the day. This hypothesis has been derived from studies with patients who had anatomical lesions in their hippocampus and did not show a CAR (Buchanan et al., 2004; Wolf et al., 2005b). More research is still needed to completely understand the function, control and regulation of the CAR.

In a similar vein, the information available about the impact of older age on the CAR is very sparse and not conclusive. Two studies did not find any age effect on the CAR (Pruessner et al., 1997; Wust et al., 2000), another study found that older age was associated with a lower CAR (Kudielka and Kirschbaum, 2003), and yet another study distinguished between two patterns of CAR in older people: a group with normal CAR (prevalence of 73%) and a group who showed a larger CAR, a larger diurnal cortisol output, and a flatter pattern of cortisol release during the day (27%) (Kumari et al., 2010). It is possible that, as in the case of the impact of age on basal cortisol levels, the impact of age on the CAR would not be strong, but large individual differences would exist. Finally, it appears that sex has a small impact on the CAR (Fries et al., 2009).

To date, only two published studies have investigated whether there is a relationship between the magnitude of the CAR and memory performance in older people. Franz et al. (2011) concluded that the CAR did not appear to contribute meaningfully to the association between HPA-axis activity and memory performance. Howev-

er, Evans et al. (2011) found in elderly people that a larger CAR was associated with better performance on memory tests that mainly depend on prefrontal cortex functioning (e.g. working memory and verbal fluency). However, this relationship was small and disappeared after controlling for age. In my opinion, the CAR is a clearly different facet of the HPA-axis activity, and it is interesting to further explore whether it would be related to the individual differences observed in the memory performance of older people. Therefore, the fourth study of this dissertation further explores this issue by investigating whether the CAR is related to the declarative and working memory performance of older men and women.

4. Aims and Hypothesis

The overview of the literature presented in this first chapter has shown that the HPA-axis activity and regulation can be related to the individual differences observed in the memory performance of older people. But what I have also shown is that there are some important gaps and mixed results in the scientific literature on this topic. To address them, I designed and performed a total of four studies. Each study had the following aims and hypotheses:

Study 1: The aim of this study was to investigate the impact of age on the HPA-axis and ANS responses to acute psychosocial stress. The HPA-axis activity was evaluated through the measurement of the salivary cortisol response. The ANS activity was evaluated through the measurement of both the salivary alpha amylase (sAA) and heart rate (HR) responses to stress. Following the results of previous studies that also employed a psychosocial stressor, we expected to find lower sAA and HR responses to stress in the older group. I had no specific hypothesis on the impact of age on the salivary cortisol response to stress, due to the mixed results in the literature.

Study 2: The aim of this study was to investigate the moderating role of sex in the HPA-axis response to an acute psychosocial stressor and its relation to memory performance in older people. Based on the results of other studies using a psychosocial stressor, we expected a greater cortisol response in men than in women. In addition, we hypothesized that the impact of cortisol reactivity to stress on memory would be different between men and women.

Study 3: The aim of this study was to investigate whether the magnitude of the HPA-axis reactivity to acute stress is related to memory performance in older people when memory is measured under non-stressful conditions. I expected that a higher cortisol response to stress would be related to poorer declarative and working memory performance.

Study 4: The aim of this study was to investigate whether, among older adults, individual differences in the CAR are related to individual differences in memory performance. This topic has been under-studied and results are contradictory; therefore, I did not have a specific hypothesis.

Chapter 2

Study 1: Impact of Age on the Psychophysiological Response to Acute Stress[1]

[1] The main results of this study have been published in: Almela, M., Hidalgo, V., Villada, C., van der Meij, L., Espín, L., Gómez-Amor, J., Salvador, A. (2011). Salivary alpha-amylase response to acute psychosocial stress: The impact of age. *Biological Psychology* 87 (3), 421– 429.

2.1 Introduction

Lifetime exposure to stress can have important consequences for health. Stress has been related to a large number of pathologies that have a higher incidence in old age, such as cardiovascular disease, atherosclerosis, cancer or Type 2 diabetes (Chrousos and Kino, 2007; Steptoe 1991). Aging is associated with several psychobiological changes, such as increased vulnerability to oxidative stress, imbalances in central neurotransmitter pathways, and changes in emotional regulation (Ferrari et al., 2008; Salmon et al., 2010; Alameida et al., 2011), which could limit our ability to cope with stressors (Pardon, 2007). Therefore, it is important to clarify the physiological mechanisms that underlie the stress response, as well as the changes that occur in them as we age.

Two main body systems are involved in the stress response, the autonomic nervous system (ANS) and the hypothalamus-pituitary-adrenal axis (HPA-axis). Recently, salivary alpha amylase (sAA), an oral cavity enzyme, has been identified as a possible biomarker of ANS reactivity to stress (for reviews see: Nater and Rohleder, 2009; Rohleder and Nater, 2009). This enzyme increases rapidly in response to physiological and psychosocial stress conditions, such as exercise and written examinations (Chatterton et al., 1996), the cold pressor stress test (van Stegeren et al., 2008), and the Trier Social Stress Test (Rohleder et al., 2004; Nater et al., 2005; 2006). Several studies have been performed to profile the sAA response to stress mainly in children (Granger et al., 2006; Spinrad et al., 2009; Raikkonen et al., 2010), adolescents (Gordis et al., 2006; Sumter et al., 2010; Susman et al., 2010) and young adults (Nater et al., 2005; 2006; Rohleder et al., 2006; Schoofs et al., 2008). However, data available on older people are very sparse and the results are mixed. Previous studies have suggested that old age has no effect on basal sAA levels (Aguirre et al., 1987; Pajukoski et al., 1997; Salvolini et al., 1999), although a more recent study has shown that older adults have higher overall sAA output throughout the day (Strahler et al., 2010a). To our knowledge, only one published study investigated the effects of age

on the acute sAA response to stress, finding an attenuated response in older adults (59-61 years) compared to young adults (20-31 years) (Strahler et al., 2010b).

More studies have been performed to examine the impact of age on other ANS biomarker responses to stress, such as heart rate, heart rate variability or plasma epinephrine and norepinephrine. According to an exhaustive review by Seals and Dinenno (2004), it appears that the primary effect of aging on the human ANS is an elevation in the tonic sympathetic activity. However, the influence of aging on the ANS response to stress remains controversial. For example, several studies have reported no changes with age (Esler et al., 1995; Wood et al., 2002), a decreased response (Kudielka et al., 2004a; Strahler et al., 2010b), or even an enhanced response (Pascualy et al., 1999; Uchino et al., 1999).

In contrast to sAA, the impact of age on the end product of HPA-axis activation, cortisol, has been investigated more extensively (for reviews and a meta-analysis see: Seeman and Robbins, 1994; Otte et al., 2005; Kudielka et al., 2009). Studies using pharmacological stimulation of the HPA-axis have consistently shown that elderly people have an elevated HPA-axis response compared to young adults (e.g. Heuser et al., 1994; Born et al., 1995; Luisi et al., 1998; Kudielka et al., 1999). Nevertheless, results are mixed when studying age differences in the cortisol response to different kinds of stressors (Kudielka et al., 2009). While several studies did not find any age effect on the cortisol responses to psychosocial stressors (Nicolson et al., 1997; Kudielka et al., 1999; 2000; Rohleder et al., 2002), others found a higher cortisol response with increasing age (Gotthardt et al., 1995; Seeman et al., 2001; Kudielka et al., 2004b; Traustadottir et al., 2005; Strahler et al. 2010b).

In the current study, we subjected a group of young and older participants to both a psychosocial stressor (TSST, Kirschbaum et al., 1993) and a control situation in a crossover design. Although no sex differences have been found in basal sAA levels (Rantonen and Meurman, 2000; Nater et al., 2007), or in the acute sAA response to stressors (Kivlighan and Granger, 2006; Takai et al., 2007), the HPA-axis shows sexual

dimorphism. In fact, the cortisol response to stress is up to twice as high in men as it is in women, regardless of age (Kudielka et al., 2009). Furthermore, this response is dependent on the phase of women's menstrual cycle (Kirschbaum et al., 1999). For these reasons, the current study included men and women in equal numbers in each age group. Additionally, as it has been shown that taking oral contraceptives does not alter basal sAA levels or sAA responses to stress (Laine et al., 1991; Schoofs et al., 2008), and in order to avoid the effect of menstrual cycle phase on cortisol concentrations, we decided to select only young women taking oral contraceptives. Before, during and after the stress task, we measured cortisol and sAA concentrations and, as a complementary measure of the ANS, heart rate (HR). Following the only study that has assessed age differences in the sAA response to stress (Strahler et al., 2010b), we expected attenuated sAA and HR responses to stress in the older group. Furthermore, due to the mixed results regarding age differences in basal sAA levels and cortisol reactivity to stress, we investigated whether the sAA overall output and cortisol stress-induced increase were different between age groups.

2.2 Methods

Participants

The sample was composed of sixty-two participants divided into two age groups: Older adults (N = 31; 16 men and 15 women; age range: 54-71 years) and Young adults (N = 31; 16 men and 15 women; age range: 18-35 years). Within both age groups, there were no sex differences in age, body mass index (BMI), subjective socioeconomic status (Subjective SES scale: Adler et al., 2000) or education level (for all $p > 0.11$) (See Table 1).

Most of the young participants (90%) were university students from a wide range of college studies, such as Psychology, Medicine, History, etc., and unem-

ployed (90%). Most of the older participants were retired (90%) and belonged to a study program at the University of Valencia for people over 50 years of age (84%). For subject recruitment, announcements were posted and informative talks were held in the various departments of the University campus. Volunteers were interviewed and completed an extensive questionnaire to check whether they met the study prerequisites. The criteria for exclusion were: alcohol or other drug abuse, dental, visual or hearing problems, presence of cardiovascular, endocrine, neurological or psychiatric disease, and the presence of a stressful life event during the last year. Participants were excluded if they were using any medication directly related to emotional or cognitive function, or one that was able to influence hormonal and sAA levels, such as glucocorticoids, β – blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, and psychotropic substances. Vitamins, sporadic use of painkillers, and natural therapies were allowed. All the older women were postmenopausal, having had their last menstrual period at least four years before, and none of them were receiving estrogen replacement therapy. All the young women were taking oral contraceptives (monophasic formulas). The use of contraceptives is widespread in Western society; therefore, women using this medication constitute an interesting research group in itself. None of the participants were habitual smokers, although in each age group two participants reported sporadic smoking (less than 10 cigarettes a week).

Participants meeting the criteria were contacted by telephone and asked to attend two sessions that took place in a laboratory at the Faculty of Psychology. Before each session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to drink only water, not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session, and not brush their teeth at least one hour prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the

Ethics Research Committee of the University of Valencia. All the participants received verbal and written information about the study and signed an informed consent form.

Procedure

This study employed a within-subject design with two completely randomized and counterbalanced conditions in two separate sessions: a stress condition and a control condition, with about two weeks between sessions. The sessions consisted of several phases of equal duration for both conditions. Each session took 1 hour and 15 minutes to complete, and they were always held between 16.00 and 20.00 hours. Each participant started his or her two sessions at the same hour. Upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked to see whether they had followed the instructions given previously (see *Participants*).

Stress Condition To produce stress, we subjected the participants to the Trier Social Stress Test (TSST). The stress task consisted of 5 min of free speech (job interview) and a 5 min arithmetic task, performed in front of a committee composed of a man and a woman. The participants remained standing at a distance of 1.5 meters from the committee. Additionally, a video camera and a microphone were clearly visible. Both the speech and arithmetic tasks were filmed.

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated. Five minutes after the start of this phase, they completed the mood questionnaire (PANAS Pre-Task). After the habituation phase, the introduction phase started (duration 5 min). In this phase the participants were informed about the procedure for the stress task. They received the instructions in front of the committee in the same room where the task took place. Next, the participants had 10 minutes to

prepare for the speech at hand. Following the preparation phase, the stress task was carried out. Subjects had 35 minutes to recover afterwards, during which they answered some questionnaires, including the mood questionnaire (PANAS Post-task). Participants were not allowed to distract themselves during this period, and when they had completed the questionnaires they waited alone for the remainder of the time.

The timing of the saliva sampling was different for the cortisol and sAA samples according to the time course of their responses to stress induction. The cortisol response to stress, as a reflection of HPA-axis activation, is slower than that of sAA, which reflects ANS activation (see Granger et al., 2007). Therefore, the first saliva sample employed to measure cortisol was taken 25 minutes after the participant's arrival at the laboratory (0 min pre-task). The second cortisol sample was collected 20 min after the onset of the stress task, and the third one 45 min after. To measure sAA the first saliva sample was collected 10 minutes before the onset of the stress task (-10 min), with the second one taken immediately before the speech (0 min), followed by one sample every five minutes after the onset of the task (5 min, 10 min and 15 min).

Control condition The control condition was similar to the experimental condition, except that the stressful task was replaced by a control task. The control task was designed to be similar to the stress task in mental workload and global physical activity, but without the main components capable of provoking stress, such as evaluative threat and uncontrollability (Dickerson and Kemeny, 2004). The control task was composed of 5 minutes of reading aloud and 5 minutes of counting without being in front of an audience. In the preparation phase, the participants read a book with a neutral content. To provoke the same orthostatic stress as in the stress condition, participants had to stand and walk at the same time points and for the same amount of time as in the stress condition. The timing of the saliva samples, the questionnaires used, and the phase durations were the same for the two conditions.

Affect questionnaire

Affect was evaluated by the Spanish version (Sandín et al., 1999) of the PANAS (Positive and Negative Affect Schedule, Watson et al., 1988). This 20-item questionnaire assesses affect according to two dimensions: Positive affect (PA: *interested, excited, strong, enthusiastic*, etc.) and Negative affect (NA: *distressed, upset, guilty, scared*, etc.), with 10 items measuring each state. Participants were asked to complete the questionnaire twice, immediately before (Pre-task) and immediately after the stress/control task (Post-task). They gave their answers based on how they felt at that particular moment. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). Sandín et al. (1999) reported a high internal consistency for the Spanish version, with a Cronbach's alpha for PA ranging from 0.87 to 0.89 and for NA from 0.89 to 0.91.

Heart Rate

Heart rate was measured using an HR monitor (Suunto, model T6, Suunto Oy, Vantaa, Finlandia), which consists of a chest belt for detection and transmission of the heartbeats and a "watch" for collection and storage of the data. The heartbeat detection is performed with an accuracy of 1 ms, and these types of monitors have shown good validity (Radespiel-Troger et al., 2003; Roy et al., 2009). Every heartbeat is transmitted and stored in the flash memory of the watch. HR was monitored continuously during the entire session, but the recorded periods when the participants were changing their positions (sitting/standing up) and walking were removed. After eliminating the artifacts, the HR mean for each phase was computed. The HR monitor failed to register the heart rate of one participant in the older group.

Biochemical analyses

Cortisol Participants provided three saliva samples by depositing 3 ml of saliva in plastic vials. They took approximately 5 minutes to fill the vial. The samples were frozen at -80º C until the analyses were done. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated) using the commercial kit Coat-A-Count Cortisol (DPC, Siemens Medical Solutions Diagnostics). For each subject, all the samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%.

Alpha-Amylase Saliva was collected using salivettes (Sarstedt, Nümbrecht, Germany). Participants were instructed to introduce the cotton swab into their mouths for exactly 1 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all the salivary glands (Rohleder and Nater, 2009). The samples were frozen at -20º C from the completion of the session until the analyses took place. The samples were shipped to Dresden and analyzed at the Kirschbaum lab, Technical University of Dresden. Concentration of alpha-amylase in saliva was measured by an enzyme kinetic method according to the protocol specified in Rohleder et al. (2006). Inter- and intra-assay variation was below 10%. Analyses of sAA failed to detect the sAA concentrations in the samples of one young woman, one older woman and one older man; therefore, these people were excluded from the statistical analyses regarding sAA.

Statistical Analyses

Data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests before statistical procedures were applied. These analyses revealed significant deviations of some sAA and cortisol values; therefore, they were square root transformed. We analyzed whether the application

of the TSST in the first or second session had an effect on all the variables measured, by conducting repeated-measures ANOVAs with Order as a within-subject factor. Only in cortisol analysis did Order have a significant effect ($p < 0.05$); therefore, we included Order as a covariate in all the statistical analyses involving the cortisol variables.

Student's t-tests were used to investigate age and sex differences in the demographic variables. We used repeated-measures ANOVAs with Condition (stress vs. control) as a within-subject factor to evaluate baseline differences between the stress and control conditions in all the variables measured.

For sAA, cortisol and HR we calculated the areas under the total response curve with respect to the ground (AUCG) and with respect to the increase (AUCi), using the trapezoid formula specified in Pruessner et al. (2003). We also computed the delta change in PANAS positive and negative affect scores (PostTask – PreTask).

Repeated-measures ANOVAs were used to investigate the effect of the TSST on the summary indices (i.e. AUCg, AUCi) of sAA, cortisol, heart rate and positive and negative affect. We used Condition (stress vs. control) as a within-subject factor and Age and Sex as between-subject factors. We investigated whether sAA indices (AUCg and AUCi) were related to cortisol and HR indices, using hierarchical regression and controlling for age, sex, BMI, order and delta change in negative affect.

We used the Greenhouse-Geisser procedure when the requirement of sphericity in the ANOVA for repeated measures was violated. Post hoc planned comparisons were performed using the Bonferroni adjustments for the *p*-values. All *p*-values reported are two-tailed, and the level of significance was marked at <0.05. For significant results, partial eta squared is reported as a measure for effect size. When not otherwise specified, results shown are means ± standard error of means (SEM). We used SPSS 17.0 to perform the statistical analyses.

2.3 Results

Positive and Negative Affect

The participants' positive affect was not influenced by the experimental procedure (all $p > 0.17$) (see figure 1-A). However, the stress induction had an effect on their negative affect (NA) (Condition: F (1,58) = 32.595 $p < 0.001$, $\eta_p^2 = 0.360$; Condition×Time: F (1,58) = 30.890 $p < 0.001$, $\eta_p^2 = 0.348$). Baseline NA was similar between conditions ($p = 0.745$). The TSST provoked an increase in NA ($p = 0.001$, $\eta_p^2 = 0.183$), while after the control task the NA scores decreased, $p < 0.001$, $\eta_p^2 = 0.357$. Consequently, the NA scores were higher after the stress than after the control task, $p < 0.001$, $\eta_p^2 = 0.493$.

A main effect of Age was found for NA scores (F (1,58) = 5.892 $p = 0.018$, $\eta_p^2 = 0.092$), showing that the younger group had higher NA scores than the older group across both conditions (see figure 1-B). However, the stress-induced NA increase was not different between the two age groups ($p > 0.30$). The factor Sex was not significant, nor were there any interactions between Sex and the other factors, for all $p > 0.5$.

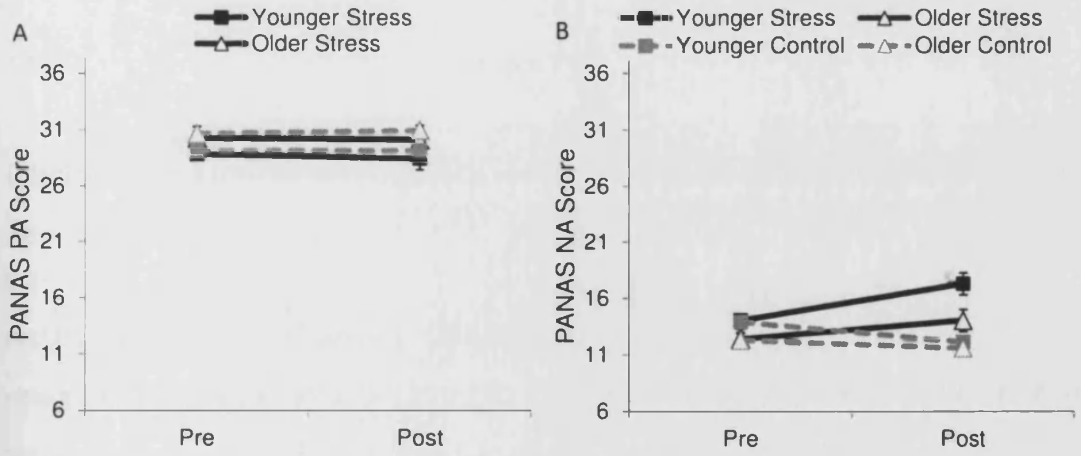

Figure 1 Means of (A) positive and negative affect (B) scores (PANAS) (±SEM) before and after the TSST and the control task in the age groups (younger: $N = 31$; older: $N = 31$).

Salivary Alpha-Amylase

Figure 2 shows the means of sAA concentrations (±SEM) for both age groups in the stress and control conditions. Baseline sAA concentrations did not differ between conditions, $p > 0.5$. The total sAA increase (AUCi) and the overall sAA output (AUCg) were higher in the stress than in the control condition (AUCi: F (1,55) = 17.084 $p < 0.001$,$\eta_p^2 = 0.237$, AUCg: F (1,55) = 21.512 $p < 0.001$, $\eta_p^2 = 0.281$).

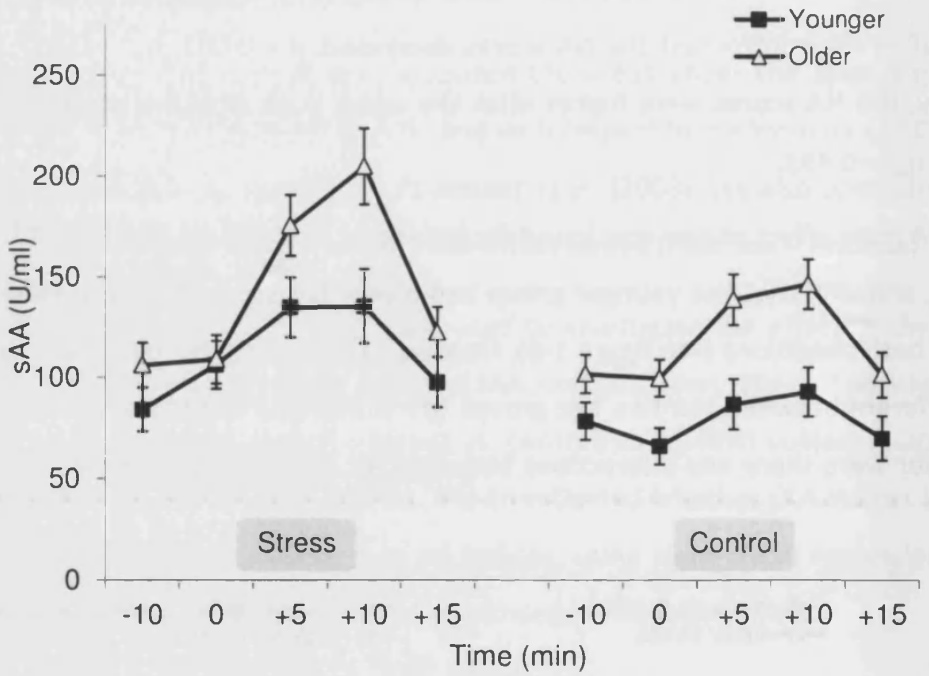

Figure 2 Means of salivary alpha amylase concentrations (±SEM) in the stress (left) and control (right) conditions in the age groups (younger: $N = 30$; older: $N = 29$).

A main effect of Age was found for both AUCi and AUCg (F (1,55) = 4.620 $p = 0.036$, $\eta_p^2 = 0.077$; and F (1,55) = 4.374 $p = 0.041$, $\eta_p^2 = 0.074$, respectively). Regardless of the condition, the older participants increased their sAA concentrations more, and their overall sAA output was higher, than the younger participants (see figure 3). The interaction between Condition and Age was not significant, $p > 0.2$. The factor

Sex was not significant nor were there any interactions between Sex and the other factors, for all $p > 0.2$.

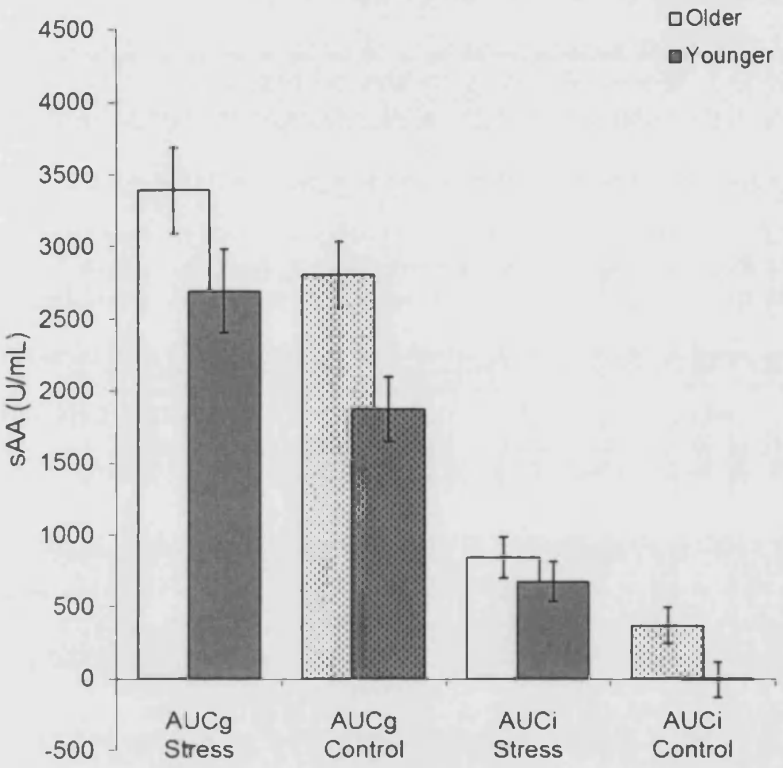

Figure 3 Means of salivary alpha amylase AUCg and AUCi in the stress and control conditions (younger: $N = 30$; Older: $N = 29$).

Salivary Cortisol

Figure 4-A shows the means (±SEM) of cortisol released by men and women from both age groups in the stress and control conditions. Baseline cortisol concentrations did not differ between conditions, $p > 0.6$. The cortisol concentrations increased in the stress condition but decreased in the control condition (AUCi: F (1,54) = 12.501 p = 0.001, η_p^2 = 0.188). Consequently, the overall cortisol output (AUCg) was higher in the stress condition than in the control condition, F (1,54) = 13.122 p = 0.001, η_p^2 = 0.195.

A main effect of Age was found for cortisol AUCi (F (1,54) = 4.537 p = 0.038, η_p^2 = 0.077), showing that regardless of the condition the older group had higher cortisol AUCi than the younger group (see figure 4-B). The interaction between Condition and Age was not significant, $p > 0.2$.

An interaction between Condition and Sex was found for both AUCi and AUCg (F (1,54) = 4.336 p = 0.042, η_p^2 = 0.074 and F (1,54) = 6.038 p = 0.017, η_p^2 = 0.101, respectively). Men had higher overall cortisol output in the stress condition than women (p = 0.010, η_p^2 = 0.117), but not in the control condition, p = 0.290. Furthermore, although non-significant, on average the stress-induced cortisol increase was higher in men than in women (p = 0.091), and men had a higher decrease in their cortisol concentrations in the control condition than women did, p = 0.064.

For the above analysis the cortisol data of 3 participants (2 young men and 1 older man) were removed, as their concentrations differed by more than 3 standard deviations from the rest of the sample. However, including them did not change the statistical conclusions in any way.

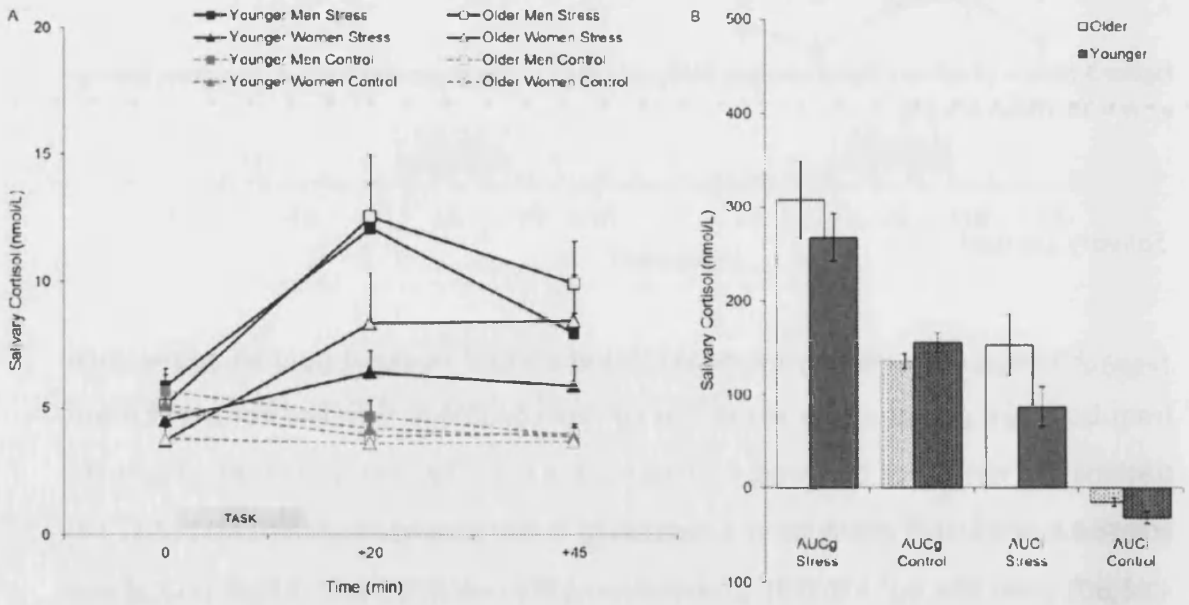

Figure 4 Means of (A) salivary cortisol concentrations (±SEM) and cortisol AUCg and AUCi in the stress and control conditions, in the younger men (N = 14), younger women (N = 15), older men (N = 15) and older women (N = 15).

Heart Rate

Figure 5 shows the means of HR (±SEM) in the stress and control conditions for both age groups. Baseline HR did not differ between conditions, $p > 0.5$. The total HR increase, as well as the overall HR output, were higher in the stress condition than in the control condition (AUCi: F (1,57) = 75.815 $p < 0.001$, $\eta_p^2 = 0.571$; AUCg: F (1,57) = 42.091 $p < 0.001$, $\eta_p^2 = 0.425$).

The stress-induced HR increase and overall output showed no differences between young and older participants or between men and women, for all $p > 0.18$. An interaction between Age and Sex was found for the AUCg, F (1,57) = 3.955 $p = 0.052$, $\eta_p^2 = 0.065$, showing that overall young women had higher HR than young men, $p = 0.008$, $\eta_p^2 = 0.118$.

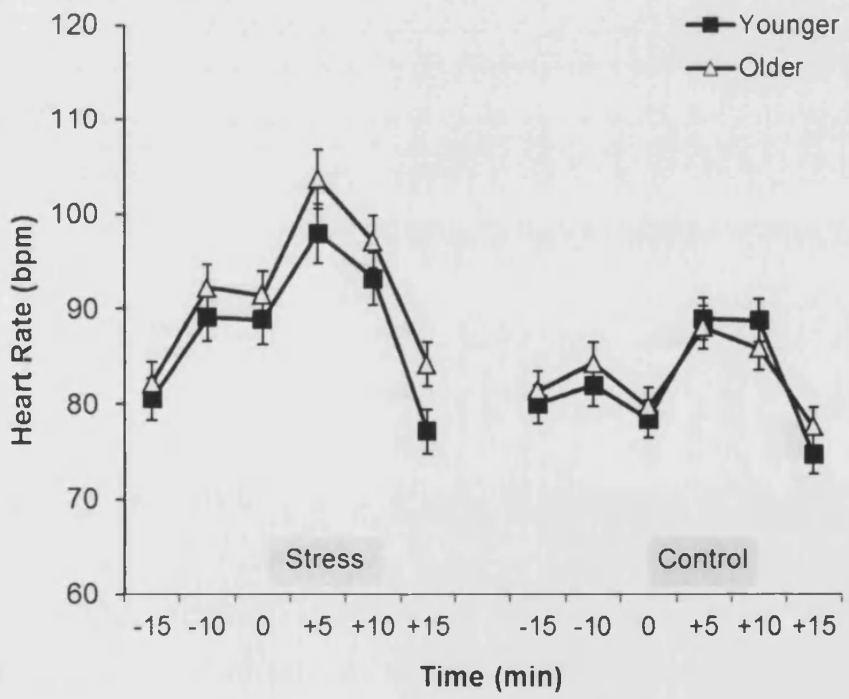

Figure 5 Means of heart rate (±SEM) in the stress (left) and control (right) conditions in the age groups (Younger: $N = 31$; Older: $N = 30$).

Relationships between sAA and the other physiological indices

We computed hierarchical linear regression analyses to test whether the sAA summary indices were related to HR and cortisol indices. For each analysis, in Step 1 we controlled for age, sex, BMI, delta change of negative mood, and order (whether the stress or control condition was performed first).

In the regression analysis with $AUCg_{sAA}$ as a dependent variable, entering $AUCg_{HR}$ in Step 2 did not increase the amount of variance explained, $\Delta F(1,49) = 2.397$, $p = 0.128$. However, entering $AUCg_{Cortisol}$ in Step 3 increased the amount of variance explained, $\Delta F(1,48) = 8.425$, $p = 0.006$, adjusted $R^2 = 15.5\%$, $\Delta R^2 = 12.9\%$. In this model, only $AUCg_{Cortisol}$ was significantly related to $AUCg_{sAA}$, $\beta = 0.423$, $p = 0.006$.

In the regression analysis with $AUCi_{sAA}$ as a dependent variable, entering $AUCi_{HR}$ in Step 2 increased the amount of variance explained, $\Delta F(1,49) = 5.595$, $p = 0.022$, adjusted $R^2 = 2.2\%$, $\Delta R^2 = 9.9\%$. In this model, only $AUCi_{HR}$ was significantly related to $AUCi_{sAA}$, $\beta = 0.324$, $p = 0.022$. Entering $AUCi_{Cortisol}$ in Step 3 did not increase the amount of variance explained in $AUCi_{sAA}$, $\Delta F(1,48) = 0.074$, $p = 0.787$.

2.4 Discussion

In the current study, a group of young and older adults was exposed to psychosocial stress and a control situation in a crossover design. The experimental procedure was indeed able to induce stress, since the exposure to the TSST produced an increase in negative mood, cortisol, sAA and HR. We did not find evidence of an attenuated ANS response to stress in older adults in either sAA or HR. However, regardless of the condition, the older group had a higher sAA global output and increased their sAA concentrations more than the younger group. Regarding the impact of age on the cortisol response to stress, the stress-induced increase in cortisol levels was

higher in the older group, and in the control condition they decreased less their cortisol levels than the younger group. Finally, we found that in the stress condition, the total amount of cortisol released was positively related to the total sAA released, and, interestingly, the HR increase was positively related to the sAA increase.

In contrast with our hypothesis, we did not find age differences in HR and sAA reactivity to stress. Our results coincide with other studies that did not find age differences in HR reactivity to psychosocial stress (Esler et al., 1995; Uchino et al., 1999). However, using the same procedure to provoke stress (i.e. TSST), but without a control condition, it has been shown that HR (Kudielka et al. 2004a) and sAA (Strahler et al. 2010b) responses to stress were attenuated in elderly people. Although the TSST is a standardized procedure to provoke stress, small modifications in this procedure could partially explain this discrepancy. We employed a preparation period of 10 minutes, while this period in the other studies lasted only 3 minutes. It is possible that having more time to prepare and think about the speech could be more stressful and provoke a higher ANS response in the older participants. The time of sAA sampling was also different. Strahler et al. (2010b) took one saliva sample one minute after the arithmetic task and, therefore, measured sAA reactivity of both the speech and arithmetic tasks combined. However, in our study we took one sample immediately after the speech task and another one immediately after the arithmetic task, while both of these saliva samples were provided in front of the committee. It is likely that we more accurately captured the individual profile of sAA secretion. In fact, 24 participants (41 % of the current sample) had their maximum sAA concentration after the speech task; therefore, these participants started to recover to baseline sAA levels in subsequent samples, including the sample taken after the arithmetic task.

Moreover, age differences in the relevance of laboratory-based stressors may affect physiological reactivity (Uchino et al., 2010). For this reason, we tried to maximize the stressfulness of the TSST by making the committee up of university professors older than 50 years of age. In addition, the interaction between the committee and the participant was always performed by the committee member of the opposite

sex. Unfortunately, information about the composition of the committee in other studies (Kudielka et al., 2004a; Strahler et al., 2010b) is not available, and, therefore, we are hesitant to draw any conclusions.

Although we did not find age differences in the stress-induced sAA increase, overall older adults had higher sAA global output (AUCg) irrespective of psychosocial stress. It has been well established that age increases basal sympathoneural activity, while sympathoadrenomedullary activity appears to decrease or remain unchanged (Seals and Dinenno, 2004). In our opinion, the observed higher sAA concentrations among the older participants across both conditions could reflect this increased basal sympathoneural activity. This finding reinforces the suggestion made by Ehlert et al. (2006) that sAA secretion reflects central norepinephrine release instead of peripheral norepinephrine secretion. They conclude this after showing that the increase in sAA after yohimbine infusion (an alpha-2 adrenoreceptor antagonist) was not related to plasma levels of cathecolamines. Furthermore, concentrations of cerebrospinal fluid norepinephrine are higher in older people (Raskind et al., 1988; Elrod et al., 1997), and older people react with greater increases in cerebrospinal fluid norepinephrine after yohimbine challenge than younger people (Peskind et al., 1995; Raskind et al., 1999). Research in the future should help clarify this matter by studying whether sAA is more reactive to yohimbine challenge in older people than in younger people.

Our findings regarding the increase in sAA (AUCi) were similar to the results regarding sAA global output. Irrespective of psychosocial stress, the older group increased their sAA levels more than the younger one. This difference was more evident when looking at the control condition (see figure 3). In this condition, the total amount of sAA secreted was higher in the older group than in the younger one, and while the younger participants hardly changed their sAA levels, among the older participants the sAA concentrations increased. Since negative affect and cortisol declined in the older group after the control task, it is not likely that this increase in sAA concentrations was provoked by stress. Moreover, the control task also provoked an increase in HR that was similar in both age groups. Another possible explanation for

these changes in sAA and HR during the control condition could be related to orthostatic challenge. During the control condition, the participants were asked to stand up and walk at the same moments and for the same length of time as in the stress condition. In line with our results, Nater et al. (2006), using a similar experimental design that emulated a comparable orthostatic challenge in the control and stress conditions, found that the sAA of a young sample was not affected by the control condition, but plasma norepinephrine release was. The results of our study suggest that aging increases the sensitivity of sAA to postural and small changes in global physical activity.

Regarding HPA-axis activity, age was related to a higher increase in cortisol (AUCi) across both conditions. In the stress condition cortisol levels increased, but in the control condition they decreased following the natural cortisol circadian rhythm. In this particular case, the AUCi is an index of decrease (Pruessner et al., 2003, p. 921). Therefore, in our study, the older group increased their cortisol levels more in the stress condition, but at the same time they decreased them less in the control condition than the younger group (See Figure 4-B). Previous studies have related increasing age with both increased stress-induced cortisol responses and decreased cortisol variability in the late afternoon (Gotthardt et al., 1995; van Cauter et al., 1996; Deuschle et al., 1997; Seeman et al., 2001; Kudielka et al., 2004b; Traustadottir et al., 2005; Strahler et al., 2010b). Both effects are consistent with the loss of HPA-axis feedback sensitivity that has been hypothesized to occur with aging (Seeman and Robbins, 1994). In addition to the effects of age on cortisol release, we found sex differences in the cortisol response to stress. Men responded to stress with a higher increase in cortisol concentrations than women, as has been shown consistently in the literature (Kudielka et al., 2009). Several attempts to explain these sex differences have pointed to sexual dimorphisms in brain structures and functioning, but they have also pointed to the circulating levels of corticosteroid binding globulin protein (CBG) (Kudielka and Kirschbaum, 2005). The use of oral contraceptives has been shown to produce an increase in CBG release (Wiegratz et al., 2003), and in the same

way, Kudielka et al. (2004b) found higher CBG levels in older women compared to older men. As the cortisol concentrations measured in saliva reflect the free portion of cortisol (not bound with CBG), the blunted cortisol response observed in women can partly be explained by higher concentrations of CBG, leading to a greater percentage of secreted cortisol bound with it (Kajantie and Phillips, 2006; Kumsta et al., 2007).

The overall stress-induced cortisol output ($AUCg_{Cortisol}$) was associated with more overall sAA release ($AUCg_{sAA}$), but it was not related to the overall sAA increase ($AUCi_{sAA}$). However, the overall sAA increase ($AUCi_{sAA}$) was associated with a higher HR increase ($AUCi_{HR}$). Previously, correlations between stress-induced sAA and cortisol responses had rarely been reported (Gordis et al., 2006), and the majority of studies did not find any correlation between sAA and cortisol responses to stress (Nater et al., 2005, 2006; Strahler et al., 2010b). The relationship observed between the HR increase and the sAA increase in response to stress is in some way not surprising, as both are biomarkers of the ANS. Associations between sAA and HR or other cardiovascular indices have been reported previously. For example, sAA reactivity to stress has been positively related to HR reactivity and the low frequency/high frequency ratio (an index of sympathetic tone), and negatively with the left ventricular ejection time and the RMSSD (root mean square of successive differences of normal-to-normal intervals, an index of parasympathetic tone) (Bosch et al. 2003; Nater et al., 2006). Some studies have also found positive relationships between sAA and other ANS biomarkers, such as plasma norepinephrine and epinephrine (Chatterton et al., 1996; Rohleder et al., 2004), although others failed to find such associations (Nater et al., 2006).

The positive relationship found between the cortisol and sAA total output reflects the coordination between the two main physiological stress systems. As Chrousos and Gold (1992) discuss, the HPA-axis and ANS could interact at many potential central sites to coordinate the stress response, leading the activation of one system to produce the activation of the other as well. The neurons that release corti-

cotropin releasing factor (CRF) project from the lateral paraventricular nuclei (PVN) in the hypothalamus to sympathetic hindbrain regions (Nauta and Feirtag, 1986; Saper et al., 1976), and, conversely, catecholaminergic fibers from the locus coeruleus (LC)-noradrenergic system project to the PVN (Cunningham and Sawchenko, 1988; 1990; Saper and Loewy, 1980). Furthermore, the administration of CRF onto LC neurons increases the LC firing rate (Dunn and Berridge, 1990; Jedema and Grace, 2004), while norepinephrine is a potent stimulus for the release of CRF (Calogero et al., 1988; Cunningham et al., 1990). Our findings support the coordination of both the HPA-axis and the ANS in generating the physiological stress response (Granger et al., 2007).

Some limitations have to be considered in order to interpret the results of the current study. The use of sAA as a biomarker of ANS activation is relatively novel, and the biochemical and physiological properties of this enzyme are still under investigation. The saliva in this study was collected using salivettes, and recently it has been shown that unstimulated and stimulated saliva collection yield different sAA concentrations (DeCaro, 2008). Although we did not instruct the participants to chew the cotton roll, we did ask them to move it around in their mouths in order to collect saliva from all the salivary glands (Rohleder and Nater, 2009). It has been shown that sAA stress-induced increases are independent of salivary flow rate (Rohleder et al., 2006); however, the results observed in the current study cannot be compared with studies that have collected saliva using non stimulating methods. In addition, age could affect salivary gland physiology and, therefore, saliva composition, since the parenchyma of the salivary glands is gradually replaced by fat, connective tissue and oncocytes with age (Nagler, 2004). However, until now, there has been no evidence that aging per se leads to a reduction in the capacity of salivary glands to produce saliva (Dobrosielski-Vergona, 1993) or produce changes in the saliva composition in healthy subjects (Aguirre et al., 1987; Fox et al., 1987). Moreover, it is important to note that all the young women in this study were taking oral contraceptives. Although these pills have not been reported to affect sAA release, they do have an effect on salivary free cortisol. The use of oral contraceptives is very widespread, and, thus, the women using

this medication constitute an interesting research group in itself. However, our results cannot be generalized to premenopausal women with natural cycles, and it would, therefore, be interesting to include them in future research. Finally, we used a stressor (TSST) that is mainly based on social-evaluative threat, and it is plausible that previous experiences with social evaluative challenges moderated the observed stress response. However, it seems unlikely that previous experiences with social stress influenced our main findings since our age groups were quite homogenous. For example, participants were all recruited within university programs, and for all participants it was their first exposure to a standardized lab stressor. Nevertheless, future research should control for previous experiences with social evaluative challenges, and investigate whether it affects age differences in the psycho-physiological response to lab stressors.

Taken together, our results add new knowledge about the effect of age on stress-induced sAA and cortisol release. Our results do not support the existence of an attenuated ANS response to psychosocial stress in older adults, but rather a heightened sympathetic tone. Our results support the existence of a decreased negative feedback sensitivity in the HPA-axis with older age. Furthermore, our findings give support to the coordination between the two main stress systems, the HPA-axis and ANS, beyond kinetic differences in their responses to psychosocial stress.

Chapter 3

Study 2: The impact of cortisol reactivity to acute stress in the memory performance of older people[2]

[2] The main results of this study have been published in: Almela, M., Hidalgo, V., Villada, C., Espín, L., Gómez-Amor, J., Salvador, A. (2011). The impact of cortisol reactivity to acute stress on memory: Sex differences in middle-aged people. *Stress: The international journal on the biology of stress*, 14 (2), 117-127.

3.1 Introduction

The aging process is characterized by large individual differences; some older individuals show small cognitive changes over time, whereas others deteriorate dramatically. Stress has been noted as a key factor related to these individual differences, and sex could moderate the relationship between stress and the cognitive decline during aging (McEwen, 2002). Women are overrepresented in diseases, such as depression or PTSD (Desai and Jann, 2000; Keane et al., 2006), that have a close relationship with both cognitive impairments and the Hypothalamus-Pituitary-Adrenal axis (HPA-axis), the most important system in the control of the stress response (Sapolsky, 2000; Young, 2009). Moreover, emphasizing the interaction between glucocorticoids and sex hormones, timing of sexual maturation has been related to both the HPA-axis activity (Lupien et al., 2009; McCormick and Mathews, 2009; Romeo, 2010) and risk of depression later in life (Harlow et al., 2004).

HPA-axis activity and regulation change differently for men and women with old age (Seeman and Robbins, 1994; Otte et al., 2005). Elderly persons have a stronger cortisol response to challenge than younger persons, and, interestingly, this age effect is especially strong among women (Otte et al., 2005). However, studies specifically investigating sex differences in the cortisol response to psychological challenge among elderly people have found mixed results (for reviews see: Seeman and Robbins, 1994; Kudielka and Kirschbaum, 2005; Kajantie and Phillips, 2006). Greater cortisol reactivity in women has been reported in several studies (Seeman et al., 1995; 2001), although more recently other studies have found higher reactivity in older men (Traustadóttir et al., 2003; Kudielka et al., 2004b) .

Two main brain structures involved in HPA-axis function and regulation are the hippocampus and the prefrontal cortex (Patel et al., 2000; Herman et al., 2005) which are both related to several types of memory, such as declarative and working memory (Scoville and Milner, 1957; Galloway et al., 2008). A large body of research, usually carried out with young participants, has demonstrated that stress can in-

fluence memory processes, although this influence depends on several factors, such as the type and phase of the memory process tested or the emotional valence of the material to be remembered (for reviews see: McEwen, 2002; Lupien et al., 2005; 2007; Sandi and Pinelo-Nava, 2007). Furthermore, studies using acute administration of synthetic glucocorticoids have described an inverted U-shaped dose response curve between glucocorticoids concentrations and declarative memory (de Kloet et al., 1999; Domes et al., 2005) or working memory (Lupien et al., 1999).

With stress, many other psychological and physiological changes occur that do not happen with artificial glucocorticoid intake, including mood changes or autonomic activation (Lupien and Schramek, 2006). Therefore, standardized laboratory procedures to provoke a consistent stress response have been used to study the effects of stress on memory function, but they have not always yielded consistent results. Most of these studies have been performed with young participants, and they have found worsening effects (Jelicic et al., 2004; Payne et al., 2006; Smeets et al., 2006), no-effects (Domes et al., 2004), and even enhancing effects (Smeets et al., 2007; Schwabe et al., 2008), on memory when stress was provoked prior to learning. To our knowledge, few studies have been performed with older people, and the results of these studies are also unclear. When declarative memory was tested after exposure to a stress task, no effects were found in women from 41 to 69 years of age (Bohnen et al., 1990), and from 32 to 68 years of age (Domes et al., 2002). In contrast, Lupien et al. (1997) reported that the stress induced a decrease in memory performance in elderly men and women (62-83 years old). These studies, examined only women or a mixed-sex group, so that it was not possible to detect sex differences in the impact of stress on memory. However, it has been proposed that sex hormones could moderate the glucocorticoid effects on memory (McEwen, 2002; Shors, 2006; Andreano et al., 2008), and the amygdala, a brain structure with estrogen receptors (Alves and McEwen, 1999), has also been associated with the effects of glucocorticoids on memory (de Quervain et al., 2009). In fact, evidence suggests that sex differences in the relationship between stress and memory may be espe-

cially important when studying elderly persons in particular. For example, in a four-year cross-sectional study, Seeman et al. (1997) found that only elderly women, and not men, with increasing baseline cortisol concentrations over time had poorer declarative memory performance. Furthermore, Wolf et al. (2005) found that elderly women with subjective memory complaints had higher 12h urinary cortisol concentrations than those without memory complaints, while no such differences were observed among elderly men. Previously, Wolf et al. (1998) reported that the exposure to a laboratory stressor impaired recall more for elderly women than for elderly men.

The focus of the current study was to investigate the moderating role of sex on cortisol responses to an acute psychosocial stressor and its relation with memory performance in middle-aged persons. The participants were exposed to two conditions in a crossover design. In the stress condition, the Trier Social Stress Test (TSST, Kirschbaum et al., 1993) was used. In the control condition, the participants were asked to solve a task designed to induce a similar mental workload and global physical activation to the stress task. In order to investigate the impact on specific processes of memory performance, we employed the Rey Auditory Verbal Learning Test (RAVLT), which provides several memory indicators (Lezak et al., 2004). Based on the results of other studies in aging populations using the TSST (for reviews see: Kudielka et al., 2005; 2009) we expect a higher cortisol response among men than among women. In addition, we hypothesized that the impact of cortisol reactivity to stress on memory would be different for men and women.

3.2 Method

Participants

The final sample was composed of 32 participants (16 men and 16 women) from 54 to 72 years of age (Total sample: M = 62.09, SEM = 0.85; Men: M = 60.50, SEM = 1.23; Women: M = 63.69, SEM = 1.07). Most of them (91%) had an educational level beyond high school, and their subjective socioeconomic status (Subjective SES scale: Adler et al., 2000) was medium-high. All the men were married, while the women were either married (50%) or widowed (50%). The mean body mass index (BMI) was 26.49, SEM = 0.54 (Men: M = 27.05, SEM = 0.48; Women: M = 25.93, SEM = 0.96). There were no sex differences in age, educational level, SES or BMI (all $p > 0.1$). All the female participants were postmenopausal and had had their last menstrual period at least four years before. None of these women were receiving estrogen replacement therapy, and none of the men were using anti-androgens or undergoing androgen replacement therapy.

Participants belonged to a study program at the University of Valencia for people older than 50 years of age. For subject recruitment, announcements were posted and informative talks were held in the various departments of the University campus. One hundred thirteen persons were interviewed and completed a questionnaire to check whether they met the study prerequisites. In order to avoid the large number of potentially confounding factors that could interfere with the stress response or with the cognitive functioning, we selected a homogeneous healthy sample using very restrictive criteria. The criteria for exclusion were: smoking more than 5 cigarettes a day, alcohol or other drug abuse, visual or hearing problems, presence of a cardiovascular, endocrine, neurological or psychiatric disease, having been under general anesthesia once or more than once in the past year, and the presence of a stressful life event during the last year. Participants were excluded if they were using any medication directly related to cardiac, emotional or cognitive

function, or one that was able to influence hormonal levels, such as glucocorticoids or β – blockers. In addition, women completed a few questions concerning reproductive lifetime events (e.g. age at menarche, gynecological problems).

Participants meeting the criteria were contacted by telephone and asked to attend two sessions that took place in a laboratory at the Faculty of Psychology. No payment was made for participation. Before each session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to drink only water and not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the University of Valencia Ethics Research Committee. All the participants received verbal and written information about the study and signed an informed consent form.

Procedure

This study employed a within-subject design with two completely randomized and counterbalanced conditions in two separate sessions: a stress condition and a control condition, with about two weeks between sessions. The sessions consisted of several phases of equal duration for both conditions, and their sequence is presented schematically in figure 1. Both sessions took 1 hour and 50 minutes to complete, and they were always held between 16.00 and 20.00 hours. Each participant started their two sessions at the same hour. Upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked to see whether they had followed the instructions given previously (see Participants).

Stress Condition To produce stress, we subjected the participants to the Trier Social Stress Test (TSST). The stress task consisted of 5 min of free speech (job in-

terview) and a 5 min arithmetic task, and it was performed in front of a committee composed of a man and a woman. The participants remained standing at a distance of 1.5 meters from the committee. Additionally, there was a video camera and a microphone clearly visible. Both the speech and arithmetic tasks were filmed.

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated. Five minutes after the start of this phase, baseline measures were obtained for anxiety (Stai-S) and mood (PANAS). After the habituation phase, the introduction phase started (duration 5 min). In this phase the participants were informed about the procedure for the stress task. They received the instructions in front of the committee in the same room where the task took place. Next, the participants had 10 minutes to prepare for the task at hand. The first saliva sample (0 min pre-stress) was taken 25 minutes after their arrival at the laboratory, trying to minimize an anticipatory cortisol increase, since elderly people are very reactive to a testing environment (Lupien et al., 2007).

Following the preparation phase, the stress task was carried out. Subjects had 20 minutes to recover after the stress task, and they answered three questionnaires (Situational Appraisal, Stai-S and PANAS, see Questionnaires and scales) and provided the second saliva sample (20 min post-stress). Then each participant performed a standardized memory test which consisted of 8 trials (RAVLT, see Questionnaires and scales). The participants completed the first six trials between 30 to 40 minutes after the TSST. After trial six, they waited 30 minutes (delay period) before they continued with the memory test. During this waiting period, they provided the last saliva sample (45 minutes post-stress). After the delay period, they finished the memory test with trials 7 and 8 and, finally, were debriefed.

Control condition The control condition was similar to the experimental condition, except that the stressful task was replaced by a control task. This task was designed to be similar to the stress task in mental workload and global physical activity, but without the main components capable of provoking stress, such as evaluative

threat and uncontrollability (Dickerson and Kemeny, 2004). The control task consisted of 5 minutes of reading aloud and 5 minutes of counting without being in front of an audience. In the preparation phase, the participants did not prepare for their task, but instead they read a book with a neutral content. The timing of the saliva samples, the questionnaires used, and the phase durations were the same for the two conditions.

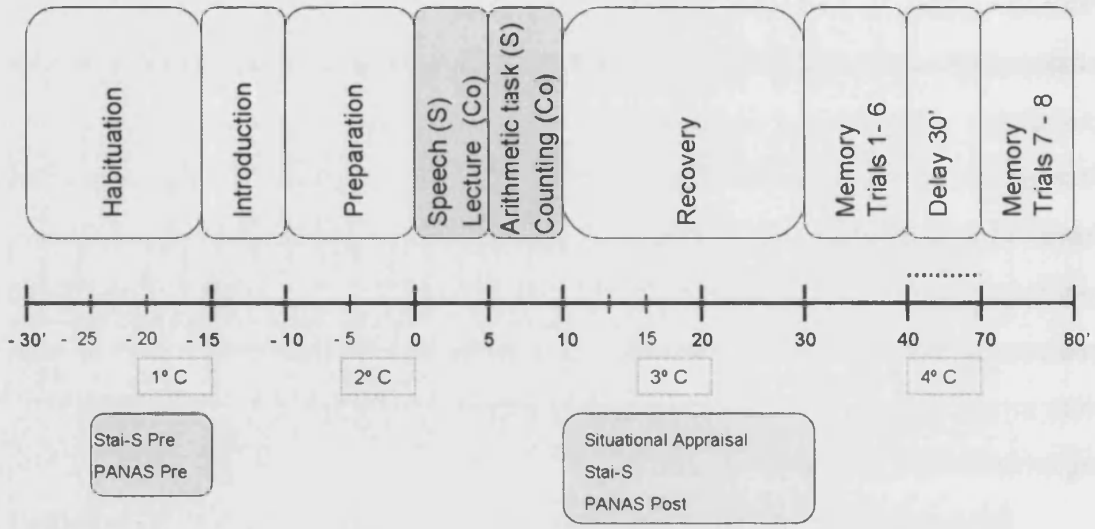

Figure 1. Schedule for the stress (S) and control (Co) conditions. (18, 28, 38 C: sequential salivary cortisol sampling; STAI-S, State Anxiety Inventory form S; PANAS, Positive and Negative Affect Schedule).

Questionnaires and Scales

Situational Appraisal Participants were asked about the stress task according to the five following aspects: stress, difficulty, frustration, effort and motivation (e.g. *How much effort did the task require?*). The questions used were formulated based on previous studies on this topic (Baggert et al., 1996; Gonzalez-Bono et al., 2002). Subjects responded to each question on a 5-point Likert scale (not at all = 1, to extremely = 5).

Mood The mood was evaluated by the Spanish version (Sandín et al., 1999) of the PANAS (Positive and Negative Affect Schedule; Watson et al., 1988). This 20 item

questionnaire assesses mood according to two dimensions: Positive affect (PA: *interested, excited, strong, enthusiastic*, etc.), and Negative affect (NA: *distressed, upset, guilty, scared*, etc.) with 10 items measuring each state. Participants were asked to complete the questionnaire based on how they felt at that particular moment. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). Sandín et al. (1999) reported a high internal consistency for the Spanish version, with a Cronbach's alpha for PA ranging from 0.87 to 0.89 and for NA from 0.89 to 0.91.

Anxiety To assess state anxiety, the Spanish version of the State Anxiety Inventory was used (STAI form S; Spielberger et al., 1970). It consists of 20 phrases (e.g. *'I feel at ease', 'I feel upset'*) with a 4-point Likert scale ranging from 0 (not at all) to 3 (extremely) to evaluate how the participants felt at the moment they gave the answers. The Spanish version of the scale had a Cronbach's alpha ranging from 0.90 to 0.93 (Seisdedos, 1988).

Memory To measure declarative memory, the Spanish version of Rey's Auditory-Verbal Learning Test (RAVLT) was used (Miranda and Valencia, 1997). This test has several versions, and for each participant a different version of the RAVLT was used in their second session to avoid learning effects. The order of the two versions was randomized and counterbalanced. The RAVLT is composed of different trials. In the first five trials, the experimenter read aloud a target list of 15 neutral words, and each participant had to repeat as many words as possible in each of the five trials. The performance on these first five trials reflects the rate of learning (Trials 1 to 5: *Learning curve*). After trial 5, the experimenter read aloud an interference list of 15 words and tested the retention of these new words. Following this step, the participants were requested to recall the words from the target list (Trial 6: *Recall after interference*); after a delay of 30 minutes, they had to recall them a second time (Trial 7: *Delayed recall*). In trial 8 (*Recognition*), the participants had to recognize the memorized words from a list presented verbally containing 15 new and 15 previously learned words. Trial 8 was divided into two different scores: *Hits*, the number of

words correctly recognized as being on the target list; and *False alarms*, the number of words incorrectly recognized as being on the target list.

Hormonal assays

Participants provided three saliva samples by depositing 3 ml of saliva in plastic vials. They took approximately 5 minutes to fill the vial. The samples were frozen at - 80º C until the analyses were done. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Coat-A-Count Cort (DPC, Siemens Medical Solutions Diagnostics). Assay sensitivity was 0.5 ng/ml. For each subject, all the samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%.

Statistical Analyses

Student's *t*-tests were used to investigate sex differences in the demographic variables. ANOVA's for repeated measures were used to assess differences in the appraisal of the two tasks, differences in the baseline of the variables measured, and the effects of both the stress and control tasks on mood, anxiety, and cortisol release. We employed Condition (stress vs. control) as a within-subject factor. For the changes in cortisol concentrations, we added Time (0, 20 and 45 min) as a within-subject factor. To assess sex differences, we included Sex as a between-subject factor.

The memory test used (RAVLT) provides one score for each trial performed, which consists of the number of correct words recalled in each trial. In trials 1 to 7, the words from the same target list have to be recalled; for this reason, we performed an ANOVA for repeated measures. We used Condition (stress vs. control) and Trials (trials 1 to 7) as within-subject factors and Sex as a between-subject factor. To

analyze the effects on recognition (trial 8), we used d-prime (d'), which is the difference between the standardized proportion of correct hits and the standardized proportion of false alarms. An ANOVA for repeated measures was performed using d-prime as a dependent variable, Condition (stress vs. control) as a within-subject factor, and Sex as a between-subject factor.

To assess whether the cortisol response to the stress task was related to the memory performance, we correlated the *Cortisol Reactivity* to stress with the number of words the participants could recall in the RAVLT trials of the stress condition and the control condition. To take into account the individual differences in the cortisol reactivity to stress, as well as in the pattern of cortisol release in a control situation (Lovallo et al., 2010), the *Cortisol Reactivity* to stress was defined as the difference between the area under the curve with respect to increase (AUCi) in the stress condition, and the AUCi in the control condition (in this case 'index of decrease', see Pruessner et al., 2003). We also correlated the age at menarche of the women with the *Cortisol Reactivity* to stress and memory performance in both conditions. Since a normal distribution could not be expected in a small sample size, Spearman's rank correlation tests were used.

One male participant was removed from the statistical analyses on anxiety, and one female participant was removed when analyzing the memory data, due to problems in the application of the respective tests. In addition, one multivariate outlier (male participant) was removed on the basis of the $p < 0.001$ criteria for Mahalanobis distance in the cortisol samples.

We checked for order effects (whether the stress or control condition was first) using an ANOVA for repeated measures, which did not reveal any effect of order (all $p > 0.2$). We used Greenhouse-Geisser when the requirement of sphericity in the ANOVA for repeated measures was violated. Post hoc planned comparisons were performed using the Bonferroni adjustments for the *p*-values. All *p*-values reported are two-tailed, and the level of significance was marked at <0.05. When not oth-

erwise specified, results shown are means ± standard error of means (SEM). We used SPSS 15.0 to perform the statistical analyses.

3.3 Results

Psychological response

Situational Appraisal The stress task was perceived as more stressful (F (1,29) = 55.242, $p < 0.001$), difficult (F (1,29) = 106.436, $p < 0.001$) and frustrating (F (1,29) = 43.948, $p < 0.001$), and as requiring more effort (F (1,29) = 113.361, $p < 0.001$) than the control task. There were no differences in motivation for the stress and control tasks, F (1,29) = 0.574, $p = 0.455$. No interaction was found between Sex and Condition in any of the variables evaluated (for all $p > 0.3$), although men perceived both tasks as more stressful than women, F (1,29) = 7.600, $p = 0.010$.

Mood and Anxiety There were no baseline differences between the stress and control conditions for mood and for anxiety (all $p > 0.6$). Positive affect was not different after the two tasks, F (1,29) = 1.234, $p = 0.276$, but participants did report a stronger negative mood after the stress task than after the control task (PANAS NA score after the stress task: 14.35 ±0.94, and after the control task: 11.37 ±0.44), F (1,29) = 12.416, $p = 0.001$. Furthermore, anxiety scores after the stress task were higher than after the control task (STAI-S score after the stress task: 13.49 ±1.90, and after the control task: 8.87 ±0.91), F (1,28) = 9.903, $p = 0.004$. No sex differences were found for mood and anxiety (for all $p > 0.1$).

Salivary Cortisol Response

The repeated measures ANOVA with cortisol concentration as the dependent variable showed main effects for Condition (stress vs. control): F (1,29) = 22.389, p < 0.001, Time (0, +20 and +45 min): F (1.39,40.21) = 13.879, p < 0.001, and their interaction: Condition*Time: F (1.29,37.54) = 21.874, p < 0.001. Post hoc analyses showed that baseline concentrations of cortisol were similar for both the stress and control conditions (p = 0.481). In the stress condition, cortisol increased after exposure to the TSST (p < 0.001), and it remained higher than baseline up until 45 minutes after the onset of the stress task (p < 0.001). For the control condition, cortisol concentrations decreased during the consecutive measures according to the cortisol circadian rhythm.

The factor Sex did not reach statistical significance (F (1,29) = 3.101, p = 0.089), nor did the three factor interaction (Condition*Time*Sex: F (1.29,37.54) = 2.767, p = 0.095). Based on the sex differences observed in the literature and on our own hypothesis, we did post hoc planned comparisons that revealed different patterns of cortisol release for both men and women in the stress and control conditions (see Fig. 2). For men, cortisol concentrations were higher 20 minutes after the onset of the stress task compared to the baseline (p = 0.001). Following this increase, their cortisol started to decrease (p = 0.047), although without reaching baseline in the last saliva sample (p = 0.002). In the control condition, the cortisol decreased from baseline to the last saliva sample (p = 0.001). On the other hand, women had a different cortisol release pattern than men. In the stress condition, their cortisol concentrations on average rose from baseline to 20 minutes after the onset of the stress task, but this increase did not reach statistical significance (p = 0.138). However, forty-five minutes after the stress task, their cortisol concentrations were higher than baseline (p = 0.014). In the control condition, the cortisol concentrations of the women did not change for any of the three saliva samples (for all p > 0.99). In addition, men and women differed in their baseline cortisol concentrations. Men had

higher baseline than women in the stress condition (p = 0.045) and the control condition (p = 0.007).

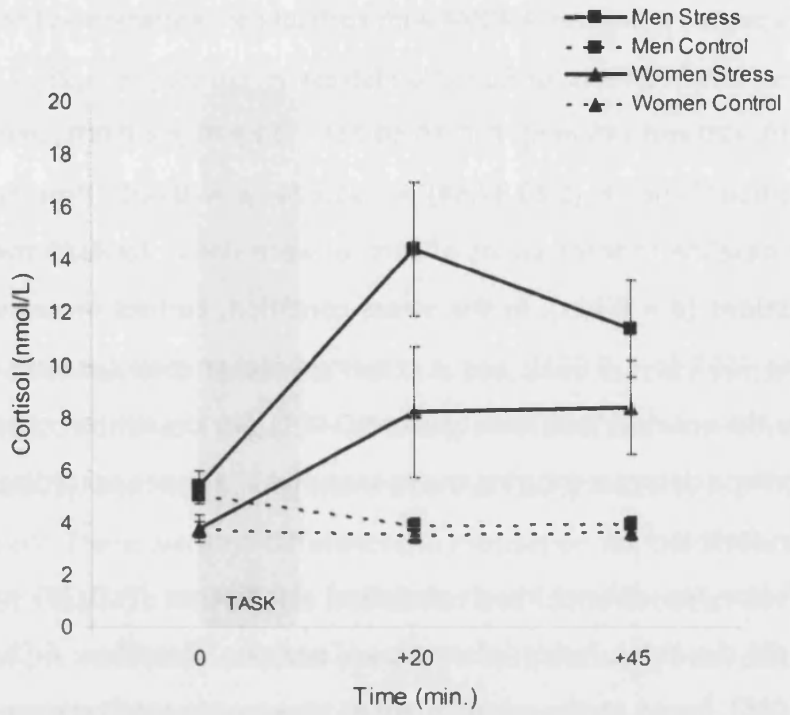

Figure 2. Salivary cortisol concentrations in the stress (TSST) and control conditions.

Memory Performance

Stress vs. Control condition The repeated measures ANOVA with memory as the dependent variable revealed the main effect of Trials, F (3.17,88.66) = 73.461, p < 0.001, and although marginally significant, an interaction between the three factors, Condition, Trials, and Sex, F (6,168) = 2.070, p = 0.059. Post hoc analyses showed that regardless of the condition, there was a positive learning curve across the first five trials. In almost every consecutive trial more words were remembered (p < 0.001), except between trials 3 and 4, (p = 0.086). The participants could recall fewer words in the trial performed after the interference list (trial 6) than before the interference list (trial 5), p < 0.001. The delay period did not affect the recollection of

words, since the participants could recall a similar number of words after the 30 min delay (trial 7) as before the delay period (trial 6), $p = 0.596$.

The interaction between the three factors was further investigated (see Fig. 3). We found that women recalled more words in Trial 1 of the stress condition than in the same trial of the control condition ($p = 0.008$), but they recalled fewer words in Trial 6 of the stress condition compared to the same trial of the control condition ($p = 0.029$). However, for men there were no differences between the stress and control condition trials ($p > 0.2$).

Finally, the repeated measures ANOVA with recognition (trial 8) as the dependent variable did not show main effects for Condition and Sex, nor was there an interaction between these two factors (all $p > 0.3$).

Cortisol Reactivity to Stress and Memory Performance The correlations between Cortisol Reactivity to the stress task and memory performance are shown in Table 1. Among men, no significant correlations were found for memory performance in the stress or control condition, for all $p > 0.2$. However, among women Cortisol Reactivity to the stress task was negatively correlated with memory performance in the stress condition, and, interestingly, also in the control condition. In other words, the women who reacted to the stress task with high increases in cortisol concentrations had a worse memory performance in both conditions.

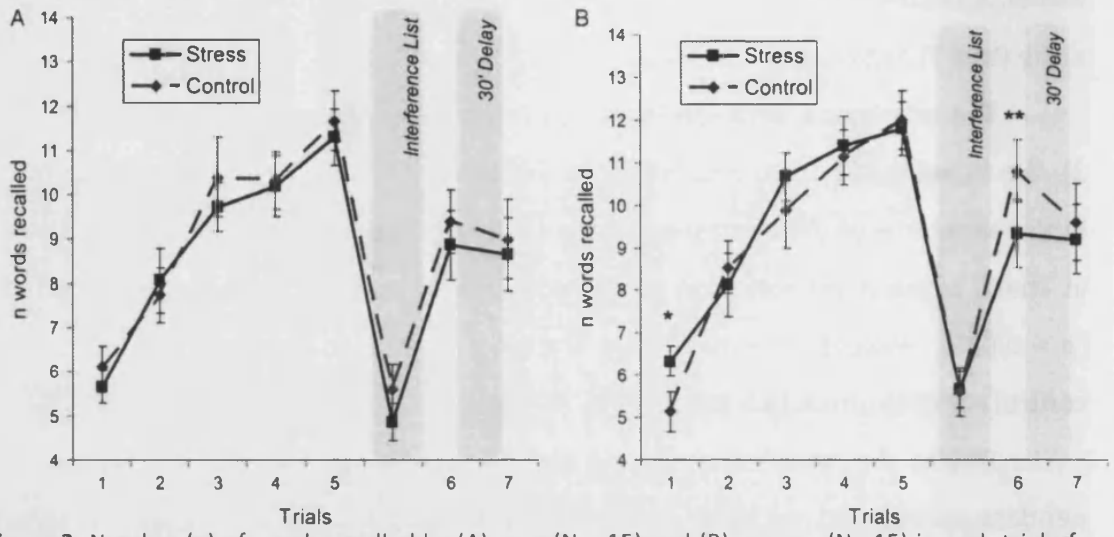

Figure 3. Number (n) of words recalled by (A) men (N = 15) and (B) women (N =15) in each trial of the RAVLT for the stress and control conditions.

Table 1. Spearman correlations between cortisol reactivity to the stress task and the number of words recalled in RAVLT trials in the stress and control conditions for men (N = 15) and women (N = 15).

	Cortisol Reactivity to the stress task							
	Men				Women			
	Stress		*Control*		*Stress*		*Control*	
RAVLT Trials	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Trial 1	- 0.02	*ns*	0.10	*ns*	- 0.44	0.10	- 0.50	0.05
Total Learning (∑T1 to T5)	- 0.13	*ns*	0.05	*ns*	- 0.52	0.05	- 0.54	0.04
Trial 6	0.20	*ns*	0.34	*ns*	- 0.60	0.02	- 0.25	*ns*
Trial 7	0.06	*ns*	0.08	*ns*	- 0.59	0.02	- 0.22	*ns*
Total Trials (∑T1 to T7)	- 0.01	*ns*	- 0.02	*ns*	- 0.53	0.04	- 0.43	*ns*
Recognition d-prime	0.07	*ns*	0.13	*ns*	- 0.33	*ns*	0.05	*ns*

Notes: Cortisol reactivity was the difference between the AUCi in the Stress condition, and the AUCi in the Control condition.

In addition, we found positive correlations between age at menarche (*M* = 12.25, *SEM* = 0.57; *Range:* 9 to 17 years old) and memory performance in the stress condition (Trial 1, Trial 6, Trial 7, Total Trials and Recognition d-Prime: *r* between 0.535 and 0.602, *p* between 0.01 and 0.04) and in the control condition (Trial 6, Trial 7, Total Trials: *r* between 0.532 and 0.556, *p* between 0.03 and 0.04) (see Fig. 4). The correlation between age at menarche and cortisol reactivity was negative, although non-significant (*r* = - 0.317, *p* = 0.2). However after excluding one woman who had an

unusually late menarche (17 years old), the correlation was marginally significant (r = - 0.484, p = 0.06).

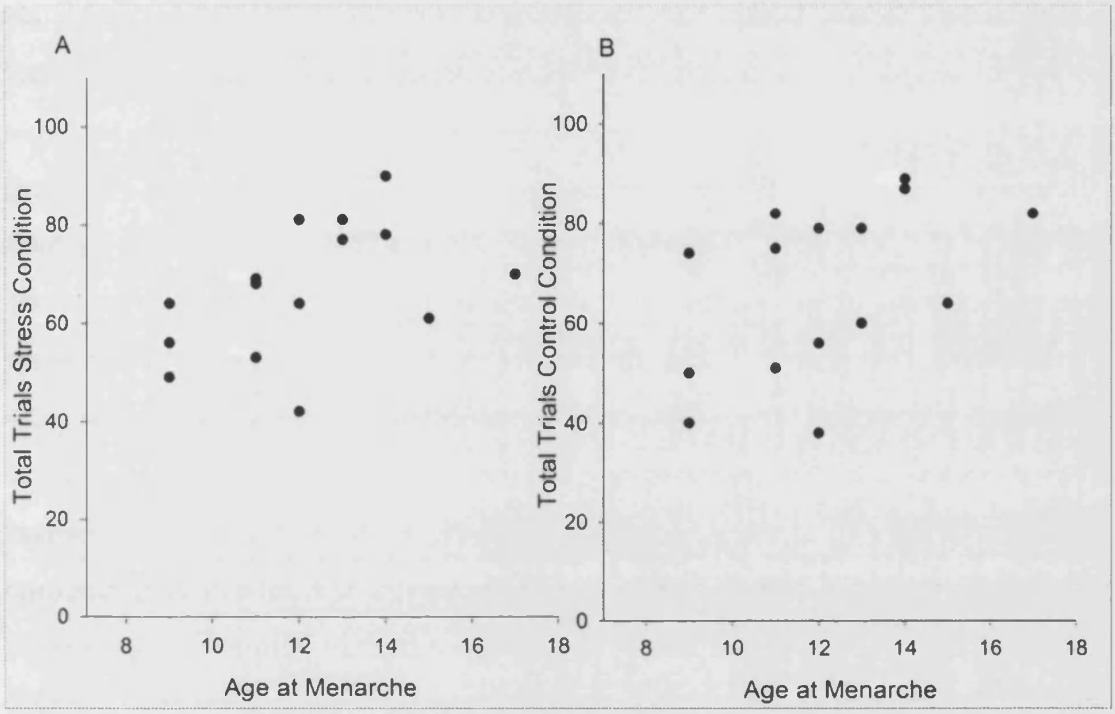

Figure 4. Scatter plot of timing of sexual maturation (age at menarche) and total trials (∑T1 to T7) of the RAVLT (N = 15).

3.4 Discussion

This study compared the performance on a declarative memory test when learning occurred after a stress task (TSST) or after a control task in a group of healthy middle-aged men and women. The main findings were threefold. First, the stress had an acute impact on memory processes only among women. Second, and independently of the acute effects of stress on memory, we found a negative relationship between cortisol reactivity to stress and declarative memory performance again only in women. Finally, the timing of the women's sexual maturation (age at

menarche) was related to their memory performance, and, as a trend, to their cortisol reactivity to stress.

The psychosocial stress test (TSST) was perceived as stressful, and it provoked psychological changes, because the anxiety and negative mood of both men and women increased. Moreover, the TSST triggered an increase in cortisol release that was marginally different for men and women. Based on previous studies that reported sex differences in the cortisol response to TSST and other kinds of stressors (Seeman et al., 1995, 2001; Traustadóttir et al., 2003; Kudielka et al., 2004), we further explored this difference. In our study, the cortisol concentrations of men increased sharply in response to the stress task, but they also started to decrease at the end of the session. The women responded differently, since their cortisol increased more slowly after the stress task, but they maintained the increased concentrations until the end of the schedule. Additionally, in the control condition, while cortisol concentrations decreased in men, women maintained similar concentrations from the beginning to the last saliva sample. These cortisol differences agree with the notion that elderly women display a more prolonged HPA-axis response to challenge than men because they are more predisposed to the loss of HPA-axis resiliency with age and, therefore, show a decrease in HPA-axis feedback sensitivity (Seeman and Robbins, 1994). However, in our study neither men nor women had recovered the basal concentrations 45 minutes after the onset of the TSST. Hence, to confirm this hypothesis, it will be necessary in future research to examine a longer recovery time.

Concerning our main goal, we found that the stress-induced response had an acute impact on memory performance, but only in women. Most interestingly, among the women the stress induction had two different effects. First, in the stress condition women could recall more words in the first trial of the RAVLT than in the control condition. Second, the recall of words was actually impaired when it was tested on trial 6. The first trial is a measure of immediate word span under overload conditions, because the number of words presented (15) greatly exceeds the num-

ber a person can retain at once. The score achieved on this first trial has an important attention component (Lezak et al., 2004). By contrast, trial 6 is the first trial that measures recall without the target list being presented immediately before the onset of the trial, and it takes place after the presentation of an interference list. Thus, both effects appear to be within the domain of working memory, even though the cognitive demands of these two trials are very different. According to the original proposal of the Yerkes and Dodson law, a high level of arousal can enhance learning on an easy task but impair learning on a difficult task (Yerkes and Dodson, 1908; see also Diamond et al., 2007). Trial 1 requires only the storage of words for a short period of time. Trial 6, however, requires storage and executive processes, because the interference list has to be inhibited, while the target list is recalled. The effect observed in trial 1 was not correlated with the cortisol increase provoked by the stress task. We consider that this effect could be explained by an enhancing effect of stress on attention that improved the number of words retained. However, the effect observed in trial 6 was related to the women's cortisol response to the stress induction. This response coupled with a more complex task could have impaired the executive processes of working memory by worsening the inhibition of the interference list and the retrieval of the target list. When new learned material interferes with the recall of material previously learned, retroactive interference occurs, which has been linked to prefrontal cortex functioning (Dewar et al., 2007). Elderly people seem to be especially vulnerable to this type of interference, since they show a sustained activation of irrelevant stimuli that enter their working memory (Hedden and Park, 2001). We found that under high concentrations of cortisol, this failure to inhibit could be heightened in middle-aged women, but not in men. Previous studies also failed to find any acute effect of stress or glucocorticoid administration on working memory among middle-aged men. For example, Wolf et al. (2001) found that cortisol administration decreased the performance on a working memory task (Digit Span) in young men, but not in elderly men.

Furthermore, when we explored the individual differences in cortisol reactivity to stress and its influence on memory, we found that regardless of the condition, only among women was a high cortisol response to the stress associated with a poorer memory performance. The effect in the control condition cannot be explained by the concentrations of cortisol at the moment of the memory testing, because cortisol was not elevated. These findings coincide with those of Lupien et al. (1997), who found in a sex-mixed group (7 men and 7 women) that high responders had worse memory performance than non-responders both before and after the exposure to stress. These findings contrast, however, with Domes et al. (2002), who found better memory performance in female high cortisol responders as compared with low responders. This divergence could be explained by methodological differences, since Domes et al. also included premenopausal women in their study, and age and menstrual cycle can be important confounding factors in the relationship between cortisol reactivity and memory performance.

Apart from sex differences in HPA-axis feedback sensitivity, other biochemical mechanisms could underlie the sex effects observed in our study. For example, recent research has shown sex differences in the activity of 11 β-hydroxysteroid dehydrogenase type 1 (Vierhapper et al., 2007), an enzyme that metabolises glucocorticoids and modulates tissue exposure to glucocorticoid activity (Holmes and Seckl, 2006). Therefore, cortisol concentration may not be the only factor determining the effects of HPA-axis reactivity on memory performance. Furthermore, it has been hypothesized that estrogens could work to contain the HPA-axis and counteract some of the potentially damaging actions of glucocorticoids on nerve cells (McEwen, 2002). However, menopause is characterized by a dramatic reduction in estrogen production, and no such drastic change occurs in men. Indeed, the relationship between sex hormones and the HPA-axis could be more extensive. Timing of sexual maturation is being considered as an important predictor of adult and postmenopausal health (Peeters et al., 1995; Laitinen et al., 2001; Mucci et al., 2001) and it has been related with allostatic load in adulthood (Allsworth et al., 2005). In the current

study, early age at menarche was associated with poorer declarative memory performance and, as a trend, with higher cortisol reactivity to stress. Early age at menarche has been associated with early childhood stress (Ellis and Garber, 2000) and according to Lupien et al. (2009), the impact of early adversity when the brain is developing could explain some of the differences observed during the aging. However, other explanations are possible, such as life-time exposure to estrogens. To disentangle the ultimate mechanisms of these relationships more research is clearly warranted.

In the current study, the stressor was applied prior to learning, similarly to other studies performed mainly with young adults (Jelicic et al., 2004; Domes et al., 2004; Payne et al., 2006; Smeets et al., 2006, 2007; Schwabe et al., 2008). Hence, this design does not make it possible to distinguish between the effects of cortisol on the different phases of the memory process. It is possible that the enhancing effects of cortisol on consolidation may have been nullified by the impairing effects on retrieval. Another limitation of our study was the sample size. Trying to avoid as many confounding factors as possible, we were conservative and we selected a homogeneous healthy sample for their age. Consequently, the number of participants was considerably reduced. It would be advisable to extend this research to a more general population including various types of aging-related diseases and medication use.

Increased basal cortisol levels over time have been associated with cognitive decline (Lupien et al., 1998). The present study further extends these findings by showing that individual differences in the cortisol reactivity to stress have a strong link to memory performance in later life, and that sex is a critical moderating factor of this relationship.

Chapter 4

Study 3: Cortisol reactivity to acute stress and memory performance in non-stressful conditions in older people[3]

[3] The main results of this study are being prepared for submission to a journal:

- Almela, M., Hidalgo, V., van der Meij, L., Villada, C., Salvador, A. Is cortisol reactivity to acute stress related to memory performance in non-stressful conditions in older people?
- Almela, M., Hidalgo, V., van der Meij, L., Villada, C., Salvador, A. General self-efficacy is related to the cortisol response to the TSST in healthy older adults.

4.1 Introduction

There is great heterogeneity in the age-related cognitive decline among healthy people (Rabbitt, 1993; Christensen et al., 1999), which means that some people maintain their memory relatively well as they age, while others experience a dramatic memory deterioration. It is crucial to unravel the causes for these individual differences in order to develop interventions that can improve quality of life among the elderly. One of the main body systems that has been associated with this heterogeneity is the activity and regulation of the hypothalamus-pituitary-adrenal axis (HPA-axis). The aim of this study was to further untangle the relationship between HPA-axis integrity and memory capacity in older people. To do so, we took a novel approach, which consisted of investigating whether the magnitude of cortisol reactivity to acute stress is related to memory performance when memory is measured under non-stressful conditions.

The HPA-axis participates in the control of the stress response, and the end-product of its activation is the release of glucocorticoids, of which cortisol is the most notable in humans (Ulrich-Lai and Herman, 2009). When facing a stress challenge, the release of cortisol induces a series of actions that are essential for our survival, such as the deviation of energy to our muscles, the enhancement of our cardiovascular tone, and the suppression of functions that are not essential at that moment, like digestion, growth or reproduction (Sapolsky et al., 2000b). However, an increase in cortisol secretion prolonged in time, such as that caused by chronic stress or specific diseases like Cushing's disease, is neurotoxic, since it has been shown to cause atrophy of the neuronal processes and inhibition of neurogenesis (Sapolsky, 1999). The hippocampus and the prefrontal cortex are especially vulnerable to these neurotoxic effects of stress because they have a high density of receptors for cortisol due to their involvement in HPA-axis negative feedback (Diorio et al., 1993; Lupien and Lepage, 2001; Crane et al., 2003; Herman et al., 2005; McEwen, 2008). These same brain areas are also pivotal for some cognitive processes such as declarative and working

memory (Scoville and Milner, 1957; Galloway et al., 2008). Therefore, it has been proposed that stress can be related to age-related memory impairment through the detrimental effects of increased cortisol levels on the hippocampus and the prefrontal cortex. Indeed, animal research has shown memory impairments and reduced hippocampal volume in aged rats with increased basal glucocorticoid secretion (Issa et al., 1990). Conversely, maintaining low glucocorticoid levels in middle-aged rats prevents memory deficits and hippocampal atrophy later on (Landfield et al., 1981).

This evidence led to the formulation of the glucocorticoid cascade hypothesis (Sapolsky et al., 1986), which postulates that accumulated exposure to glucocorticoids reduces the ability of neurons to resist insults, increasing the rate at which they are damaged by other toxic changes or ordinary attrition. Therefore, memory decline would be the end product of years of exposure to elevated cortisol levels due to, for example, chronic stress or diseases like depression. More recently, the vulnerability hypothesis (Charney and Manji, 2004) has indicated that some predisposition is necessary for suffering the neurotoxic effects of enhanced cortisol levels on brain tissue. This predisposition could be represented by genetics, but it has also been pointed out that there are crucial stages in the life span, such as infancy and aging, when the impact of stress could be more detrimental (Lupien et al., 2009).

In fact, research in humans has shown that aging produces changes in the activity of the HPA-axis that have been related to memory impairments. However, there is great heterogeneity in these changes, and we know more about their correlates in memory performance in some facets of HPA-axis activity than in others. Thus, aging has a heterogeneous impact on basal cortisol levels, with some individuals showing increases in basal cortisol levels over time, and others showing decreases (Lupien et al., 1996). Interestingly, in the series of studies of Lupien et al. (1994; 1996; 1998), people who showed increasing basal cortisol levels over time and high cortisol levels at the end of the study also showed poorer memory performance and 14 % less hippocampal volume (Lupien et al., 1994; 1998). Similarly, cross-sectional studies

have shown poorer memory performance in older individuals with enhanced basal cortisol levels (MacLullich et al., 2005; Comijs et al., 2010).

Less is known about the relationship between other markers of HPA-axis activity, such as the cortisol awakening response (CAR) or the acute cortisol response to stress, and memory performance in older people. Comparable to the impact of age on basal cortisol levels, it looks like age does not have a big impact on the CAR (Pruessner et al., 1997; Wust et al., 2000; Edwards et al., 2001), although a subgroup of elderly people (prevalence of 27%) showed an enhanced CAR (Kumari et al., 2010). Again, having an enhanced CAR has been related to worse declarative memory performance among older people (Almela et al., under review) and, although contradictory, to better working memory performance (Evans et al., 2011; Almela et al., under review).

Concerning acute manipulations of HPA-axis activity, aging is associated with a greater reactivity to pharmacological challenge that has been explained as an age-related impairment in HPA-axis negative feedback sensitivity (for reviews see: Seeman and Robbins, 1994 and Kudielka et al., 2009). However, when facing psychological stressors, age differences in cortisol reactivity appear small and depend on other factors such as sex and the nature of the stimulus used to provoke stress. Thus, while several studies did not find any effect of aging on the cortisol response to psychosocial stressors (Nicolson et al., 1997; Rohleder et al., 2002), others found a higher cortisol response with increasing age (Seeman et al., 2001; Kudielka et al., 2004b; Traustadóttir et al., 2005; Strahler et al., 2010b; Almela et al., 2011b).

However, whether the magnitude of the stress-induced HPA-axis reactivity is related to 'basal' memory capabilities is a question that remains unanswered, because research has focused mainly on the acute effects of stress on memory (for reviews see: Heffelfinger and Newcomer, 2001; Wolf, 2009). In our opinion, this is an important question to answer, because the magnitude of the cortisol response to acute stress could be a measure of the HPA-axis status, which could be related to the

memory decline observed in older people. To our knowledge, to date no study has been specifically designed to study whether a large cortisol response to psychosocial stress is also related to worse memory performance under non-stressful conditions. However, some preliminary evidence comes from two studies originally designed to investigate the acute impact of stress on memory. They found that high-cortisol responders to stress had poorer memory performance than non-responders both before and after the exposure to stress (Lupien et al., 1997), and that only in women, greater cortisol reactivity to psychosocial stress was related to poorer memory performance on a control day (Almela et al., 2011a). This preliminary evidence suggests that responding to stress with a large cortisol increase is an indicator of faulty HPA-axis regulation, and that this relationship could be stronger in women than in men.

The goal of our study was to fill this gap in the literature and investigate among older people whether the magnitude of the cortisol response to stress is related to basal declarative and working memory performance as measured in a non-stressful condition. To do so, we selected a homogeneous healthy sample from 55 to 77 years of age. We included both men and women because we were interested in investigating the sex effects, since it has been shown that sex can be an important moderator of both the cortisol response to acute stress (Kudielka et al., 2004b) and the relationship between HPA-axis activity and memory performance (Almela et al., 2011a). The study included two sessions. In the first session, a neuropsychological assessment of the participants was performed using two standardized tests for measuring declarative memory and another two for measuring working memory. In the second session, the participants were exposed to the Trier Social Stress Test (TSST, Kirschbaum et al., 1993), which has consistently been shown to provoke endocrine, cardiovascular, immune and subjective stress responses (Kudielka et al., 2007), as well as producing a stronger cortisol response compared to other laboratory procedures (Dickerson and Kemeny, 2004). We expected that a higher cortisol response to stress would be related to poorer declarative and working memory performance. Finally, we included three questionnaires to investigate whether the magnitude of the

cortisol response to stress is related to the participants' self-esteem, locus of control, and general self-efficacy.

4.2 Methods

Participants

The sample was composed of 66 participants (31 men and 35 women) aged from 55 to 77 years old (men: M = 63.29, SD = 5.21; women: M = 63.54, SD = 3.66). Most of them (85%) had an educational level beyond high school and were retired (91%). Men reported slightly higher subjective socioeconomic status (Adler et al., 2000) than women (SES scale: 1 = lowest to 10 = highest: Men: M = 6.52, SD = 1.12; Women: M = 6, SD = 0.84, p = 0.037), and men had a higher body mass index than women (BMI men: M = 27.71, SD = 3.92; BMI women: M = 25.65, SD = 3.47, p = 0.026). There were no sex differences in age or educational level (for both $p > 0.3$). All female participants were postmenopausal and had had their last menstrual period more than one year before. None of these women were receiving estrogen replacement therapy.

Participants belonged to a study program at the University of Valencia for people older than 50 years of age. One hundred sixty-six persons volunteered to participate. These volunteers were interviewed and completed an extensive questionnaire to check whether they met the study prerequisites. In order to avoid a large number of potentially confounding factors that could interfere with HPA-axis activity or cognitive functioning, we selected a homogeneous healthy sample using very restrictive criteria. The criteria for exclusion were: smoking more than 5 cigarettes a day, alcohol or other drug abuse, visual or hearing problems, presence of a cardiovascular, endocrine, neurological or psychiatric disease, having been under general anesthesia once or more than once in the past year, and the presence of a stressful life event during

the last year. Volunteers were excluded from participation when they met the criteria for dementia as defined by the DSM-IV and the NINCDS-ADRDA criteria for Alzheimer's disease, and when they were using any medication directly related to emotional or cognitive function, or were medication that could influence hormonal levels, such as glucocorticoids, antidiabetic medication, antidepressants, benzodiazepines, and psychotropic substances. Vitamins, sporadic use of painkillers, and natural therapies were allowed.

Procedure

Participants meeting the criteria were contacted by telephone and asked to attend two different sessions that took place in two different places at the School of Psychology. Both sessions were carried out individually, and the time elapsed between the first and second sessions had a mean of 7 (± 0.42) days. The first session consisted of a neuropsychological assessment, and the second was a laboratory procedure employed to provoke an acute cortisol stress response (TSST). We decided that the neuropsychological assessment would always precede the stress session because we wanted to avoid any stress-related activation of the HPA-axis during the memory assessment due, for example, to the anticipation and recall of the TSST. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and procedure were approved by the Ethics Research Committee of the University of Valencia. All the participants received verbal and written information about the study and signed an informed consent form.

Neuropsychological Assessments

The neuropsychological assessments were all conducted by the same experimenter and lasted 1 hour and 30 minutes. Half of the sample began the session at 10 h (16 men and 18 women) and the other half at 12 h (15 men and 17 women). The

neuropsychological assessment was composed of tests that have been shown to be valid and reliable (Sahakian and Owen, 1992; Lezak et al., 2004). The neuropsychological assessment consisted of two tests of declarative memory (Logical Memory and Auditory Verbal Learning Test) and two tests of working memory (Spatial Span and Spatial Working Memory).

Logical Memory The Spanish version of this subtest from the Wechsler Memory Scale III was administered (Pereña et al., 2004). The administration of this test was performed according to the manual. The experimenter read aloud two brief narratives, and afterward participants had to recall as many memory units or "ideas" as possible. After a delay of 25 minutes, the participants were asked again to recall as many "ideas" from the two narratives as possible. From this test, two outcomes were used in the subsequent analyses: (i) Immediate Recall: total "ideas" recalled from the two narratives immediately after having heard them, and (ii) Delayed Recall: total "ideas" recalled from the two narratives after the 25-minute delay.

Auditory Verbal Learning Test The Spanish version of the WHO-UCLA Auditory Verbal Learning Test was used (AVLT, Maj et al., 1994). This test consists of a five-trial presentation of a 15-word list (learning trials), followed by a single presentation of an interference list. After that, there were two recall trials; the first one was done right after the recall of the interference list (immediate recall after interference), and the second one after a delay of 30 minutes (delayed recall). Three outcomes were used in subsequent analyses: (i) Learning Slope: total number of words recalled on the first five trials, (ii) Immediate Recall: total number of words recalled after the interference trial and (iii) Delayed Recall: total number of words recalled after the 30-minute delay.

Spatial Span This test from the Cambridge Neuropsychological Test Automated Battery was applied (CANTAB, Cambridge Cognition, Cambridge, United Kingdom, www.cantab.com, 2006). This test measures short term memory capacity and is based on the Corsi Block Tapping Task (Milner, 1971). Participants were presented

with a set of nine white squares on a tactile computer screen. In each trial, a number of squares changed their colour, starting with 2 squares in the first trial which increased to all 9 squares in the last trial. At the end of each trial, a tone indicated that the participant had to touch the squares that had changed their colour, either in the same order (forward) or in the reverse order (backward) as they were presented. Two outcome measures were obtained: (i) Span Length Forward: the last number of squares that the participants were able to touch correctly in the same order as they were presented and (ii) Spatial Length Backward: the last number of squares that the participants were able to touch correctly in the reverse order of their presentation.

Spatial Working Memory We also measured working memory with the Spatial Working Memory test of the CANTAB (Owen et al., 1995). In this test, several boxes were presented on a tactile computer screen. Participants were instructed to find a token that was hidden beneath one of these boxes. By touching a box they could reveal if there was a token under it, after which the box returned to its original state. Once a token was located, the participants had to locate another token in the same set of boxes. However, they were told that in every trial the token would never be under a box where a token had been found in a previous trial. As an outcome measure, we used the total errors committed, which includes returning to a box in which a token had already been and returning to a box that had already been found to be empty.

Cortisol Stress Response: Trier Social Stress Test

To provoke stress, we subjected the participants to the Trier Social Stress Test (TSST). The session took 1 hour and 50 minutes to complete, and it was always carried out in the afternoon in three different shifts starting at 16 h, 17.15 h or 18.30 h. The distribution of men and women was similar in all three shifts ($p > 0.3$). Upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked to see whether they had followed the instructions given to

them previously: the day before the session they had to maintain their general habits, sleep as long as usual, and refrain from heavy physical activity; since the night before the session they could not consume alcohol, and two hours prior to the session they could not drink (except water), eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate.

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated. Five minutes before this phase ended, the first saliva sample was provided (-20 min). After the habituation phase, the introduction phase started (duration 5 min). In this phase the participants were informed about the procedure for the stress task. They received the instructions in front of the committee in the same room where the task took place. Next, the participants had 10 minutes to prepare for the task at hand. Just before the beginning of the stress task, the second saliva sample was provided (0 min). The stress task consisted of 5 min of free speech (job interview) and a 5 min arithmetic task, and it was performed in front of a committee composed of a man and a woman. The participants remained standing at a distance of 1.5 meters from the committee. Additionally, a video camera and a microphone were clearly visible. Both the speech and arithmetic tasks were filmed. Subjects had 60 minutes to recover after the stress task, during which they answered some questionnaires and rested. Four saliva samples were provided during the recovery time: +25 min, +40 min, +55 min and +70 min. After the last saliva sample, the protocol ended and the participants were debriefed.

Heart Rate

Heart rate (HR) was measured using an HR monitor during all the entire stress session (Polar, model S810i, Electro Ltd., Kempele, Finland). The monitor consists of a chest belt for detection and transmission of the heartbeats and a "watch" for data

collection and storage. The heartbeat detection is performed with an accuracy of 1 ms, and these types of monitors have shown good validity (Radespiel-Troger et al., 2003). Every heartbeat is transmitted and stored in the flash memory of the watch. HR was monitored continuously during the entire session, but the recorded periods when the participants were changing their positions (sitting/standing up) and walking were removed. After artefact elimination, the HR mean for each phase was computed.

Saliva Sampling & Biochemical Analyses

Participants provided saliva samples by depositing 3 ml of saliva in plastic vials. They took approximately 5 minutes to fill the vial. The samples were frozen at -20º C until the analyses were performed. The samples were analyzed at our lab in the Central Research Unit of the Faculty of Medicine, University of Valencia. The samples were analyzed by competitive solid phase radioimmunoassay (tube coated) using the commercial kit Spectria Cortisol RIA kit (cat. nº 06119) from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/l. For each subject, all the samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8 %.

Questionnaires

Self-Esteem To measure global self-esteem participants filled in the Rosenberg Self-Esteem Scale (Rosenberg, 1965). This scale is the most widely used and validated measure of self-esteem. The scale consisted of 10-items that measure general feelings of self-worth and self-acceptance. Item examples are: "I feel that I'm a person of

worth, at least on an equal plane with others" and "All in all, I am inclined to feel that I am a failure".

Locus of control To measure locus of control participants filled in the Locus of Control of Reinforcement questionnaire (Rotter, 1966). This questionnaire measures generalized expectancies of internal versus external control of reinforcement. People with an internal locus of control believe that their own actions determine their own success, while those with an external locus of control believe that rewards in life are generally outside of their control. The questionnaire had 29 items, for every item participants were presented with two sentences of which one sentence expressed an internal belief and the other an external belief. Participants had to indicate which sentence was closest to their own personal belief.

General Self-Efficacy To measure self-efficacy participants filled in the General Self-Efficacy Scale (Schwarzer and Jerusalem, 1995). This scale measures the belief to cope with difficult situations in life. Perceived self-efficacy facilitates goal-setting, effort investment, persistence when faced problems and recovery from setbacks. The scale had 10 items that were answered on a 4 point Likert scale. Each item refered to successful coping and implied an internal-stable attribution of success. Examples of items are: "I can always manage to solve difficult problems if I try hard enough" and "It is easy for me to stick to my aims and accomplish my goals".

Statistics

Student's *t*-test and Chi-square analyses were used to investigate sex differences in the demographic variables and psychological traits. Cortisol and HR values were square root transformed because they did not have a normal distribution. Two participants were excluded from the analyses involving HR (two women) and cortisol

(a man and a woman) because their HR and cortisol concentrations differed more than 3 S.D. from those of the rest of the sample.

To analyze the cortisol and HR responses to the stress induction, we performed ANOVAs for repeated measures. As men and women differed slightly in their subjective socio-economic status (SES) and BMI, we explored whether these variables were related to their cortisol and HR responses to the TSST using Spearman's Rho correlation. We used the Greenhouse-Geisser procedure when the requirement of sphericity in the ANOVA for repeated measures was violated. Post-hoc planned comparisons were performed using the Bonferroni adjustments for the *p*-values.

To analyze whether the memory performance was different depending on the cortisol response to stress, we first created the following summary indices: AUCg (total cortisol secretion during the stress session) and AUCi (change in total cortisol levels from baseline), see Pruessner et al. (2003) for the specific formulas. With these summary indices, we performed regression analyses, and following Aiken and West (1991), we performed a moderator regression analysis to investigate whether sex was a moderator. Scatterplots were inspected to investigate whether the relationships were linear or curvilinear.

We performed Spearman's Rho correlations to analyze whether the magnitude of the cortisol response to stress was correlated to the participants' self-esteem, locus of control and general self-efficacy. All *p*-values reported are two-tailed, and the level of significance was marked at <0.05. When not otherwise specified, values are mean ± standard error of mean (SEM). We used SPSS 19.0 to perform the statistical analyses.

4.3 Results

Heart Rate response to the TSST

A repeated-measures ANOVA was used to analyze the HR response to the TSST. Phase was entered as a within-subject factor (Habituation, Preparation, Speech, Arithmetic and Recovery), and Sex was entered as a between-subject factor. SES and BMI were not related to the HR response to stress (for all $p \geq 0.117$); therefore, these variables were not included as covariates.

The results showed a main effect of Phase, F (2.80, 165.31) = 68.401, $p < 0.001$ (see Fig. 1-A). The factor Sex was not significant, nor was there an interaction between Sex and Phase, both $p > 0.3$. The participants' HR increased from baseline to the speech phase ($p < 0.001$), and steadily decreased from the speech phase to baseline levels in the recovery phase (recovery phase vs. baseline, $p > 0.8$), see figure 1.

Acute cortisol response to the TSST

A repeated-measures ANOVA was used to analyze the acute cortisol response to the TSST. Time was entered as a within subject factor (-20, 0, +25, +40, +55, +70) and Sex as a between-subject factor. SES and BMI were not related to the cortisol response to stress (for all $p \geq 0.132$); therefore, these variables were not included as covariates. There was only a main effect of Time, F (1.78, 108.42) = 32.003, $p < 0.001$. The factor Sex was not significant, nor was there an interaction between Sex and Time (for both $p > 0.2$).

Figure 2 shows the means (±SEM) of cortisol released by men and women before and after the stress induction. Cortisol concentrations were not different in the two samples provided before the stress task ($p > 0.9$). The peak of cortisol concentra-

tion was reached 25 min after the onset of the stress task (+25 vs. -20: $p < 0.001$). Afterwards, cortisol concentrations decreased until reaching similar levels to those of the baseline sample in the last saliva sample (+70 vs. -20: $p > 0.9$).

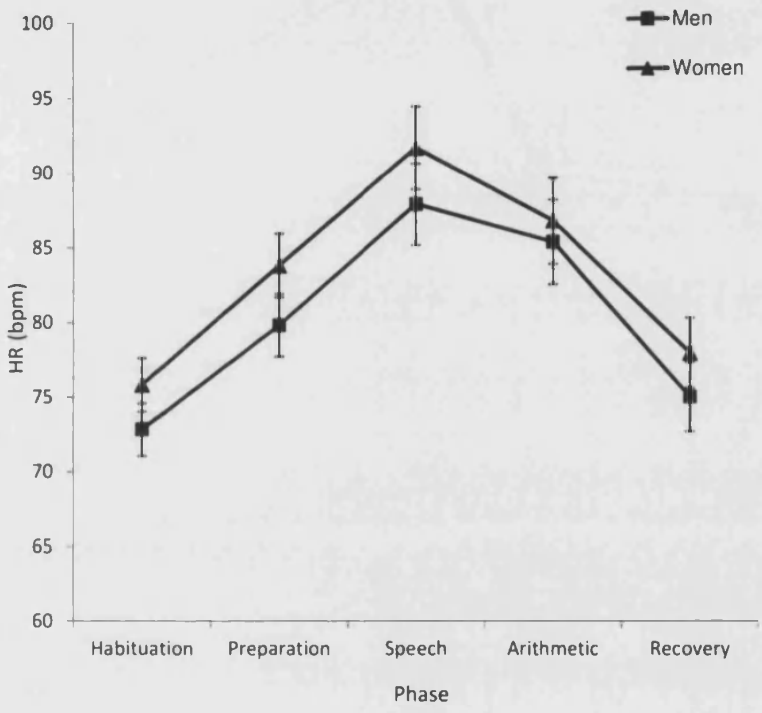

Figure 1. Heart Rate response to the TSST.

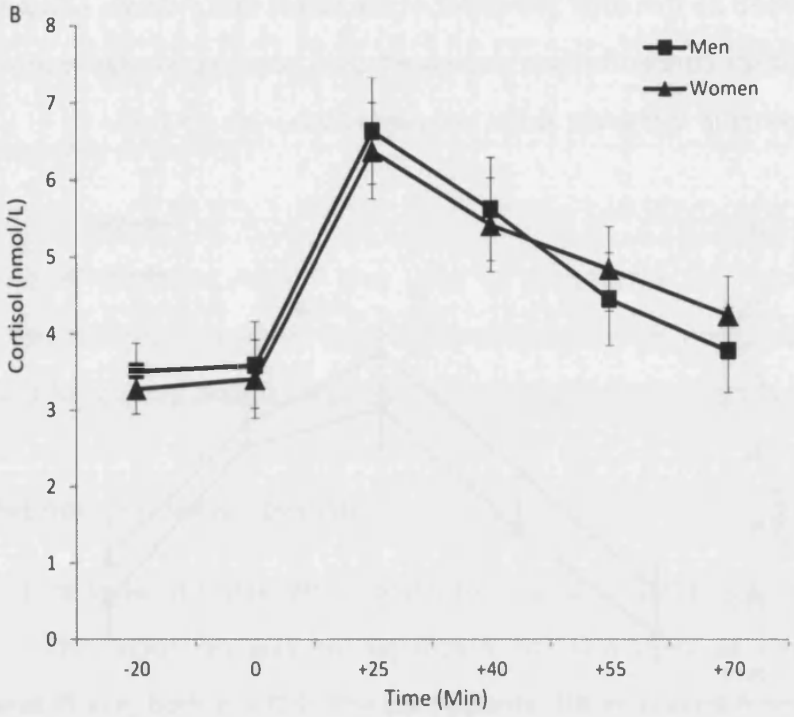

Figure 2. Cortisol response to the TSST.

Relationship between the stress-induced cortisol response and memory performance

To test whether there was a relationship between the stress-induced cortisol response and memory performance, we performed hierarchical regression analyses (Aiken and West, 1991). The inspection of the scatterplots suggested that some relationships could be curvilinear; therefore, a curvilinear term was added in the analyses. We performed separate analyses for each memory outcome being predicted by either AUCi or AUCg. In step 1, we added the following control variables: Age, BMI, SES and sex (0 = women, 1 = men). In step 2, we added AUCg or AUCi to investigate a linear relationship between memory outcome and cortisol secretion. In step 3, we added the square of AUCg or AUCi to investigate a curvilinear relationship between memory outcome and cortisol secretion. In step 4, we added the interaction term

Sex*AUCg or AUCi, and in step 5 we added the interaction term Sex*AUCg^2 or AUCi^2 to investigate whether the relationship between the cortisol response and memory was moderated by the sex of the participants. All predictors were standardized prior to entry into the regression analyses to facilitate the interpretation of first-order terms and reduce multicollinearity. When a significant curvilinear relationship is found, a positive β represents a concave upward relationship (U-shaped form) and a negative β represents a concave downward relationship (inverted U-shaped form).

Auditory Verbal Learning Test

Results from the regression analyses are shown in table 1. The regression analyses performed with cortisol AUCg showed that, only for the *learning slope* (∑T1 to T5), the interaction between sex and the AUCg linear term was significant. Post hoc analyses showed that, only among men, a larger cortisol output was linearly related to better performance on these first five trials of the AVLT ($p = 0.027$). This relationship was negative but non-significant among women. Additionally, among the total sample there was a marginal curvilinear relationship (U-shaped form) between cortisol AUCi and both *immediate recall* ($p < 0.09$) and *delayed recall* ($p < 0.08$).

Logical Memory

Results from the regression analyses are shown in table 2. Results show that there was a curvilinear relationship (U-shaped form) between cortisol AUCi and performance on both the immediate and delayed recall trials of the paragraph recall test. Therefore, those participants who increased their cortisol levels either more or less performed better on this test, while participants who only moderately increased

their cortisol levels performed worse, see figure 3. Sex did not moderate the relationship between cortisol release and performance on this test.

Table 1. Results of the hierarchical regression analyses with the AVLT outcomes as dependent variables and the cortisol indices (AUCg or AUCi) as independent variables.

AVLT		AUCg		AUCi	
Learning Slope (∑T1 to T5)	Step 2 AUC	*ns*		*ns*	
	Step 3 AUC^2	*ns*		*ns*	
	Step 4 Sex Interaction AUC	Adj R^2= 0.02, ΔR^2= 0.09 $F_{1,55}$= 5.71*		*ns*	
		β	*p*		
	Sex*AUC	-0.557	0.020		
	Men	0.539	0.024		
	Women	-0.137	*ns*		
	Step 5 Sex Interaction AUC^2	*ns*		*ns*	
Immediate Recall	Step 2 AUC	*ns*		*ns*	
	Step 3 AUC^2	*ns*		Adj R^2= 0.05, ΔR^2= 0.05 $F_{1,56}$= 3.11#	
				β	*p*
	AUC			0.008	*ns*
	AUC^2			0.236	0.083
	Step 4 Sex Interaction AUC	*ns*		*ns*	
	Step 5 Sex Interaction AUC^2	*ns*		*ns*	
Delayed Recall	Step 2 AUC	*ns*		*ns*	
	Step 3 AUC^2	*ns*		Adj R^2= 0.03, ΔR^2= 0.05 $F_{1,56}$= 3.25#	
				β	*p*
	AUC			-0.034	*ns*
	AUC^2			0.244	0.077
	Step 4 Sex Interaction AUC	*ns*		*ns*	
	Step 5 Sex Interaction AUC^2	*ns*		*ns*	

*: $p < 0.05$, #: $p < 0.10$

Table 2. Results of the hierarchical regression analyses with the Logical Memory outcomes as dependent variables and the cortisol indices (AUCg or AUCi) as independent variables.

Logical Memory		AUCg	AUCi	
Immediate Recall	Step 2 AUC	*ns*	*ns*	
	Step 3 AUC^2	*ns*	Adj R^2= 0.06, ΔR^2= 0.08 $F_{1,56}$= 5.58*	
			β	*p*
	AUC		-0.149	*ns*
	AUC^2		0.315	0.022
	Step 4 Sex Interaction AUC	*ns*	*ns*	
	Step 5 Sex Interaction AUC^2	*ns*	*ns*	
Delayed Recall	Step 2 AUC	*ns*	*ns*	
	Step 3 AUC^2	*ns*	Adj R^2= 0.05, ΔR^2= 0.07 $F_{1,56}$= 4.58*	
			β	*p*
	AUC		-0.023	*ns*
	AUC^2		0.286	0.037
	Step 4 Sex Interaction AUC	*ns*	*ns*	
	Step 5 Sex Interaction AUC^2	*ns*	*ns*	

*: $p < 0.05$, #: $p < 0.10$

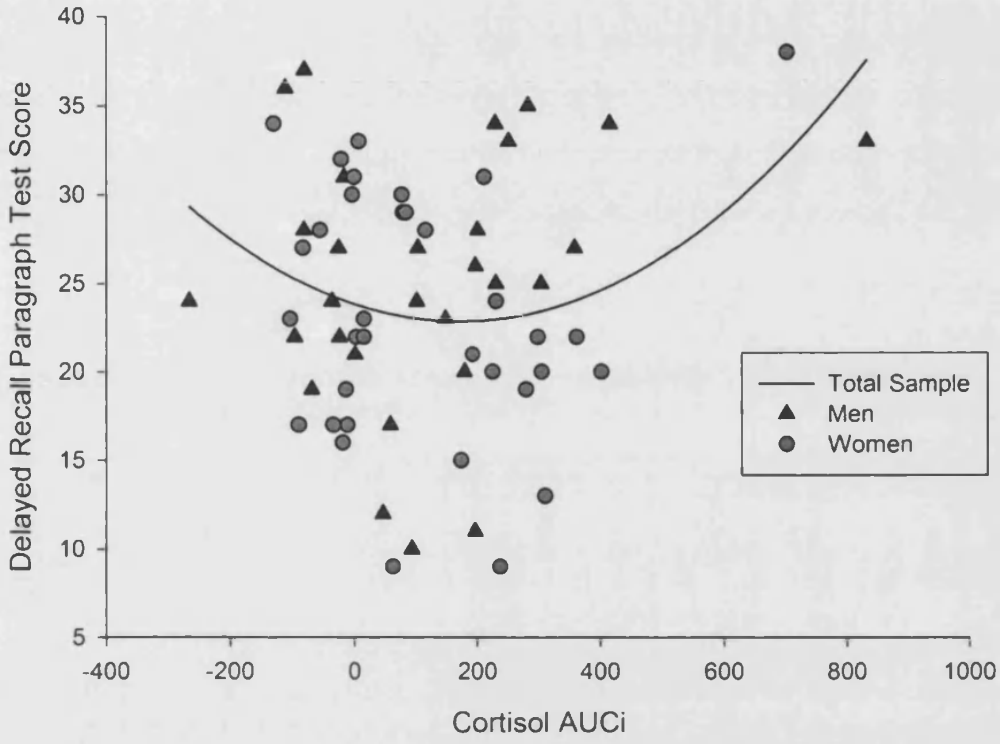

Figure 3. Relationship between cortisol AUCi and delayed recall score of the logical memory test.

Spatial Span & Spatial Working Memory

Results from the regression analyses are shown in table 3. The regression analyses performed with cortisol AUCg as predictor showed an interaction between sex and the AUCg curvilinear term, but only for the backward span length. Post hoc analyses showed that, only among men, those who either had less or greater cortisol output had a larger backward span length than those who had a moderate cortisol output.

The regression analyses performed with cortisol AUCi as predictor showed, in the total sample, a curvilinear relationship between cortisol AUCi and performance on the spatial working memory test (total errors: inverted U-shaped form). Therefore,

the better performance on this test was achieved by those participants who increased their cortisol levels either less or more, while those participants who increased their cortisol levels only moderately performance worse (figure 4). This curvilinear relationship was also found for the forward spatial length, but only in women, and for the backward spatial length, but only in men.

Table 3. Results of the hierarchical regression analyses with the working memory outcomes as dependent variables and the cortisol indices (AUCg or AUCi) as independent variables.

Working Memory		AUCg		AUCi	
Span Length Forward	Step 2 AUC	*ns*		*ns*	
	Step 3 AUC^2	*ns*		Adj R^2= 0.16, ΔR^2= 0.05 $F_{1,56}$= 3.84*	
				β	*p*
	AUC			0.074	*ns*
	AUC^2			0.247	0.055
	Step 4 Sex Interaction AUC	*ns*		*ns*	
	Step 5 Sex Interaction AUC^2	*ns*		Adj R^2= 0.20, ΔR^2= 0.05 $F_{1,54}$= 4.23*	
				β	*p*
	Sex*AUC^2			0.331	0.045
	Men			0.103	*ns*
	Women			0.716	0.009
Span Length Backward	Step 2 AUC	*ns*		*ns*	
	Step 3 AUC^2	*ns*		*ns*	
	Step 4 Sex Interaction AUC	*ns*		*ns*	
	Step 5 Sex Interaction AUC^2	Adj R^2= 0.13, ΔR^2= 0.14 $F_{1,54}$= 9.76*		Adj R^2= 0.11, ΔR^2= 0.09 $F_{1,54}$= 6.35*	
		β	*p*	*β*	*p*
	Sex*AUC^2	-1.001	0.003	-0.427	0.015
	Men	0.806	0.007	0.349	0.022
	Women	-0.208	*ns*	-0.443	0.118
Spatial Working Memory (Total Errors)	Step 2 AUC	*ns*		*ns*	
	Step 3 AUC^2	*ns*		Adj R^2= 0.16, ΔR^2= 0.07 $F_{1,56}$= 5.24*	
				β	*p*
	AUC			-0.045	*ns*
	AUC^2			-0.289	0.026
	Step 4 Sex Interaction AUC	*ns*		*ns*	
	Step 5 Sex Interaction AUC^2	*ns*		*ns*	

*: $p < 0.05$, #: $p < 0.10$

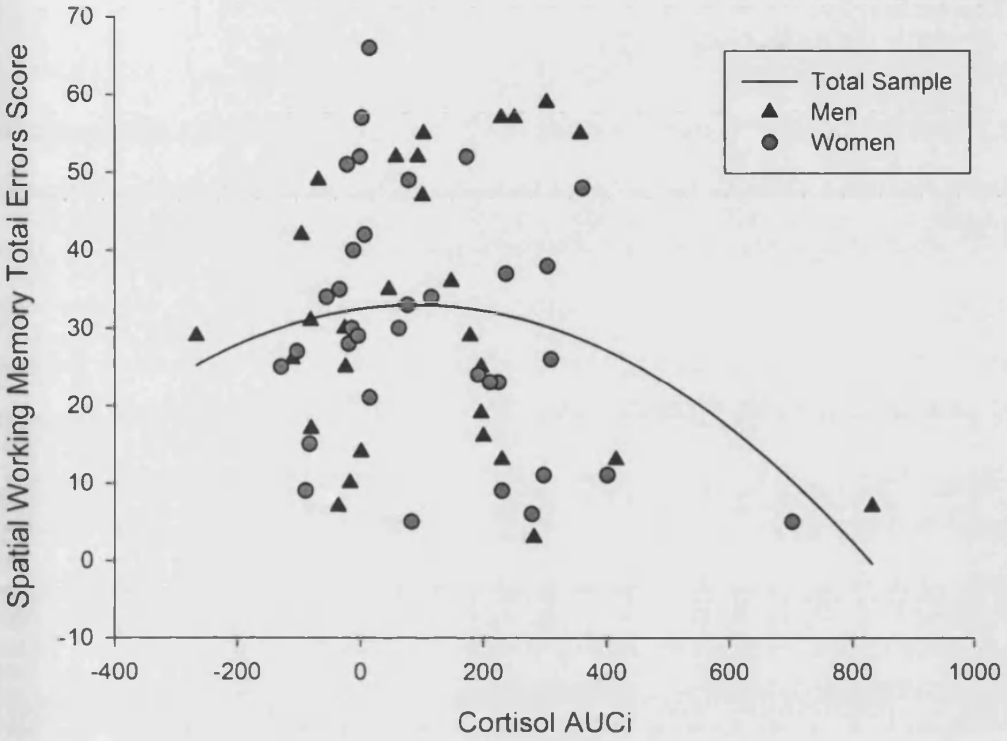

Figure 4. Relationship between cortisol AUCi and total errors score of the spatial working memory test.

Relationship between the stress-induced cortisol response to stress and the psychological variables

There were no sex differences in self-esteem, locus of control, and general self-efficacy ($p > 0.17$). The release of cortisol in response to the TSST was not related to self-esteem and locus of control (for both $p > 0.1$). However, a higher score on general self-efficacy was associated with lower cortisol levels in all the salivary samples provided after the TSST (r_s between - 0.308 and - 0.380, p between 0.013 and 0.002; AUCg: $r_s = - 0.351$, $p = 0.005$; AUCi: $r_s = - 0.258$, $p = 0.041$).

4.4 Discussion

This study investigated whether the magnitude of the HPA-axis response to stress is related to 'basal' memory capabilities among men and women between 55 and 77 years of age. We measured the participants' declarative and working memory performance in a non-stressful condition. Then, on a different day, the participants were exposed to a standardized laboratory procedure (TSST) to measure the magnitude of their cortisol response to a stressful situation. Indeed, the TSST produced increases in cortisol concentrations and HR that were not different between men and women. Furthermore, a higher self-efficacy was negatively related to the cortisol response to the TSST. However, self-esteem and locus of control were not related to the stress-induced cortisol response. When we investigated the relationship between the cortisol response to stress and memory performance, we found that a larger cortisol response to stress was not related to poorer memory performance. Instead, a moderate cortisol response to stress was related to poorer declarative and working memory performance, whereas those participants who did not increase their cortisol levels and those who had the largest cortisol increase had better declarative and working memory capabilities. Additionally, sex did not appear, overall, to moderate the relationship between the stress-induced cortisol response and declarative and working memory performance.

These results do not agree with our hypothesis, as we expected a poorer memory performance among those who reacted to the TSST with larger cortisol increases. Nevertheless, our hypothesis was based on other studies that found that an enhanced cortisol secretion, either in basal activity or in the CAR, was linked to worse memory performance among older people (e.g. MacLullich et al., 2005; Comijs et al., 2010; Almela et al., under review). Furthermore, previous studies had found that non-cortisol responders had better memory performance than cortisol responders, both before and after a stress induction procedure (Lupien et al., 1997), and that only among women, a higher cortisol response to stress was related to worse perfor-

mance on the first trials of the AVLT in a control session (Almela et al., 2011a). However, these last two studies were originally designed to investigate acute effects of stress on memory performance, their samples were relatively small, and curvilinear effects were not explored.

It is possible that our hypothesis was over-simplified. The release of cortisol when facing a situation that is evaluated as a threat has an adaptive function (Sapolsky et al., 2000b), regardless of whether the threat is to our physical or our social well-being (Dickerson and Kemeny, 2004). In fact, the release of cortisol initiates a series of physiological changes that have the purpose of improving our chances of survival in the short and long-term (Sapolsky et al., 2000b). Therefore, it seems plausible that once a stimulus has been evaluated as threatening, having a blunted cortisol response can constitute a maladaptive response because it can reduce our chances of survival. In support of this, a reduced HPA-axis response to stress has been observed in several stress-related pathologies such as atopic dermatitis (Buske-Kirschbaum et al., 2002), asthma (Buske-Kirschbaum et al., 2003) and, interestingly, also in depression, which in turn is accompanied by declarative and working memory deficits and a decrease in hippocampal and prefrontal cortex volumes (Burke et al., 2005; Savitz and Drevets, 2009a).

Hence, the results of our study suggest that a blunted cortisol response to stress is evidence of an HPA-axis malfunction accompanied by poorer memory performance among healthy older people. The literature on this matter is quite scarce, but a recent study supports this notion by showing that having a blunted cortisol response to the TSST, also among healthy older people, was associated with the presence of the G allele on the serotonin receptor gene 1A (HTR1A G) and the occurrence of more stressful life events during childhood and adolescence (Armbruster et al., 2011). Both factors, i.e. HTR1A G allele and more stressful life events during childhood, have been associated with a large number of stress-related pathologies, such as depression, PTSD or anxiety (Heim and Nemeroff, 2001; Savitz et al., 2009b), and

enhanced stressful life events during childhood have also been associated with reduced hippocampal and prefrontal cortex volumes (Stein et al., 1997; Dannlowski et al., 2011).

A possible mechanism through which a blunted cortisol response to stress can be a risk factor for memory problems can be related to cortisol's long-term effects. Thus, cortisol shapes and restrains other stress-related physiological processes that can have detrimental effects on our organism if they continue to be activated once the stressor has been overcome, such as immune responses and the release of the corticotrophin releasing hormone (CRH) (Sapolsky et al., 2000b; Raison and Miller, 2003). In fact, unrestrained inflammation and CRH release secondary to insufficient cortisol-mediated feedback inhibition influence cell survival in the central nervous system and contribute to neuronal degeneration (Allan and Rothwell, 2001; Brunson et al., 2001; Nadeau and Rivest, 2003).

Apart from those who had the largest cortisol response to the TSST, those who did not increase their cortisol levels in response to the stressful situation also had better memory performance. It is possible that the TSST was not stressful enough to trigger a cortisol response among these participants. The fact that these participants reported higher levels of general self-efficacy coincides with this notion. Therefore, a protective trait against memory deficits would be to have a higher threshold for triggering stress-related HPA-axis activation, which in turn would reduce the chances of being exposed to the damaging effects of stress. Having a lower HPA-axis reactivity to the TSST has been related to higher self-esteem and higher internal locus of control, both of which have been shown to be protective against age-related decreases in hippocampal volume and memory performance (Pruessner et al., 2004).

In our study we did not find sex differences in the cortisol response to the stress induction. In our opinion, this finding warrants further discussion because it contrasts with other studies that have shown that men reacted to the TSST with a larger cortisol increase than women (Kudielka et al., 2004b; Strahler et al., 2010b;

Almela et al., 2011b). Additionally, we consider that the overall cortisol response to the TSST was moderate because the mean peak of cortisol was 6.5 nmol/l, whereas in other studies cortisol concentrations have reached 10 nmol/l and more (Kudielka et al., 2004b; Strahler et al., 2010b; Almela et al., 2011b). Comparing the protocol of the current study with our previous one (Almela et al. 2011b), several variations can contribute to explaining these discrepancies. The age of the TSST committee was different in the two studies. The age of the audience in the current study was between 25 and 30 years old, whereas in Almela et al. (2011b) the age of the audience was similar to that of the participants. We consider that performing the speech and arithmetic task in front of a younger audience could reduce its stressfulness. Moreover, the exposure to the TSST was always carried out after the neuropsychological assessment. It is likely that having participated in a neutral session before the stress session had an effect on the expectations of the participants, as well as reducing the novelty effect. We tried to differentiate between the two sessions by performing them in different laboratories and having different experimenters lead them. In our opinion, men were more sensitive to these small variations than women, and these variations have to be taken into account in future studies where strong cortisol responses to stress are sought.

Some limitations have to be considered in interpreting the results of the current study. The sample consisted of people who were very healthy for their age, because we wanted to avoid as many confounders as possible by selecting the participants using very restrictive health- and medication-related criteria. However, this is a problem for the generalizability of the results. Therefore, future research could use other populations composed of people with specific age-related health problems. Additionally, we have to mention the correlational nature of this study, which does not allow for endorsing causal relationships.

Taken together, our results show that among healthy older individuals, both declarative and working memory capabilities are maintained better when, facing a

stressor, they do not respond with a cortisol increase or their cortisol response is large than if their cortisol response is moderate. This finding suggests that a large cortisol response to acute stress reflects a well-functioning HPA axis, and that not responding with HPA-axis activation when facing a potential stressful stimulus may protect against memory deficits in later stages of human life.

Chapter 5

Study 4: The cortisol awakening response and memory performance in older people [4]

[4] The main results of this study are under review in *Psychoneuroendocrinology*: Almela, M., van der Meij, L., Hidalgo, V., Villada, C., Salvador, A. The cortisol awakening response and memory performance in older people: the impact of sex.

4.1 Introduction

There are large individual differences in the patterns and magnitudes of cognitive decline during the aging process. This is illustrated by the fact that in older people there is a larger variability in declarative memory and working memory performance than in young adults (Rabbitt, 1993; Christensen et al., 1999). This variability is important since it may mean the difference between maintaining independence or becoming dependent on others for daily activities. Being able to predict the magnitude of the age-related cognitive decline is essential for those affected, their families, health care specialists, and society as a whole. To do so, it is necessary to understand the main mechanisms involved in these individual differences, especially when considering the advantages of the treatments and interventions that could be derived from this knowledge. The aim of the current study is to untangle the influence of one of the most important mechanisms that has been linked with age-related cognitive decline: the Hypothalamus-Pituitary-Adrenal axis (HPA-axis).

The HPA-axis is the body's main mechanism for controlling the stress response through the release of glucocorticoids, with cortisol being the most noteworthy in humans (Sapolsky et al., 2000). Aging has been shown to produce changes in the activity of the HPA-axis. Indeed, older age is associated with an average elevation of 20-50% in basal cortisol levels (for a review see: Chahal and Drake, 2007) and to a greater cortisol reactivity to pharmacological or psychological challenges (for a meta-analysis see: Otte et al., 2005). Age-related cognitive decline has been linked to HPA-axis activity, mainly because of the role that the hippocampus and the prefrontal cortex play in the regulation of the HPA-axis. These two brain structures are crucial for declarative memory (Scoville and Milner, 1957) and working memory (Galloway et al., 2008). Moreover, they are exposed to the action of glucocorticoids, since they have a high density of mineralocorticoid and glucocorticoid receptors (Lupien and Lepage, 2001), and they are involved in HPA axis negative feedback (Diorio et al., 1993; Crane et al., 2003; Herman et al., 2005).

Several other studies have examined the existence of a relationship between the HPA-axis and memory performance among older people. These studies have measured HPA-axis activity using different methodologies, such as: (i) measuring basal cortisol levels in longitudinal or cross-sectional designs; (ii) measuring the acute cortisol changes elicited by pharmacological or psychological challenges; or more recently, (iii) measuring the cortisol awakening response (CAR). Results from longitudinal studies measuring basal cortisol levels have been mixed. Several studies have shown that risk of memory impairment increases after years of cumulative increases in basal cortisol levels (Lupien et al., 1994; Seeman et al., 1997; Karlamangla et al., 2005; Li et al., 2006). However, others have not found a relationship between basal cortisol changes over time and cognitive decline (Fonda et al., 2005; Peavy et al., 2009; Comijs et al., 2010). One possible explanation for these discrepancies is that cortisol levels around the moment of memory testing could influence the relationship between long-term changes in basal cortisol levels and cognitive decline. Along these lines, cross-sectional studies have shown that acute high cortisol levels are associated with poorer cognitive performance in older people (MacLullich et al., 2005; Comijs et al., 2010). In fact, Lupien et al. (1994) found that impairment in memory performance only occurs when an increase in cortisol levels over the years is accompanied by high cortisol levels around the moment when the memory is tested.

Concerning acute manipulations of the HPA-axis activity, research has focused mainly on the acute modulation of memory performance by increases in cortisol levels elicited by pharmacological or psychological challenges (for reviews see: Heffelfinger and Newcomer, 2001; Wolf, 2009). However, fewer published studies have investigated whether the magnitude of the cortisol reactivity is related to 'basal' memory abilities. Among them, Lupien et al. (1997) found that high-cortisol responders had poorer memory performance than non-responders, both before and after exposure to stress, and Almela et al. (2011) showed that, only in women, greater cortisol reactivity to psychosocial stress was related to poorer memory performance. In sum, data from longitudinal and cross-sectional studies and from studies acutely manipulat-

ing HPA-axis activity seem to indicate that greater HPA-axis activity is related to worse memory performance among older adults.

The relationship between the magnitude of the CAR and memory performance has hardly been investigated among older adults. The CAR is a period of increased cortisol secretory activity initiated by morning awakening that typically peaks between 30 and 45 min post-awakening. It has been identified as a crucial point of reference within the cortisol circadian rhythm (Pruessner et al., 1997; Wilhelm et al., 2007). The CAR is a complex physiological phenomenon that is not completely understood, as it seems to be independent from the cortisol release during the rest of the day (for reviews see: Fries et al., 2009; Clow et al., 2010). However, it is clear that the hippocampus is a key brain structure in the integrity of the CAR, since hippocampal volume and the magnitude of the CAR are positively related (Pruessner et al., 2007; Bruehl et al., 2009), and the CAR is attenuated in patients with structural damage in their hippocampus (Buchanan et al., 2004; Wolf et al., 2005). Two recent studies have assessed the relationship between the CAR and cognitive performance among older adults. Franz et al. (2011) reported that, in men between 51 to 60 years of age, a greater CAR was related to poorer visual spatial memory, poorer working memory and poorer short term memory. However, after controlling for several confounds and the overall cortisol outcome throughout the day, these relationships were non-significant. Therefore, Franz et al. concluded that the CAR did not appear to contribute meaningfully to the association between cortisol and memory performance. Conversely, Evans et al. (2011), in an older sample (60 to 91 years old) of men and women, found that a larger CAR was associated with better performance on memory tests mainly dependent on prefrontal cortex functioning (Trail making-B and Verbal fluency). However, these relationships disappeared after controlling for age. In our opinion, the main reason for these weak and mixed results could be related to methodological issues when measuring the CAR.

Our study investigated whether, among older adults, individual differences in the CAR are related to individual differences in memory performance. To obtain a re-

liable CAR measure, it is crucial for participants to provide the first saliva sample directly after waking, since the CAR involves a fast release of cortisol immediately after awakening. The saliva collection is usually done at home, which introduces the possibility of non-adherence to the saliva sampling protocol. In fact, adherence can be difficult because the post-awakening period is characterized by deficits in alertness, psychomotor performance and cognitive performance, referred to as 'sleep inertia effects' (Lubin et al., 1976; Balkin et al., 2002). To control for this non-adherence to the protocol, in our study we followed the method proposed by Thorn et al. (2006) for identifying those participants who most likely did not adhere to the saliva sampling protocol and, therefore, should be treated with caution. Thorn et al. proposed that those participants with a flat or negative CAR should be identified as suspected non-adherents to the protocol, since EEG and mobility data have shown that a flat or negative CAR in healthy people is most likely an artifact caused by saliva sampling after the actual awakening response has occurred (Kupper et al., 2005). Additionally, our sample was composed of an equal number of men and women, as it has been shown that sex can be a crucial factor in the relationship between HPA-axis activity and cognitive performance among older people (Seeman et al., 1997; Almela et al., 2011). In sum, we investigated whether the magnitude of the CAR is related to declarative memory and working memory performance and also explored if sex moderated these relationships.

4.2 Methods

Participants

The sample was composed of 88 participants (44 men and 44 women) from 55 to 77 years old (Men: M = 63.41, SD = 4.91; Women: M = 63.73, SD = 3.90). Most of them (86%) had an educational level beyond high school, and were retired (91%). Men reported a slightly higher subjective socioeconomic status (Adler et al., 2000)

than women (SES scale, from 1 -lowest SES- to 10 points -highest SES-: Men: M = 6.52, SD = 1.19; Women: M = 5.98, SD = 1, p = 0.022), and men had a higher body mass index than women (Men: M = 27.71, SD = 3.49; Women: M = 25.62, SD = 3.34, p = 0.005). There were no sex differences in age or educational level (for both $p > 0.7$). All female participants were postmenopausal and had had their last menstrual period more than one year before the testing time. None of these women were receiving estrogen replacement therapy.

Participants belonged to a study program at the University of Valencia for people older than 50 years of age. One hundred sixty-six persons volunteered to participate. These volunteers were interviewed and completed an extensive questionnaire to check whether they met the study prerequisites. In order to avoid a large number of potentially confounding factors that could interfere with the CAR or with the cognitive functioning, we selected a homogeneous healthy sample using very restrictive criteria. The criteria for exclusion were: smoking more than 5 cigarettes a day, alcohol or other drug abuse, visual or hearing problems, presence of a cardiovascular, endocrine, neurological or psychiatric disease, having been under general anesthesia once or more than once in the past year, and the presence of a stressful life event during the last year. Volunteers were excluded from participation when they met the criteria for dementia as defined by the DSM-IV and the NINCDS-ADRDA criteria for Alzheimer's disease, and when they were using any medication directly related to emotional or cognitive function, or medication that was able to influence hormonal levels, such as glucocorticoids, anti-diabetic medication, antidepressants, benzodiazepines, and psychotropic substances. Vitamins and sporadic use of painkillers were allowed.

Procedure

Participants meeting the criteria were contacted by telephone and asked to attend a neuropsychological assessment which took place in a laboratory at the Faculty of Psychology. The study was conducted in accordance with the Declaration of Hel-

sinki, and the protocol and conduct were approved by the Ethics Research Committee of the University of Valencia. All the participants received verbal and written information about the study and signed an informed consent form.

The neuropsychological assessments were done individually and were all conducted by the same experimenter. One half of the sample began the session at 10 h (21 men and 23 women) and the other half at 12 h (23 men and 21 women). The distribution of men and women between the two turns was not different ($p > 0.6$). The neuropsychological assessment was composed of tests that have proved to be valid and reliable (Sahakian and Owen, 1992; Lezak et al., 2004). The neuropsychological assessment consisted of two tests of declarative memory (Logical Memory and Auditory Verbal Learning Test), and two tests of working memory (Spatial Span and Spatial Working Memory). Each participant's answers on the declarative memory tests were audio recorded and corrected twice by two persons previously trained for this.

Logical Memory The Spanish version of this subtest from the Wechsler Memory Scale III was administered (Pereña et al., 2004). The administration of this test was performed according to the manual. The experimenter read aloud two brief narratives, and afterward participants had to recall as many memory units or "ideas" as possible. After a delay of 25 minutes, the participants were asked again to recall as many "ideas" from the two narratives as possible. The final part of the test consisted of a recognition trial composed of several questions related to the two narratives. From this test, four outcomes were used in the subsequent analyses: (i) Immediate Recall: total "ideas" recalled from the two narratives immediately after having heard them, (ii) Delayed Recall: total "ideas" recalled from the two narratives after the 25-minute delay, (iii) Percentage of Delayed Retention: percentage of "ideas" recalled after the 25-minute delay from the "ideas" of the two narratives that were recalled immediately after having heard them, (iv) Recognition: total number of correct answers on the recognition trial.

Auditory Verbal Learning Test The Spanish version of the WHO-UCLA Auditory Verbal Learning Test was used (AVLT, Maj et al., 1994). This test consists of a five-trial

presentation of a 15-word list (learning trials), followed by a single presentation of an interference list. After that, there are two recall trials, the first one is done right after the recall of the interference list (immediate recall after interference), and the second one after a delay of 30 minutes (delayed recall). The test finishes with a recognition trial of the target words presented together with new words. Four outcomes were used in subsequent analyses: (i) Learning Slope: total number of words recalled on the first five trials, (ii) Recall after Interference: total number of words recalled after the interference trial, (iii) Delayed Recall: total number of words recalled after the 30-minute delay, and (iv) Recognition: the difference between the standardized proportion of correct hits and the standardized proportion of false alarms (d-Prime).

Spatial Span This test from the Cambridge Neuropsychological Test Automated Battery was applied (CANTAB, Cambridge Cognition, Cambridge, United Kingdom, www.cantab.com, 2006). This test measures short term memory capacity and is based on the Corsi Block Tapping Task (Milner, 1971). Participants were presented with a set of nine white squares on a tactile computer screen. In each trial, a number of squares changed their colour, starting with 2 squares in the first trial which increased to all 9 squares in the last trial. At the end of each trial, a tone indicated that the participant had to touch the squares that had changed their colour, either in the same order (forward) or in the reverse order (backward) as they were presented. Two outcome measures were obtained: (i) Span Length Forward: the last number of squares that the participants were able to touch correctly in the same order as they were presented , (ii) Spatial Length Backward: the last number of squares that the participants were able to touch correctly in the reverse order of their presentation.

Spatial Working Memory We also measured working memory with the Spatial Working Memory test of the CANTAB (Owen et al., 1995). In this test, several boxes were presented on a tactile computer screen. Participants were instructed to find a token that was hidden beneath one of these boxes. By touching a box they could reveal if there was a token under it, after which the box returned to its original state. Once the first token was located, the participants had to locate another token in the

same set of boxes. However, they were instructed that in every trial the token would never be under a box where a token had been found previously. As an outcome measure, we used the total errors committed, which includes returning to a box in which a token was already found and returning to a box that was already found to be empty.

Preliminary analyses: factor analysis with memory tests outcomes

To reduce the number of memory variables, we performed an exploratory factor analysis with varimax rotation. The Kaiser criterion (dropping all components with eigenvalues < 1.0) and scree plot inspection were used to determine the number of factors. Three factors were identified: (i) Paragraph Recall, which explained 36.28 % of the total variance, (ii) Word List Learning, which explained 18.61 %, and (iii) Working Memory, which explained 16.15 % (total variance explained: 71.04%). Table 1 shows the factor loadings of the variables on the three factors. The Kaiser-Meyer-Olking (KMO) indicated a satisfactory relationship between sample size and the number of variables (0.668), and Bartlett's test indicated that the correlations between variables were sufficient to warrant a factor analysis, $C^2(66) = 637.148$, $p < 0.001$. The participant's performance on each factor was not different between those who began the neuropsychological assessment at 10 h or 12 h (Paragraph Recall: $p > 0.8$, Word List Learning: $p > 0.09$, Working Memory: $p > 0.8$).

Table 1. Factor loadings of the memory tests outcomes.

		Paragraph Recall	Word List Learning	Working Memory
Logical Memory WMS-III	Immediate Recall	**0.801**	0.297	0.231
	Delayed Recall	**0.923**	0.278	0.124
	% Delayed Retention	**0.742**	0.081	-0.104
	Recognition	**0.859**	0.030	-0.073
AVLT	Learning Slope	0.151	**0.804**	0.295
	Recall after Interference	0.199	**0.886**	0.010
	Delayed Recall	0.107	**0.909**	0.096
	Recognition	0.117	**0.612**	-0.224
CANTAB	Spatial Span Forward	-0.063	0.099	**0.805**
	Spatial Span Backward	0.182	-0.007	**0.736**
	TE Spatial Working Memory	0.060	0.007	**-0.817**

Legend: Numbers in bold are factor loadings higher than 0.5. WMS-III: Wechsler Memory Scale III; AVLT: Auditory Verbal Learning Test; CANTAB: CANTAB Eclipse Battery; TE: Total Errors.

Saliva sampling

To control for cortisol concentrations at the moment of memory testing, participants provided two saliva samples using salivettes (Sarstedt, Rommelsdorf, Germany) before and after the neuropsychological assessment.

To measure the CAR, participants provided in their own homes 4 saliva samples per morning for 2 consecutive mornings using salivettes. No samples were provided during the weekend. Participants provided the samples immediately after awakening, and 30, 45, and 60 min post-awakening. Additionally, they recorded in a log their awakening time and the time of each saliva collection. After providing the saliva samples, participants stored their samples in their home fridge and brought the samples to the university within three days after completion.

Participants were instructed thoroughly about how to provide the saliva samples. A demonstration was made by the experimenter, and participants were given written instructions. The instructions were as follows: (i) place the tubes, the written instructions, and the log near your bed so you do not have to stand up to provide the first saliva sample; (ii) to provide the saliva sample take the cotton roll out of the tube and place it in your mouth for 2 min and move it around while chewing it slightly; (iii) after you have provided the first saliva sample you can stand up and move around; (iv) you cannot drink (except water), eat, brush your teeth, take any medication or do any physical exercise until you have finished the fourth saliva sample. Two women did not return the saliva samples.

Once in the lab, the salivettes were centrifuged at 3000 rpm for 5 min (4000 rpm for 15 min), resulting in a clear supernatant of low viscosity that was stored at –80 ºC until assay. Free salivary cortisol levels were measured in duplicate by a competitive solid phase radioimmunoassay (tube coated) using a commercial kit Coat-ACount Cortisol (DPC, Siemens Medical Solutions Diagnostics). Assay sensitivity was 0.5 ng/ml. Each subject's samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%. Three participants (1 man and 2

women) were excluded from the analyses as their AUCs differed more than 3 S.D. from the rest of the sample.

Adherence vs. non-adherence to the saliva sampling protocol

Self-reported adherence cannot be relied upon (Kudielka et al., 2003; Broderick et al., 2004); therefore, we checked participants' adherence to the saliva sampling protocol by analyzing participants' CAR on both days (Kupper et al., 2005; Thorn et al., 2006; O'Connor et al., 2009; Walker et al., 2011). This method assumes that if there was a delay in providing the first saliva sample upon awakening, its cortisol concentrations would be too high (nearer to the peak than they should be), and too low at the 45 min sample (more post-peak than they should be). Therefore, participants were divided into two groups: the Likely Adherent (LA) group, which was composed of those who showed a positive cortisol AUCi on both days, and the Suspected Non-Adherent (SNA) group, which was composed of those who showed a negative cortisol AUCi on one or both days.

The LA group was composed of 53 participants (26 men and 27 women) who represented 62% of the total sample. The SNA group was composed of 22 participants (9 men and 13 women) who did not show CAR on one of the days (25% of the total sample) and 11 participants (9 men and 2 women) who did not show CAR on either of the two days (13% of the total sample).

Statistics

Cortisol values were square root transformed because they did not have a normal distribution. The two saliva samples taken before and after the neuropsychological assessments were averaged. The cortisol values measuring CAR were averaged across days according to their sampling time, because their values did not differ across days ($p > 0.7$) and were correlated (r between 0.3 and 0.5, for all $p \leq 0.005$). For the cortisol samples provided at home, we created two summary indices: AUCg

(total cortisol secretion during the 60 min after awakening), and AUCi (change in total cortisol levels from baseline), see Pruessner et al. (2003) for the specific formulas.

Student's *t*-tests were used to investigate sex differences in the demographic variables. To analyze the CAR profile and the adherence to the saliva sampling protocol we performed ANOVAS for repeated measures. We used the Greenhouse-Geisser procedure when the requirement of sphericity in the ANOVA for repeated measures was violated. Post hoc planned comparisons were performed using the Bonferroni adjustments for the *p*-values. We performed regression analyses to investigate whether there was a relationship between the CAR and memory outcomes, and, according to Aiken and West (1991), we performed a moderator regression analysis to investigate whether sex was a moderator.

All *p*-values reported are two-tailed, and the level of significance was marked at <0.05. Scatterplots were inspected to investigate whether the relationships were linear or quadratic. When not otherwise specified, values are mean ± standard error of mean (SEM). We used SPSS 17.0 to perform the statistical analyses.

4.3 Results

Cortisol awakening response: likely adherence vs. suspected non-adherence

A repeated-measures ANOVA was used to analyze the cortisol profile of the LA and SNA groups. Time (Awakening, +30, +45, +60) was included as a within-subject factor, and Adherence (LA vs. SNA) and Sex were included as between-subject factors. SES and BMI were included as covariates.

As expected, the cortisol release profile was different between the LA and SNA groups, see figure 1 (Time × Adherence: F (1.73, 133.59) = 41.512, $p < 0.001$). In the LA group, cortisol levels increased from awakening to 30 min later ($p < 0.001$), and started to decrease afterward, without reaching awakening levels in the last saliva sample (Awakening vs. +60 min, $p < 0.001$). In the SNA group, cortisol concentrations

were already high at the awakening sample, and they were not different from samples +30 min and +45 min, for both samples, $p > 0.999$. Cortisol levels in the +60 min sample were lower than in the awakening sample, $p = 0.036$. The SNA group had higher cortisol concentrations than the LA group at the awakening sample ($p < 0.001$), but lower concentrations 45 min after awakening, $p = 0.024$.

The factor Sex was not significant, and there were no interactions with the other factors, $p > 0.3$.

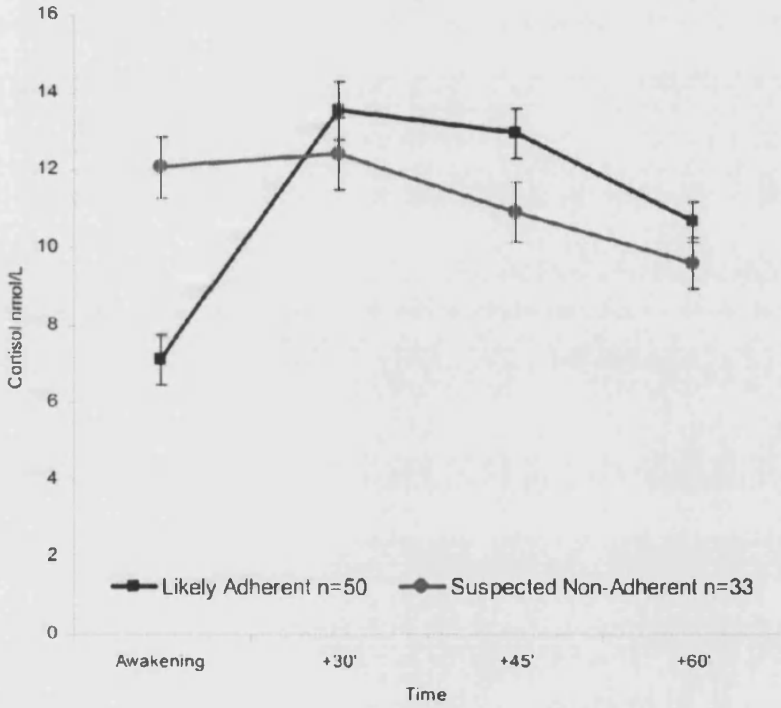

Figure 1. CAR of likely adherent and suspected non adherent groups.

Relationship between CAR and memory performance

We performed regression analyses to identify how much factor variance was explained by the CAR indices, and whether this relationship was moderated by sex. As covariates we included age, BMI, SES, and cortisol levels during the memory testing, because these variables have been related to performance on neuropsychological assessments. In Step 1, we included the covariates, one of the cortisol indices (AUCg or

AUCi) and Sex (0 = Women, 1 = Men). In Step 2, we included the interaction between sex and one of the cortisol indices. Regression analyses were performed for the complete sample and for the LA group.

Paragraph recall

Results from de regression analyses (table 2) showed that higher total cortisol output (AUCg) was related to worse paragraph recall performance (total sample and the LA group). In addition, only in the LA group was a greater cortisol increase (AUCi) related to worse paragraph recall performance. In the total sample, there was no linear relationship between cortisol AUCi and paragraph recall because the relationship was in fact quadratic (inverted U-shaped form, $F(2,80) = 3.520$, $p = 0.034$), showing that in the total sample, a large cortisol decrease and increase was related to worse paragraph recall performance (see figure 2). Sex did not moderate the relationship between AUCg or AUCi and paragraph recall performance in either of the two groups. As a complementary result, we also found that a higher subjective socioeconomic status (SES) was related to better paragraph recall.

Table 2. Regression analyses with paragraph recall as a dependent variable

	AUCg				AUCi			
	Total Sample		LA Group		Total Sample		LA Group	
Step 1	Adj R^2= 0.13		Adj R^2= 0.15		Adj R^2= 0.06		Adj R^2= 0.14	
	$F_{6,77}$= 3.10**		$F_{6,43}$= 2.48*		$F_{6,76}$= 1.85#		$F_{6,43}$= 2.34*	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Age	-.103	*ns*	-.094	*ns*	-.079	*ns*	-.049	*ns*
BMI	-.104	*ns*	-.072	*ns*	-.057	*ns*	-.031	*ns*
SES	.274	.014	.349	.021	.235	.041	.314	.036
Cortisol[1]	.187	.082	.157	*ns*	.142	*ns*	.101	*ns*
Sex	-.104	*ns*	-.099	*ns*	-.066	*ns*	-.044	*ns*
AUC	-.287	.008	-.377	.013	-.101	*ns*	-.349	.018
Step 2	*ns*		*ns*		*ns*		*ns*	

Legend: [1]Mean of cortisol levels during the neuropsychological assessment. ** $p \leq 0.01$, * $p \leq 0.05$, # $p \leq 0.10$.

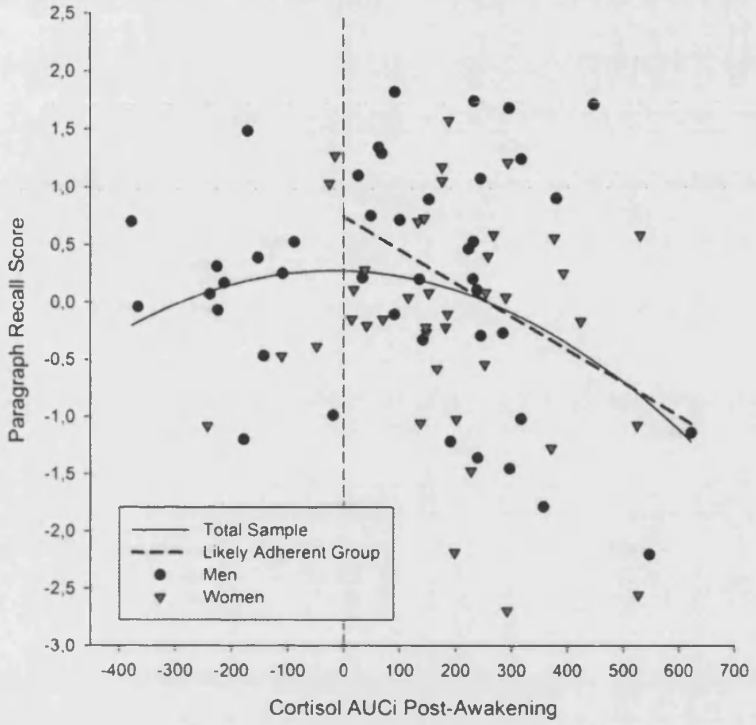

Figure 2. Relationship between cortisol AUCi post-awakening and paragraph recall performance.

Word list learning

Results from the regression analyses (table 3) showed that the relationship between global cortisol output (AUCg) and word list learning performance was moderated by the sex of the participants (total sample and LA group). Although there was a significant interaction, post hoc test of the slopes were non-significant. Nevertheless, in men, higher AUCg was related to better word list learning performance, and in women, higher AUCg was related to worse word list learning performance, see figure 3.

Regarding the cortisol increase (AUCi), only in the total sample did sex moderate the relationship between word list learning and the cortisol increase post-awakening. Although there was a significant interaction, post hoc tests of the slopes were non-significant. Nevertheless, among men the relationship was negative, while

it was positive among women. The interaction effect disappeared completely when considering only the LA group.

Table 3. Regression Analyses with Word List Learning as a dependent variable

	AUCg				AUCi		
	Total Sample		LA Group		Total Sample		LA Group
Step 1	*ns*		*ns*		*ns*		*ns*
Step 2	Adj R^2= 0.06, ΔR^2= 0.06 $F_{1,75}$= 5.54*		Adj R^2= 0.11, ΔR^2= 0.08 $F_{1,42}$= 4.46*		Adj R^2= 0.05, ΔR^2= 0.05 $F_{1,75}$= 4.44*		*ns*
	β	*p*	*β*	*p*	*β*	*p*	
AUC×Sex	-1.388	.021	-1.824	.041	2.887	.038	
Post Hoc	*β*	*p*	*β*	*p*	*β*	*p*	
Men	.322	.067	.363	*ns*	-.212	*ns*	
Women	-.196	*ns*	-.261	*ns*	.320	*ns*	

Legend: [1]Mean of cortisol levels during the neuropsychological assessment. * $p \leq 0.05$

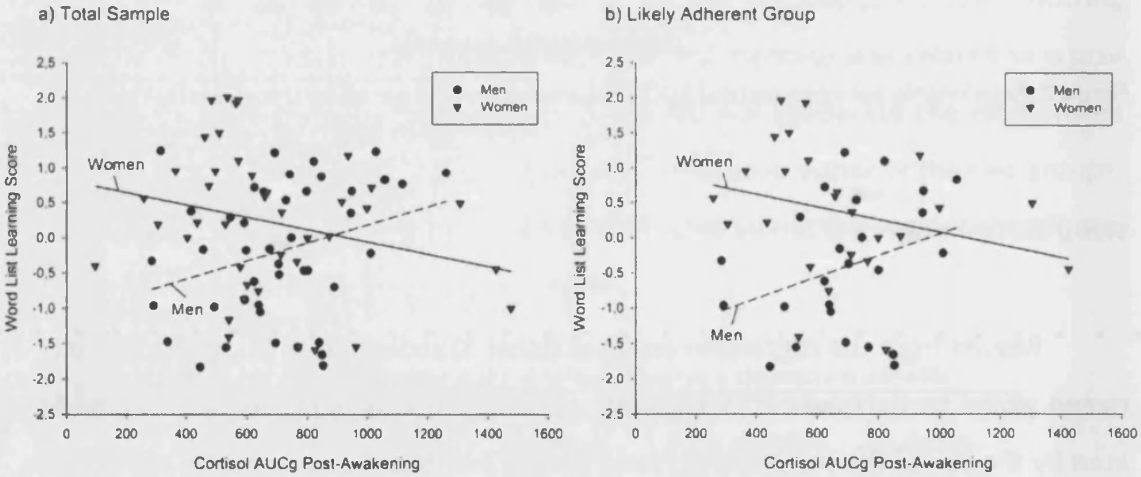

Figure 3. Relationship between cortisol AUCg post-awakening and word list performance.

Working Memory

Results from regression analyses show that the global cortisol output (AUCg) was not related to working memory performance, nor was the relationship moderated by the sex of the participants (table 4). Regarding the cortisol increase (AUCi), only

among the LA group was it related to working memory performance and moderated by the sex of the participants. Among men, a greater cortisol increase was related to better working memory performance. Among women, the relationship was negative but non-significant, see figure 4.

Table 4. Regression analyses with working memory as a dependent variable

	AUCg			AUCi		
	Total Sample		LA Group	Total Sample		LA Group
Step 1	Adj R^2= 0.20 $F_{6,76}$ = 4.33**		*ns*	Adj R^2= 0.20 $F_{6,76}$ = 4.48**		*ns*
	β	*p*		*β*	*p*	
Age	-.423	<.001		-.440	<.001	
BMI	.098	*ns*		.114	*ns*	
SES	-.161	*ns*		-.170	*ns*	
Corti-sol[1]	.071	*ns*		.050	*ns*	
Sex	-.094	*ns*		-.075	*ns*	
AUC	-.008	*ns*		-.089	*ns*	
Step 2	*ns*		*ns*	*ns*		Adj R^2= 0.17, ΔR^2= 0.10 $F_{1,42}$= 5.87*
					β	*p*
AUC×Sex					-6.725	0.020
Post hoc					*β*	*p*
Men					.459	0.022
Women					-.261	*ns*

Legend: [1]Mean of cortisol levels during the neuropsychological assessment. ** $p \leq 0.01$*, $p \leq 0.05$.

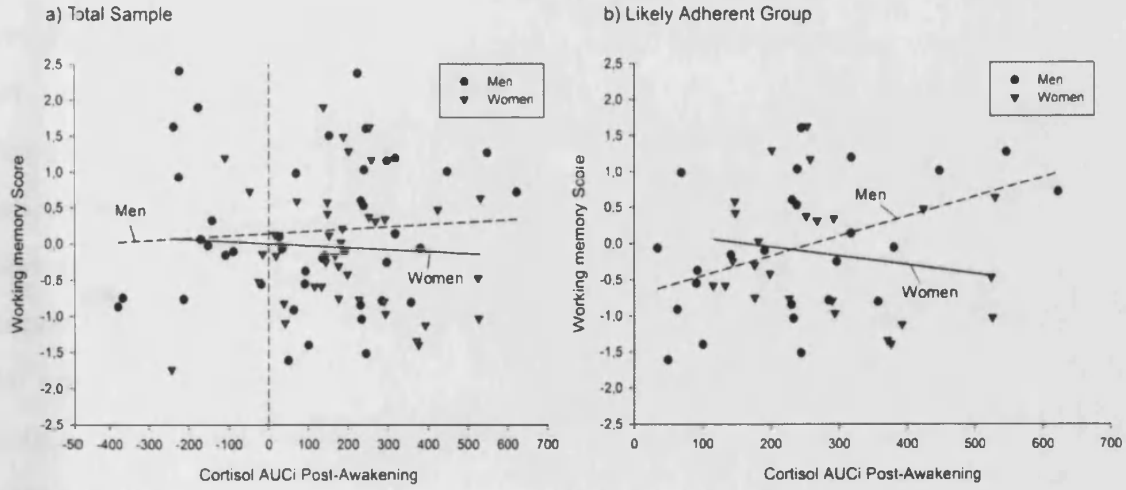

Figure 4. Relationship between cortisol AUCi post-awakening and working memory performance.

Correlational Analyses

To compare this study with other studies, we correlated, only for the likely adherent group, the raw data from the memory tests with cortisol AUCg and AUCi. For the word list learning test and working memory tests, the correlations were performed for men and women separately because the regressions showed that the relationship between cortisol indices and performance was moderated by the sex of the participants. Results from correlational analyses are shown in table 5.

Table 5. Correlation analyses between the memory tests outcomes and cortisol indices.

	Total Sample			
	AUCg	AUCi		
Paragraph Recall				
Immediate Recall				
Delayed Recall	-0.27$^{\bullet}$	-0.29$^{\bullet}$		
Percentage of Delayed Retention	-0.27$^{\bullet}$	-0.36$^{\bullet\bullet}$		
Recognition	-0.24$^{\#}$	-0.37$^{\bullet\bullet}$		
	Men		Women	
	AUCg	AUCi	AUCg	AUCi
Word List Learning				
Learning Slope (∑ Trial 1 to 5)	0.35$^{\#}$		-0.48$^{\bullet}$	
Trial 1	0.45$^{\bullet}$	0.42$^{\bullet}$	-0.40$^{\bullet}$	
Trial 2		0.35$^{\#}$	-0.48$^{\bullet}$	
Trial 3	0.35$^{\#}$	0.40$^{\bullet}$		
Trial 4			-0.47$^{\bullet}$	
Trial 5			-0.41$^{\bullet}$	
Trial 6 Recall after Interference				
Trial 7 Delayed Recall				
Recognition				
Working Memory				
Spatial Working Memory				
Spatial Span Forward		0.34$^{\#}$		
Spatial Span Backward	0.38$^{\#}$	0.47$^{\bullet}$		

Legend: ** $p \leq 0.01$, * $p \leq 0.05$, $^{\#}$ $p \leq 0.10$

4.4 Discussion

This study investigated the relationship between the cortisol awakening response (CAR) and memory performance among men and women between 55 and 77 years of age. The relationship between CAR and memory performance was analyzed in the total sample and in a subsample of participants who showed the CAR on two different days (likely adherent group). The main results of this study show that, in both men and women, a greater CAR is related to poorer declarative memory performance, and, only in men, it is related to better working memory performance.

To measure declarative memory performance we employed a paragraph recall test. We found that a greater cortisol output (AUCg) was related to poorer performance on this test. Additionally, a larger cortisol increase (AUCi) was related to poorer performance on this test only in the likely adherent group. Interestingly, for the total sample this latter relationship was quadratic (inverted U-shaped form), showing that those participants who had a larger cortisol decrease also performed worse on this test. In our opinion, the discrepancy in the results between the total sample and the likely adherent group could be explained by the possibility that some people in the total sample did not provide their first sample exactly when they awakened, but some time later. Therefore, the measurement of their CAR shifted, and instead of showing the typical cortisol increase post-awakening, their samples showed the recovery after this increase had already happened. Moreover, it seems likely that those who showed a relative large cortisol decrease had a relative large CAR (thus making a large decrease possible). This reasoning can explain the left side of the inverted U relationship (see figure 2), as those participants with a large cortisol decrease had a poorer performance just as those participants on the right side of the inverted U curve (large CAR also poorer performance). In fact, in healthy people the main reason for not showing the typical cortisol increase post-awakening has been found to be a delay in providing the first saliva sample (Kupper et al., 2005). However, based on our data, we cannot discard the possibility that a larger cortisol decrease after awakening

was due to another cause (e.g. undiagnosed pathology, larger cortisol secretion during sleep). Clearly, more research is needed in order to uncover the reasons of not showing a CAR and its relationship with declarative memory performance.

A second measure of declarative memory performance, a word list learning test, was applied. All the outcomes of this test loaded in a second factor. In the total sample and the LA group, the performance on this test was also related to the amount of cortisol secreted after awakening (AUCg), but in this case the relationship was moderated by the sex of the participants. Although post-hoc tests were non-significant, results showed that among men this relationship was positive, and among women it was negative (see fig. 3). It is worth noting that previous studies support the existence of sex differences in the performance on this test (Gale et al., 2007), and most interestingly, in the relationship between HPA-axis activity and performance on this kind of memory test. Thus, we found in another study that, among middle-aged people, and only in women but not in men, greater cortisol reactivity to acute stress was related to poorer performance on a word list learning test when it was applied on a day without stress (Almela et al., 2011).

Unexpectedly, the relationship between CAR and memory performance was not the same when using the word list learning test as when using the paragraph recall test. In our opinion, a main reason for this discrepancy is that the two tests were not measuring exactly the same. Supporting this notion, the factorial analyses revealed that the performance on each test explained unique variance, and previous research has shown that there is only a modest relationship between the outcomes on these two memory tests (Macartney-Filgate and Vriezen, 1988; Helmstaedter et al., 2009). Furthermore, it has been proposed that a paragraph recall test is the "purest" measure of episodic memory, while a word list learning test could be considered a measure of general cognitive functioning, due to its overlapping with other measures, including measures of working memory, which suggests a higher involvement of prefrontal cortex functioning in its performance (Vanderploeg et al., 1994; Woodard et al., 1999; Lezak et al., 2004). In fact, the relationship between CAR and

the performance on the word list learning test was closer to the relationship found with the performance on the working memory tests than with the performance on the paragraph recall test.

To measure working memory, two different neuropsychological tests were employed, and the factorial analyses revealed that both were measuring the same construct. We found that a larger cortisol increase post-awakening (AUCi) was related to better working memory performance, but only among men from the likely adherent group. Moreover, the correlational analyses confirmed that those men, who increased their post-awakening cortisol concentrations more, had a better performance on the first trials of the word list learning test and on the spatial span backward, all of which have an important involvement of working memory and, therefore, the prefrontal cortex functioning. The relationship found between a greater CAR and better working memory is in line with Evans et al. (2011) who found that a larger cortisol increase post-awakening was related to better performance on the Trail-making test (form B) and on a test of verbal fluency, both of which are strongly linked to executive function (Lezak et al., 2004; Kemper and McDowd, 2008). Unfortunately, they did not investigate whether there were sex differences in these relationships.

Taken together, our results suggest an opposite relationship between CAR and memory processes that are either highly dependent on the hippocampus (declarative memory) or the prefrontal cortex (working memory). However, the function and regulation of the CAR are not clearly understood. Evidence suggests that the hippocampus and the prefrontal cortex play a role in the regulation of the CAR (for a reviews see: Fries et al., 2009; Clow et al., 2010), although the final mechanism through which these brain structures influence the CAR is unknown. It has been suggested that the hippocampus, despite its general inhibitory effect on the HPA-axis activity throughout the day, could exert a permissive effect on the sharp cortisol release that occurs post-awakening. This permissive effect should happen during the pre-awakening period, because awakening has been associated with the switching off of hippocampal activation (Braun et al., 1997; Balkin et al., 2002). Less is known, however, about the

function of the prefrontal cortex on the CAR, not even whether its role is inhibitory or permissive. However, it is suspected that it plays a part because the CAR dynamic closely parallels that of prefrontal cortex reactivation and the attainment of full alertness (Clow et al., 2010). To disentangle the ultimate mechanisms in these relationships, more research is clearly warranted.

As in the current study, other studies have suggested that sex can moderate the relationship between HPA-axis activity and cognitive processes, which are dependent on prefrontal cortex functioning. For example, we found in another study that a stress-induced cortisol increase impaired working memory performance only in middle-aged women and not in middle-aged men (Almela et al., 2011). An explanation for this sex difference is that after menopause women experience a reduction in estrogen production, whereas men do not experience such a drastic change in hormonal levels. It has been hypothesized that estrogens could work to contain the HPA-axis and counteract some of the potentially damaging actions of glucocorticoids on nerve cells (McEwen, 2002). Furthermore, the prefrontal cortex and its neural circuitry are prime mediators of estrogen's role in cognition, and menopausal cognitive decline could be secondary to executive dysfunction (Keenan et al., 2001). An alternative explanation for the sex differences is that men and women use different strategies to resolve memory tests, i.e. men and women may use different neural paths to reach the same behavioural end point (Andreano and Cahill, 2009). Therefore, the sex differences found in the current study can also be explained by a different involvement of the hippocampus and prefrontal cortex to resolve the same task.

It is important to mention that the percentage of suspected non-adherent participants in our study was relatively high. To address this issue, we have presented our results for the total sample and for a subgroup consisting of participants who showed a CAR on both days. Results showed that not selecting the suspected non-adherent participants did not change the relationships between memory performance and cortisol AUCg. However, when investigating the relationships between cortisol AUCi and the three memory factors, the results changed substantially when

we eliminated the suspected non-adherent participants. This result shows that it is of extreme importance to take participants' adherence into account, and it can explain the mixed and weak results found in the literature.

The present study extends previous findings showing that HPA-axis activity and regulation are related to memory performance in older adults, and that sex is an important moderator of this relationship. Our results draw a complex picture of associations between the CAR and memory performance, and they suggest that, among people from 55 to 77 years of age, a greater CAR is related to poorer performance on hippocampus-dependent memory tasks. However, only among men, a larger CAR is related to better performance on tasks that are dependent on prefrontal cortex functioning. More research is needed to further disentangle the mechanisms in these relationships.

Chapter 6

Discussion of the main findings

The studies described in the previous chapters have shown that the activity and regulation of the HPA-axis are related to memory performance in older men and women. Furthermore, these studies show that this relationship is not limited to an acute influence of cortisol on memory performance, but also involves a more permanent effect that points to the HPA-axis as a crucial factor in the individual differences observed in the memory performance of older people. What follows is a short description and conclusion of these empirical chapters and a global reflection on their implications.

1. Summary of main findings:

1.1 Study 1

Previous studies have shown that aging is associated with psychobiological changes that could limit our ability to cope with stressors. The aim of this study was to clarify the physiological mechanisms that underlie the stress response and the changes that occur in them as we age. To do so, we investigated the impact of age on the HPA-axis and ANS responses to acute psychosocial stress. The HPA-axis activity was measured through the salivary cortisol stress response, and the ANS activity was evaluated through the measurement of HR and a novel biomarker of the ANS activity: sAA. Sixty-two participants were divided into two age groups (Younger Group: N = 31, Age Range: 18-35 years; Older Group: N = 31, Age Range: 54-71 years) and exposed to the TSST and a control condition in a crossover design. Aging was associated with a larger cortisol response to stress and to a lower cortisol decrease in the control condition. No age differences were found in the HR or sAA responses to stress. However, the sAA global output was higher in older than younger adults. Additionally, in the stress condition, the total amount of cortisol released was positively related to the total sAA released, while the HR increase was positively related to the sAA increase.

Our results support the existence of decreased negative-feedback sensitivity in the HPA-axis regulation with increasing age. However, they do not support the existence of an attenuated ANS response to stress in older adults, but rather a heightened sympathetic tone. Furthermore, we found further evidence of the coordination between the HPA-axis and the ANS in their response to acute psychosocial stress.

1.2 Study 2

Stress has been identified as a main factor involved in the cognitive changes that occur during the aging process. This study investigated sex differences in the relationship between the magnitude of the stress-induced cortisol response and memory performance among older people. To this end, 16 men and 16 women (54 to 72 years) were exposed to the TSST and a control condition in a crossover design. Afterwards, their memory performance was measured using a standardized memory test (RAVLT). Only among women, there was an acute impact of stress on memory performance and a significant relationship between a higher cortisol response to stress and a worse memory performance in both the stress and control conditions. These results confirm that sex is a critical factor in the relationship between acute cortisol increase and memory performance. Furthermore, they emphasize a strong link between the individual cortisol response to stress and memory functioning among postmenopausal women.

1.3 Study 3

The second study of this dissertation was originally designed to study the acute effects of stress on memory performance, but the results suggested that the magnitude of the cortisol response to acute stress could be related to the memory

performance of older people when memory is tested in non-stressful conditions. Additionally, sex could be a moderator of this relationship. The third study of this dissertation was designed to further investigate this. To do so, I measured the declarative and working memory performance of 31 men and 35 women between 55 and 77 years of age in a non-stressful session. On a different day, the magnitude of their cortisol response to acute psychosocial stress was assessed. The relationship between the cortisol response to stress and memory performance was U shaped: a moderate cortisol response to stress was related to poorer declarative and working memory performance, whereas those who did not increase their cortisol levels and those who had the largest cortisol increase had better declarative and working memory capabilities. Sex did not moderate these relationships. These results suggest that a moderate cortisol response to stress could reflect a defective HPA-axis response to stressors that is accompanied by poorer memory performance. In contrast, a high cortisol response seems to reflect a correct functioning of the HPA-axis and, together with a non-cortisol response, may protect against memory deficits in the later stages of human life.

1.4 Study 4

After having found that an acute cortisol response to stress was related to memory performance in older people, the fourth study in this dissertation investigated whether another facet of the HPA-axis activity, the CAR, was also associated with memory performance among older adults. To study this, I recruited 88 participants (44 men and 44 women) from 55 to 77 years old, and I assessed their memory with two tests that measure declarative memory (a paragraph recall test and a word list learning test) and two tests that measure working memory (a spatial span test and a spatial working memory test). Results showed that a greater CAR was related to a poorer declarative memory performance in both men and women, and to a better

working memory performance only in men. These results suggest that the relationship between CAR and memory performance is negative in men and women when memory performance is largely dependent on hippocampal functioning (i.e. declarative memory), and positive, only in men, when memory performance is largely dependent on prefrontal cortex functioning (i.e. working memory).

2. Some caution

The findings included in this dissertation have to be treated with caution. Although in every study I tried to control for as many potentially confounding factors as possible, no control is perfect, and frequently it is possible to come up with alternative explanations for the results. Each empirical chapter contains the main limitations of the study explained in it. Here, I will describe some general limitations that, in my opinion, apply to all of the studies and, therefore, have to be taken into account in order to properly interpret and generalize the results of this dissertation. Thus, all the participants in the studies were healthy people, and many volunteers were excluded due to very restrictive exclusion criteria that were focused, mainly, on health status and medication intake. I decided to use these restrictive exclusion criteria to avoid as many factors as possible that could interfere with the goals and variables of each study. However, the incidence of chronic and acute illnesses and the intake of medications increase with older age. Additionally, the sample of each study was composed of people who attended university lectures for people older than 50 years of age. This could have introduced some bias in the samples, related to socio-economic status and education, and due to the fact that the samples could include more people in the so-called "active aging" group, i.e. people who optimize their health opportunities, participation and security in order to enhance their quality of life. Therefore, we cannot generalize the results of this dissertation to all the elderly people in the normal population, and I strongly recommend that these studies are replicated to some extent in other populations with age-related diseases and medication use.

3. Conclusions

The results of the studies of this dissertation underscore the fact that HPA-axis functioning is related to individual differences in memory performance observed in elderly adults. In my opinion, the findings shown by these studies add new evidence to the existing literature. The results of each study have been discussed thoroughly in each empirical chapter, and below I have highlighted the most important findings of this dissertation:

- Older people react with larger cortisol increases to acute stress than younger people. Additionally, older people decrease their cortisol levels less than younger people in non-stressful conditions. These results are consistent with the loss of HPA-axis feedback sensitivity that has been hypothesized to occur with aging (Seeman and Robbins, 1994).

- Older age is associated with a larger overall sAA release. However, no effects of age were found in the sAA response to acute stress. These findings reflect a heightened basal sympathoneural activity in older people, while the response to stress remains unchanged.

- Men, regardless of age, react with larger increases in cortisol secretion to stress than women. However, this sex difference could be moderated by the characteristics of the stressor.

- Older women are more vulnerable to the acute effects of stress on their memory function than older men. Thus, only among older women, acute stress improved attention but impaired working memory executive processes.

- Declarative and working memory performance in older people is also related to the magnitude of their cortisol response to stress when memory is tested in non-stressful conditions. The relationship is U-shaped: a moderate cortisol re-

sponse is related to worse memory performance, and a larger or lower cortisol response is related to better memory performance.

- The magnitude of the CAR is related to the memory performance of older people. Among both sexes, a larger CAR is related to worse declarative memory performance and, only among men, to better working memory performance.

Taken together, these findings confirm that the relationship between the HPA-axis functioning and memory performance of older people is more complex than expected. It is not always the case that more cortisol secretion is related to worse memory performance. Instead, the relationship between the HPA-axis activity and memory performance depends on which facet of the HPA-axis activity is being measured.

4. Future

Like many other scientific studies, the questions answered by the studies described in this dissertation raise other questions that need to be answered. More research is needed to completely understand the nature of the relationship between HPA-axis activity and the memory performance in older people. Overall, the studies described in this dissertation have peripherally measured the production of cortisol, and this has been related to the central memory functioning. However, more research is needed to understand what specific biological mechanisms are through which the HPA-axis is related to memory performance. Additionally, longitudinal research is needed to answer the question of whether the observed results explain the memory performance of older people only at the moment of memory testing, or whether they are indicative of memory decline over time. In addition, it would be interesting in future research to unravel other variables that could be related to HPA-axis functioning. For example, in this dissertation I found that beliefs of self-efficacy

are related to the acute cortisol response to stress. More research is needed to identify other important variables that could become intervention targets to prevent memory deficits in older people. Finally, the results of this dissertation strongly recommend the exploration in future studies of curvilinear relationships when investigating the relationship between HPA-axis activity and memory performance.

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Spanish translation of Chapter 1

Capítulo 1

Relación entre la actividad del eje HHA y las diferencias individuales en la memoria de personas mayores

1. El eje Hipotálamo-Hipófiso-Adrenal (Eje HHA)

1.1 *Actividad y Control*

Cuando nos referimos al eje HHA, nos estamos refiriendo a un complejo sistema de comunicación que discurre a través de tres regiones diferentes de nuestro cuerpo. Este sistema de comunicación es crucial para nuestra supervivencia, porque es una parte fundamental de nuestra defensa frente a situaciones que amenazan a nuestro bienestar, así como, porque coordina el retorno al estado de homeostasis una vez que la amenaza ha pasado. Por lo tanto, la actividad del eje HHA forma parte de la respuesta de estrés de nuestro organismo, y su función principal es aumentar nuestras posibilidades de supervivencia frente a amenazas a nuestro bienestar físico y psicológico.

Esta comunicación se establece entre el hipotálamo, la hipófisis y la glándula adrenal a través de la liberación de hormonas (ver figura 1). Ante la presencia de un estresor físico o psicológico, el núcleo paraventricular del hipotálamo (NPV) libera hormona liberadora de corticotropina (CRH) y vasopresina (AVP), desde las terminaciones nerviosas neurosecretoras de la eminencia media al sistema porta hipofisario. Una vez en la hipófisis, CRH y AVP inducen la liberación de hormona adrenocorticotropa (ACTH) en el torrente sanguíneo, que estimula el córtex de la glándula adrenal donde, finalmente, los glucocorticoides son liberados al torrente sanguíneo (Ulrich-Lai y Herman, 2009). El glucocorticoide más notable en humanos es el cortisol.

Bajo situaciones no estresantes, la secreción de glucocorticoides sigue un ritmo circadiano, con niveles más altos por la mañana y niveles más bajos por la noche (Ulrich-Lai y Herman, 2009). La mayor secreción de glucocorticoides en condiciones no estresantes se produce por la mañana, unos 30 minutos después de despertar. Este aumento repentino en la secreción de cortisol es un componente discreto y distinto dentro del ritmo circadiano del cortisol, que ha sido llamado: respuesta matutina

de cortisol (CAR). La función del CAR todavía no está claramente definida, pero podría estar relacionada con la recuperación de la consciencia después de dormir (Clow et al., 2010).

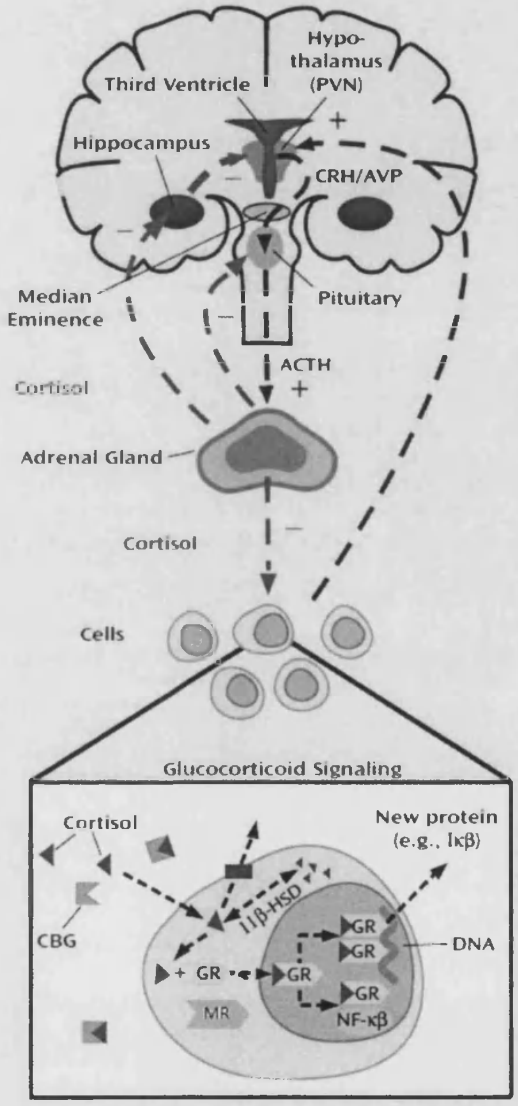

Figura 1. Eje HHA. CRH: hormona liberadora de corticotropina; AVP: arginina vasopresina; PVN: núcleo paraventricular del hipotálamo; ACTH: hormona adrenocorticotropa; CBG: globulina fijadora de corticoesteriodes; 11β-HSD: enzima 11-β-hidroxiesteroide deshidrogenasa; GR: receptor glucocorticoides; MR: receptor mineralcorticoides (cortesía: Raison and Miller, 2003).

La secreción de glucocorticoides tiene que controlarse estrechamente, porque una exposición prolongada a los mismos está relacionada con serios problemas metabólicos, inmunológicos y fisiológicos (McEwen, 2008). Para ello, la actividad del eje HHA está controlada por un sistema de feedback negativo cuyo principal objetivo es mantener los niveles de glucocorticoides dentro de unos límites tolerables (Keller-Wood y Dallman, 1984). Este sistema de feedback negativo está regulado por las neuronas del NPV del hipotálamo, que cuando detectan que los niveles de cortisol en el torrente sanguíneo han aumentado, inhiben la secreción de CRH y AVP (Whitnall, 1993). Además, hay también otros sistemas neuronales de control inhibitorio que trabajan en paralelo con el feedback hormonal. Estos sistemas neuronales complementarios están representados, fundamentalmente, por estructuras límbicas como (i) el hipocampo y (ii) el córtex prefrontal (ver figura 1).

(i) El hipocampo tiene un alto grado de receptores para glucocorticoides y ejerce una influencia principalmente inhibitoria en el NPV (Herman et al., 2005; Patel et al., 2000). Además, está implicado, no sólo en el control del ritmo circadiano de los glucocorticoides, sino también en la inhibición de la activación del eje HHA en respuesta al estrés (Fendler et al., 1961; Fischette et al., 1980). Esta función inhibitoria es ejercida a través de dos pasos. En el primer paso, las fibras glutamatérgicas del hipocampo proyectan al núcleo del lecho de la estría terminal, al área preóptica medial, al hipotálamo dorsomedial, y a otros núcleos hipotalámicos, los cuales están poblados con neuronas GABAérgicas. En un segundo paso, estas neuronas GABAérgicas proyectan directamente al NPV ejerciendo la función inhibitoria (Cullinan et al., 1993; Herman et al., 2003).

(ii) El córtex prefrontal medial procesa la información estresante, tiene una alta densidad de receptores para glucocorticoides, y también modula la activación del eje HHA en respuesta al estrés (Cullinan et al., 1995; Patel et al., 2000). De modo similar al hipocampo, la función inhibitoria del córtex prefrontal medial es ejercida a través de dos pasos. Las fibras glutamatérgicas del córtex prefrontal medial interaccionan con el área preóptica, el núcleo del tracto solitario y el núcleo del lecho de la

estría terminal, entre otros, y a través de allí, las neuronas GABAérgicas envían señales inhibitorias al NPV. Además, el córtex prefrontal medial tiene conexiones con el hipocampo y la amígdala (que tiene una función excitatoria en la actividad del eje HHA), y debido a esto, el córtex prefrontal medial es una de las áreas más importantes en la coordinación y el control de la actividad del eje HHA en respuesta al estrés (Ulrich-Lai y Herman, 2009).

1.2 *Funciones de los glucocorticoides*

La liberación de glucocorticoides tiene funciones complejas en nuestro organismo, que han sido clasificadas dentro de las siguientes dimensiones: (i) permisivas, (ii) estimuladoras, (iii) supresoras y (iv) preparativas (para una revisión extensa ver: Sapolsky et al., 2000b). Las funciones (i) permisivas y (ii) estimuladoras refuerzan los mecanismos que constituyen nuestra primera defensa frente al estrés. La diferencia entre ambas es que los efectos permisivos son ejercidos por los niveles de glucocorticoides basales, que son los que están presentes antes de la aparición del estresor, y sin embargo, los efectos estimulantes, son ejercidos por el aumento en los niveles de glucocorticoides que se produce ante la aparición de un estresor. Como ejemplo de un (i) efecto permisivo, los glucocorticoides basales facilitan la actividad simpática del sistema nervioso autónomo (SNA), porque, por ejemplo, aumentan de forma permisiva la actividad cardiovascular. Así, ante la aparición de un estresor, una de las funciones principales de nuestra primera respuesta, es optimizar los recursos disponibles y que el uso de la energía se centre en aquellas partes del cuerpo donde es más necesaria, como por ejemplo, en nuestros músculos. Para ello se produce la liberación de catecolaminas por parte del SNA, que provoca el aumento de la presión arterial, del ritmo cardíaco, y desvía la sangre a nuestros músculos. En este contexto, las acciones de los glucocorticoides facilitan que las catecolaminas y otros vasoconstrictores ejerzan sus acciones, a través de, por ejemplo, inhibir la recaptación de catecolaminas y

elevar la sensibilidad cardiovascular a las mismas, a través de aumentar la capacidad de unión de los receptores β-adrenérgicos (Sakaue y Hoffman, 1991). Como ejemplo de un (ii) efecto estimulante de los glucocorticoides, el incremento de la liberación de los mismos provocada por el estrés, aumenta la cantidad de energía (glucosa) disponible, a través de estimular la gluconeogénesis y la deposición de glucógeno en el hígado, así como de inhibir el transporte y la utilización periférica de glucosa. Todos de estos efectos tienen el propósito de poder mantener durante más tiempo la respuesta de estrés (Munk y Náray-Fejes-Tóth, 1994).

De forma complementaria, (iii) las funciones supresoras de los glucocorticoides controlan la respuesta de estrés, y favorecen el regreso a la homeostasis antes de que nuestra propia respuesta de estrés pueda dañar nuestro organismo. Como ejemplo de función supresora, los glucocorticoides suprimen la activación inmunológica e inflamatoria que se produce en respuesta al estrés. Ante un estresor, se produce una rápida activación del sistema inmune con el objetivo de poder defendernos rápidamente contra una posible causa de enfermedad. De esta forma, el sistema inmune se activa para identificar y derrotar posibles patógenos, que puedan entrar en nuestro organismo debido a posibles heridas o infecciones. Sin embargo, si este aumento en la actividad de sistema inmune se prolonga demasiado en el tiempo, puede haber graves consecuencias para nuestro organismo, como inflamaciones y enfermedades autoinmunes. Por lo tanto, en el largo plazo, los glucocorticoides tienen la función de suprimir la elevación en la actividad del sistema inmune provocada por el estrés, y así reducir la probabilidad de que una excesiva actividad autoinmune dañe nuestro organismo.

Finalmente, (iv) las funciones preparativas de los glucocorticoides no afectan a la respuesta inmediata de estrés, pero modulan nuestra respuesta ante la posible aparición de otro estresor. Como ejemplo de función preparatoria de los glucocorticoides, éstos estimulan el apetito en el plazo largo (Dallman et al., 1993). La exposición a un estresor suprime el hambre en el corto plazo, efecto que es provocado probablemente por la liberación de CRH (Arase et al., 1988). De hecho, alimentarse es un

proceso costoso que proporciona energía de forma lenta y, por lo tanto, es prescindible cuando estamos frente a un estresor. Sin embargo, los glucocorticoides estimulan el apetito en el largo plazo, función que se considera preparatoria, puesto que promueve el volver a llenar nuestras reservas de energía y prepararnos para la posible aparición de otro estresor.

1.3 *¿Cómo ejercen los glucocorticoides sus efectos?*

Los glucocorticoides son capaces de realizar todas estas complejas funciones a través de dos mecanismos diferentes. Estos dos mecanismos, se definen según el tiempo que tardan los glucocorticoides desde que son liberados hasta que ejercen sus funciones. (i) La alternativa lenta es denominada vía clásica o genómica. A través de esta vía los glucocorticoides pueden ejercer sus funciones en cuestión de minutos u horas. Esta vía requiere de la existencia de receptores intracelulares que, en su estado inactivo, están en el citosol de las células ligados a proteínas estabilizadoras (ver figura 1). Una vez los glucocorticoides han penetrado pasivamente la membrana celular, se unen a estos receptores que promueven la translocación hacia el núcleo de la célula. Una vez dentro del núcleo, regulan la expresión genética, interactuando con otros factores de transcripción o uniéndose a elementos específicos de respuesta del ADN (Ulrich-Lai y Herman, 2009).

La vía clásica o genómica comprende dos tipos de receptores específicos que se unen a los glucocorticoides: los receptores para mineralcorticoides (MRs) y los receptores para los glucocorticoides (GRs). Los MRs tienen una alta afinidad para los glucocorticoides, mientras que los GRs tienen una afinidad diez veces más baja (Reul y de Kloet, 1985). Esta diferencia parece indicar que los MRs controlan los efectos de los glucocorticoides cuando los niveles son bajos, como se da en condiciones basales. Pero, cuando los niveles de glucocorticoides aumentan, como ante situaciones estresantes, los MRs se saturan y los GRs empiezan a ser ocupados. Por lo tanto, GRs es el

tipo de receptor que probablemente está más implicado en el control de los efectos de los glucocorticoides ante situaciones estresantes, incluyendo el feedback inhibitorio de la actividad del eje HHA (de Kloet et al., 2005). El nivel más alto de expresividad de estas dos clases de receptores, se ha encontrado en las estructuras del cerebro que están más relacionadas con la apreciación cognitiva de una situación estresante, así como altamente relacionadas con el feedback inhibitorio del eje HHA, como son el hipocampo y el córtex prefrontal (Patel et al., 2000).

(ii) La alternativa rápida, es la vía no-clásica o no-genómica, a través de la cual, los glucocorticoides pueden ejercer sus efectos en un margen de segundos a minutos. Estos efectos rápidos incluyen la rápida inhibición del eje HHA, que ocurre en un margen de minutos desde que se produce el aumento del nivel de glucocorticoides en sangre, y además, efectos rápidos de los glucocorticoides en procesos cognitivos como: la evaluación de situaciones nuevas (Oitzl y de Kloet, 1992), procesos de extinción (Rodrigues et al., 2009) y procesos de recuerdo (de Quervain et al., 2009). Estos efectos ocurren demasiado rápido para ser mediados por la vía clásica o genómica (Tasker et al., 2005). El mecanismo exacto a través del que se producen estos rápidos efectos todavía no es conocido, pero las hipótesis apuntan hacia acciones de membrana de los receptores nucleares (MRs y GRs) o a un receptor de membrana que todavía no haya sido identificado (de Kloet et al., 2008; Ulrich-Lai y Herman, 2009).

Finalmente, queda por comentar que existen proteínas que interactúan con los glucocorticoides y juegan un papel importante en la disponibilidad de la hormona para ejercer sus efectos. Entre ellas, quiero destacar la globulina fijadora de corticoesteriodes (CBG) y la enzima 11-β-hidroxiesteroide deshidrogenasa. CBG es una proteína que se une al cortisol con una alta afinidad, y se ocupa de facilitar su transporte en la sangre, proporcionar una reserva de hormona inactiva, y regular la cantidad de hormona libre disponible para su difusión en los diferentes tejidos (Mendel, 1989; Henley y Lightman, 2011). Cuando el cortisol es medido en plasma, normalmente, se obtiene un valor total que es la combinación entre la porción de cortisol inactivo, que circula unido a las proteínas CBG, y la porción activa que es libre, y que

por lo tanto, no está unida a proteínas CBG. Sin embargo, cuando se mide el cortisol en saliva, sólo se mide la porción libre o activa, porque sólo esta porción puede pasar a través de las membranas lipídicas y difundir en fluidos del cuerpo como la saliva (Kirschbaum y Hellhammer, 2000). Por otro lado, la enzima 11-β-hidroxiesteroide deshidrogenasa metaboliza los glucocorticoides tras haber entrado en la célula y, por lo tanto, regula el acceso de los glucocorticoides a sus receptores.

2. Mecanismo a través del cual el eje HHA puede afectar a la capacidad de memoria

El eje HHA tiene funciones cruciales que incrementan nuestra capacidad para sobrevivir ante todo tipo de amenazas. Siendo este el caso, parece contradictorio que el mismo mecanismo que contribuye a nuestra supervivencia, también pueda provocar efectos perjudiciales en nuestro organismo, y pueda estar relacionado con el deterioro cognitivo asociado al envejecimiento. Sin embargo, numerosas investigaciones han demostrado que una exposición prolongada a altos niveles de glucocorticoides debido, por ejemplo, a situaciones de estrés crónico o a enfermedades específicas como la enfermedad de Cushing, tiene efectos perjudiciales para nuestro organismo. De hecho, una alteración en la regulación del eje HHA ha sido asociada a varias condiciones como: la depresión, la diabetes, o incluso, la enfermedad de Alzheimer (McEwen, 2008). Las estructuras cerebrales que estarían más expuestas a estos efectos nocivos serían aquellas que están más implicadas en el control de la actividad del eje, como son el hipocampo y el córtex prefrontal. Por otro lado, estas mismas estructuras tienen una implicación central en diversos procesos de memoria, sobre todo en la memoria declarativa y en la memoria de trabajo (Scoville and Milner, 1957; Galloway et al., 2008). Por lo tanto, si una exposición excesiva a altos niveles de glucocorticoides puede causar daño al hipocampo y al córtex prefrontal, esto se traduciría en problemas de memoria.

Investigaciones realizadas predominantemente en animales no-humanos, han demostrado que, en efecto, el estrés crónico afecta profundamente a la morfología del hipocampo y del córtex prefrontal. En el hipocampo, una exposición prolongada a altos niveles de glucocorticoides inhibe la neurogénesis en el giro dentado (Gould y Tanapat, 1999) y pone en peligro la supervivencia neuronal (Sapolsky, 2000). Además, causa otras alteraciones estructurales como, por ejemplo, atrofia dendrítica (Magarinos et al., 1996), pérdida de sinapsis excitatorias glutamatérgicas (Sandi et al., 2003) y reducción en el área de densidades postsinápticas (Sousa et al., 2000). En el córtex prefrontal medial se han observado alteraciones similares después de una exposición crónica a altos niveles de glucocorticoides, incluyendo atrofia dendrítica (Wellman, 2001) y pérdida de espinas dendríticas (Cerqueira et al., 2005). Todos estos efectos perjudiciales han sido relacionados con la capacidad que tienen los glucocorticoides para aumentar la vulnerabilidad de las neuronas ante amenazas para su supervivencia. Por ejemplo, niveles altos de glucocorticoides a nivel crónico, perjudican el metabolismo de la energía las neuronas, porque exacerban el estado de merma de energía que ellos mismos inducen, y aumentan el estrés oxidativo (Sapolsky et al., 1985). No es sorprendente, que todos estos cambios estructurales en el hipocampo y en el córtex prefrontal estén acompañados por deterioros en la capacidad de memoria (Sousa et al., 2000; Liston et al., 2006).

2.1 *Hipótesis sobre la relación entre el eje HHA y el deterioro de la memoria asociado a la edad*

Se han formulado varias hipótesis para intentar explicar la implicación del eje HHA, en el deterioro de la memoria que se produce como consecuencia de un envejecimiento normal. La primera hipótesis es la hipótesis de la cascada de glucocorticoides (Sapolsky et al., 1986), llamada más tarde como hipótesis de la neurotoxicidad (Gilbertson et al., 2002), que se basa, fundamentalmente, en resultados de estudios

realizados en animales no-humanos. Según esta hipótesis, el deterioro de la memoria que se observa en organismos más viejos es causado por la exposición acumulada a altos niveles de glucocorticoides, a lo largo del ciclo vital del individuo. La elevación en los niveles de glucocorticoides podría haber sido causada por diversos factores, como por ejemplo, episodios de estrés crónico o enfermedades específicas como la depresión. En condiciones normales, ante periodos de estrés excesivo y, consecuentemente, de excesiva secreción de glucocorticoides, se produce una reducción a la baja de sus receptores en el hipocampo. Una vez el episodio estresante ha pasado y, consecuentemente, el aumento en la secreción de glucocorticoides cesa, la regulación a la baja de receptores se autocorrige. Sin embargo, es posible que en un determinado momento, la regulación a la baja de receptores provoque una disminución en la capacidad del hipocampo para inhibir la actividad del eje, conduciendo a una hipersecreción de glucocorticoides más permanente. Esta situación, precipitará más regulación a la baja de receptores, y más hipersecreción de glucocorticoides, en un ciclo que, finalmente, causará daño y muerte neuronal en el hipocampo, que será traducido en problemas cognitivos como el deterioro de la memoria.

Resultados de diversos estudios, realizados predominantemente en ratas, apoyaron esta hipótesis (Sapolsky et al., 1986). Sin embargo, otros estudios, realizados también en ratas o en otras especies, no pudieron demostrar una pérdida de células en el hipocampo, como resultado de la exposición a estrés crónico o a niveles elevados de glucocorticoides (ratas: Bodnoff et al., 1995; Coburn-Litvak et al., 2004; musarañas de árbol: Fuchs et al., 2001; primates no humanos: Leverenz et al., 1999; humanos: Muller et al., 2001). Además, en humanos, la correlación entre el incremento de edad y una actividad elevada del eje HHA pareció ser débil y bastante heterogénea (Lupien et al., 1996; Seeman et al., 1997). Por otro lado, el estudio de pacientes con PTSD reveló que era posible la combinación entre un volumen hipocampal reducido e hiposecreción de glucocorticoides (Meewisse et al., 2007).

Toda la evidencia anterior, condujo a la formulación de la hipótesis de la vulnerabilidad, según la cual debe existir alguna predisposición para sufrir los efectos

nocivos de la exposición a niveles elevados de glucocorticoides. Esta predisposición, sería un factor de riesgo pre-existente para padecer enfermedades relacionadas con el estrés, y podría estar representada por factores genéticos o por la exposición al estrés en etapas tempranas o tardías de nuestro ciclo vital (Charney y Manji, 2004). Es posible que ambas hipótesis no sean excluyentes sino complementarias, puesto que, podría ser, que el impacto negativo del estrés en las estructuras cerebrales sea diferente dependiendo de la etapa del desarrollo en la que nos encontremos, y por lo tanto, este impacto negativo no sea resultado de una exposición acumulada al estrés a lo largo de todo el ciclo vital, sino, a la exposición al estrés durante ventanas específicas de vulnerabilidad a lo largo de nuestro desarrollo. Estas ventanas de vulnerabilidad vendrían representadas especialmente por la infancia, donde el hipocampo, la amígdala y el córtex prefrontal todavía se están desarrollando, y por las etapas más tardías del ciclo vital, que es cuando nuestro cuerpo tiene más dificultades para mantener la homeostasis (Lupien et al., 2009).

3. Cambios asociados al envejecimiento en la actividad del eje HHA y sus correlatos en la capacidad de memoria

Se ha sugerido que el envejecimiento y el estrés comparten procesos etiológicos y fisiopatológicos (Pardon, 2007). De hecho, varios autores han definido el envejecimiento como la pérdida gradual de la habilidad del cuerpo para mantenerse en un estado estable (homeostasis) y de su habilidad para ajustarse a situaciones cambiantes (p. ej. estresores) (Rowe y Kahn, 1987; Masoro, 2005; Pardon, 2007). Este progresivo fracaso en adaptarse a un entorno cambiante, podría estar relacionado con una alteración en el funcionamiento del eje HHA asociada a la edad, ya que, como se ha comentado anteriormente, el eje HHA tiene como función principal mantener y asegurar el regreso al estado de homeostasis después del estrés. De hecho, el envejecimiento provoca cambios en la actividad del eje HHA, y estos cambios han sido identi-

ficados como el mecanismo a través del cual el estrés está implicado en el deterioro cognitivo asociado al envejecimiento (Sapolsky et al., 1986; Lupien et al., 2005; Pardon, 2007).

En los siguientes apartados voy a exponer cuáles son los principales cambios asociados al envejecimiento que se han identificado en la actividad del eje HHA, así como, si estos cambios están relacionados con la capacidad de memoria de las personas mayores. De este modo, voy a explicar el impacto del envejecimiento en (i) los niveles de cortisol basales, en (ii) la reactividad del cortisol frente amenazas o desafíos, y en (iii) la respuesta matutina de cortisol (CAR).

3.1 *Cambios asociados al envejecimiento en los niveles de cortisol basales y sus correlatos la en memoria*

Los resultados de los primeros estudios transversales que compararon los niveles basales de cortisol entre personas jóvenes y mayores, parecían indicar que los niveles basales de cortisol no cambiaban con el incremento de edad en personas sanas (West et al., 1961; Jensen y Blichert-Toft, 1971; Waltman et al., 1991). Sin embargo, algunos estudios mostraron pequeños cambios en el ritmo circadiano de cortisol, como una amplitud reducida y mayores niveles de cortisol por la noche en las personas más mayores (Friedman et al., 1969; Jensen y Blichert-Toft, 1971; Touitou et al., 1982). Por otro lado, estudios realizados en animales no-humanos mostraban un alto grado de controversia, con algunos estudios revelando un aumento en la actividad del eje HHA con el incremento de edad (Tang et al., 1978; Angelucci et al., 1987) y otros mostrando ningún cambio (Lorens et al., 1990; van Eekelen et al., 1991). Curiosamente, los animales envejecidos y con niveles elevados de glucocorticoide basal, tenían peor memoria al compararlos con animales que no aumentaban sus niveles de glucocorticoide basal a través del tiempo (Issa et al., 1990).

En conjunto, estos primeros estudios parecían indicar que el envejecimiento no tenía un impacto importante en la actividad del eje HHA, sino que, más bien, dicho impacto podría ser heterogéneo. En este punto, estudios longitudinales podían arrojar más luz en este asunto, a través de medir los niveles de cortisol en los mismos individuos a medida que envejecían. Así, Lupien et al. (1996) en un estudio longitudinal de entre 3 y 6 años de seguimiento encontraron que, de hecho, había una gran heterogeneidad en los cambios que ocurren en el eje HHA a través de tiempo. Encontraron evidencia para tres subgrupos de personas mayores: el primer grupo mostró un incremento progresivo en los niveles de cortisol basal a través de los años y mantenía niveles altos al final del estudio (38% de la muestra), el segundo grupo mostró un incremento progresivo a través de los años y mantenía niveles moderados al final del estudio (47% de la muestra), y el tercer grupo mostró una disminución progresiva y mantenía niveles moderados al final del estudio (15%). Además, resulta interesante que, el grupo que mostró un aumento en sus niveles de cortisol a través del tiempo y que mantenía niveles altos al final del estudio, presentaba también, déficits de memoria y una reducción en el volumen hipocampal del 14%. Del mismo modo, Seeman et al. (1997) en un estudio longitudinal de 2.5 años de seguimiento encontraron un aumento en los niveles de cortisol basal en el 20% - 26% de la muestra, y una disminución en el 34% - 41 %. Además, curiosamente, sólo entre las mujeres, el aumento en la secreción de cortisol estuvo asociado con una disminución en su capacidad de memoria. Finalmente, Li et al. (2006) encontraron que, no sólo el aumento en los niveles de cortisol basal en un periodo de 3 años se asociaba con una disminución en el rendimiento de la memoria, sino que, además, niveles más elevados de cortisol al inicio del estudio predecían deterioros en la memoria en los años subsiguientes.

A modo de conclusión, parece que el envejecimiento tiene un efecto en la actividad basal del eje HHA. Sin embargo, este efecto no es universal, sino que existe una gran heterogeneidad entre individuos, con algunos mostrando aumentos en su niveles de cortisol basal a través del tiempo y otros mostrando reducciones. Además, el aumento en los niveles basales de cortisol a través del tiempo está asociado con un

deterioro de la memoria. Finalmente, a pesar de que Lupien et al. (1996) y Li et al. (2006) no evaluaron efectos de sexo, Seeman et al. (1997) encontraron que el sexo podría ser un factor moderador importante en la relación entre el incremento de cortisol a través del tiempo y el deterioro de la memoria.

3.2 *Cambios asociados al envejecimiento en la reactividad del cortisol y sus correlatos en la memoria*

El estudio del impacto del envejecimiento en la reactividad del eje HHA ante amenazas o desafíos ha recibido mucha atención, porque es crucial para poder contestar a la pregunta de si cuando envejecemos, perdemos progresivamente nuestra capacidad para adaptarnos a las condiciones de un entorno cambiante y para mantener la homeostasis. Dos aproximaciones diferentes se han utilizado para estudiar este tema. Así, se ha estudiado el impacto del envejecimiento sobre la reactividad del eje HHA ante (i) la administración de sustancias farmacológicas y (ii) ante diferentes tipos de estresores (p. ej. estresores psicosociales de laboratorio o procedimientos cognitivos).

3.2.1 *Cambios asociados al envejecimiento en la reactividad del cortisol a la administración de sustancias farmacológicas y sus correlatos en la memoria*

El uso de sustancias farmacológicas para estudiar la actividad del eje HHA ha permitido una mejor comprensión de los cambios fisiológicos que, por ejemplo, pueden estar detrás del incremento de los niveles de cortisol basal en el envejecimiento. Varios tests farmacológicos se han desarrollado para poder evaluar la actividad del eje a través de estimular o inhibir la liberación de cortisol. Entre las pruebas que se centran en la estimulación del eje HHA, el test del CRH (a veces acompañado por va-

sopresina) mide la sensibilidad de la hipófisis para producir ACTH, a través de la administración de CRH sintético o extraído, e indirectamente, también mide la sensibilidad de la glándula adrenal para producir cortisol. De modo similar, el test del ACTH evalúa la sensibilidad de la glándula adrenal para producir cortisol, a través de la administración de ACTH sintético o extraído. La mayoría de los estudios que han utilizado el test del CRH, han encontrado una liberación de ACTH y cortisol más elevada en personas mayores que en personas jóvenes (Pavlov et al., 1986; Heuser et al., 1994; Luisi et al., 1998; Kudielka et al., 1999), con algunos estudios apuntando hacia un efecto de edad más grande en las mujeres que en los hombres (Greenspan et al.,1993; Heuser et al., 1994). De forma contraria, la respuesta adrenal al ACTH no varía con el envejecimiento (Vermeulen et al., 1982; Ohashi et al., 1986b; Rasmuson et al., 1998; Martinez-Taboada et al., 2002).

Para explicar esta reactividad elevada de las personas mayores al test del CRH, se ha sugerido que, con el envejecimiento, se produce una reducción en la sensibilidad del feedback inhibitorio del eje HHA. Para estudiar esta hipótesis, la capacidad inhibitoria del eje HHA ha sido comparada entre individuos jóvenes y mayores. Estos estudios implican la utilización de sustancias que tienen el potencial de inhibir la liberación de cortisol, como, por ejemplo, la dexametasona o el propio cortisol. Una vez estas sustancias son administradas, se miden sus efectos en la liberación de ACTH y de cortisol. Los resultados de estos estudios, indican que los individuos más mayores presentan una reducción en la inhibición de ACTH y cortisol, si los comparamos con individuos más jóvenes (Wilkinson et al., 1997; Kudielka et al., 1999; Wolf et al., 2002). Esta reducción en la capacidad inhibitoria del eje HHA, ha sido atribuida a una reducción en la sensibilidad de los receptores para glucocorticoides (MRs y GRs), en el hipocampo de las personas mayores (Wilkinson et al., 1997; Otte et al., 2003).

A modo de conclusión, las personas mayores muestran una reactividad aumentada del eje HHA ante la activación farmacológica, y este incremento en la respuesta se ha relacionado, particularmente, con un descenso en la capacidad de feedback inhibitorio. Además, el sexo podría ser un factor moderador, hasta el punto

de que se ha sugerido que el impacto del envejecimiento en la reactividad del eje HHA podría ser tres veces más fuerte en las mujeres que en los hombres (Otte et al., 2005).

Además de confirmar la existencia de cambios relacionados con el envejecimiento en el feedback inhibitorio del eje HHA, el uso de sustancias farmacológicas para aumentar los niveles de glucocorticoides, ha demostrado que cambios agudos en los niveles de los mismos modulan, también de forma aguda, la capacidad de memoria. De este modo, diferentes dosis de, por ejemplo, dexametasona o prednisona (ambos son agonistas de los glucocorticoides), demostraron que podían provocar un descenso de la capacidad de memoria (Wolkowitz et al., 1990; Newcomer et al., 1994). Posteriormente, se han hecho distinciones entre los efectos que provocan los glucocorticoides, en las distintas fases del proceso memorístico (p. ej. consolidación y recuerdo) y, también, en los diferentes tipos de memoria (p. ej. memoria declarativa y de trabajo). Así, niveles altos de glucocorticoides mejoran la consolidación pero perjudican el recuerdo (para más información ver: Roozendaal et al., 2002), y afectan a tipos de memoria en los que el hipocampo y el córtex prefrontal juegan un papel fundamental, como es el caso de la memoria declarativa y de trabajo, pero no a otros tipos de memoria como la memoria procedimental (Kirschbaum et al., 1996; Lupien et al., 1999).

La mayoría de estudios que han investigado la modulación aguda de la memoria a través de incrementos farmacológicos de los niveles de glucocorticoides, han sido realizados con muestras de personas jóvenes, y pocos estudios han incluido a personas más mayores. Además, de entre los estudios que han investigado a personas mayores, la mayoría solo incluyó hombres en sus muestras (Lupien et al., 2002; Wolf et al., 2001). A pesar de ello, los resultados muestran que la capacidad de memoria de las personas mayores también es vulnerable a aumentos agudos en los niveles de glucocorticoides (Lupien et al., 2002). Sin embargo, existe la posibilidad de que las personas mayores sean menos sensibles a los efectos agudos de los glucocorticoides sobre la memoria de trabajo (Newcomer et al., 1995; Wolf et al., 2001), efecto que

ha sido explicado por una posible disminución en la sensibilidad del córtex prefrontal a niveles elevados de glucocorticoides (Wolf et al., 2001).

Los estudios farmacológicos han demostrado ser muy útiles para investigar las funciones específicas de los glucocorticoides, pero también tienen sus limitaciones. La limitación más importante es que hay diferencias neuroendocrinas importantes, entre las elevaciones de glucocorticoides inducidas farmacológicamente, y las elevaciones de glucocorticoides provocadas por el estrés (Lupien y Schramek, 2006). Tal y como se ha mencionado anteriormente, la actividad del eje HHA es dinámica, e implica una cascada de acontecimientos que, al final, conduce a la liberación de glucocorticoides. Sin embargo, si el aumento de glucocorticoides es inducido artificialmente, todo el conjunto de acontecimientos neuroendocrinos que normalmente ocurren antes de la liberación de glucocorticoides, es obviado y, en algunos casos, puede ser incluso opuesto a lo que normalmente ocurriría tras una activación natural del eje HHA. De hecho, una situación estresante provoca el aumento de CRH y ACTH, mientras que la administración exógena de glucocorticoides suprime la liberación de CRH y ACTH y, por lo tanto, disminuye los niveles circulantes de ambos. Por otro lado, hay diferencias importantes en las afinidades de los receptores entre los glucocorticoides endógenos y los sintéticos, hecho que limita la información derivada de estos estudios a la activación del receptor específico implicado (Raison y Miller, 2003). Por ejemplo, la dexametasona se une con gran afinidad a los receptores GRs mientras que el cortisol endógeno tiene mucha más afinidad por los receptores MRs y menos por los GRs. Finalmente, el estrés no es sólo el aumento de glucocorticoides, ya que con el estrés se producen muchos otros cambios psicológicos y fisiológicos que no ocurren con la administración exógena de glucocorticoides, incluyendo cambios de humor o activación autónoma, que pueden afectar también a la función cognitiva.

3.2.2 *Cambios asociados al envejecimiento en la reactividad del cortisol al estrés y sus correlatos en la memoria*

Para superar las limitaciones anteriores, y estudiar el estrés dentro de un contexto más válido ecológicamente, se han desarrollado varios procedimientos de laboratorio, con la finalidad de provocar respuestas psicológicas y fisiológicas de estrés consistentes. Estos procedimientos están dirigidos a activar el eje HHA y el SNA, e incluyen toda una serie de tareas estresantes: tareas de hablar en público, tareas cognitivas (p. ej. operaciones aritméticas, tareas de vigilancia o de tiempo de reacción), procedimientos para inducir emociones, tareas de exposición al ruido y estresores más físicos como el test de presión por frío (CPT: cold pressor test). Entre ellos, el procedimiento que ha demostrado provocar de forma más consistente respuestas endocrinas, cardiovasculares, inmunológicas y psicológicas (Kudielka et al., 2007), así como provocar el mayor incremento en la secreción de cortisol es la combinación entre una tarea de hablar en público y una tarea cognitiva (Dickerson y Kemeny, 2004).

Los estudios que han utilizado procedimientos estresantes de laboratorio, han encontrado, consistentemente, diferencias de sexo en la respuesta de cortisol en saliva al estrés. Así, las respuestas de cortisol inducidas por estrés son hasta dos veces más altas en los hombres que en las mujeres (para revisiones ver: Kajantie y Phillips, 2006; Kudielka et al., 2009). El mismo efecto del sexo puede observarse en muestras jóvenes que en muestras más mayores (Steptoe et al., 1996; Nicolson et al., 1997; Kudielka et al., 2004b; Strahler et al., 2010b). Además, en las mujeres, la fase del ciclo menstrual también tiene un efecto en la respuesta del cortisol en saliva al estrés. Por ello, las mujeres que están en la fase lutеínica tienen una respuesta de cortisol similar a la de los hombres, mientras que las mujeres en fase folicular, ovulatoria, que están tomando anticonceptivos orales o son posmenopáusicas, tienen respuestas más bajas (Kirschbaum et al., 1999; Rohleder et al., 2003; Strahler et al., 2010b). La cantidad circulante de CBG ha sido señalada como un factor importante relacionado con estas diferencias (Kudielka y Kirschbaum, 2005). Dado que, tanto las mujeres que toman an-

ticonceptivos orales, como las mujeres posmenopáusicas, tienen concentraciones más elevadas de CBG (Wiegratz et al., 2003; Kudielka et al., 2004b). Cuando las concentraciones de cortisol se miden en saliva, están reflejando la porción libre de cortisol (no unido a CBG), por lo tanto, la respuesta de cortisol reducida que se observa en estas mujeres podría ser explicada, al menos en parte, por las elevadas concentraciones de CBG, que dejarían a una porción más grande del cortisol liberado, unido a proteínas CBG e inactivo.

Los estudios que investigan el impacto del envejecimiento en la reactividad del cortisol al estrés son muy escasos, y los resultados no son concluyentes (Kudielka et al., 2009). Así, mientras que algunos estudios no han encontrado diferencias entre personas jóvenes y mayores (Kudielka et al., 1999, 2000; Nicolson et al., 1997; Rohleder et al., 2002), otros han encontrado una mayor respuesta de cortisol en las personas mayores (Kudielka et al., 2004b; Seeman et al., 2001; Traustadóttir et al., 2005; Strahler et al., 2010b;). Además, de forma similar a los estudios que han investigado diferencias de edad en la reactividad del cortisol a la administración farmacológica, los resultados indican que el sexo podría moderar el impacto del envejecimiento sobre la respuesta de cortisol al estrés. Sin embargo, en el caso de la respuesta del cortisol a estresores de laboratorio, los estudios sugieren que serían los hombres, y no las mujeres, los que tendrían una respuesta aumentada al estrés al compararlos con personas más jóvenes (Kudielka et al., 2004b; Strahler et al., 2010b).

Aparte de los efectos del envejecimiento en la respuesta del eje HHA a estresores agudos, también es importante mencionar que el envejecimiento también podría tener un impacto en la respuesta del SNA al estrés, que podría estar relacionado, por ejemplo, con la exacerbación de enfermedades cardiovasculares que se produce con el incremento de edad. Hay cierto consenso en que, bajo condiciones normales, las personas mayores presentan una elevación en la actividad simpática basal (para una revisión ver: Seals y Dinenno, 2004). Sin embargo, los resultados de los estudios que han investigado si el envejecimiento influye en la respuesta del SNA al estrés son contradictorios. Así, mientras varios estudios no han encontrado ninguna diferencia

en la respuesta del SNA entre personas jóvenes y mayores (Esler et al., 1995; Wood et al., 2002), otros han encontrado un descenso en la respuesta de las personas mayores (Kudielka et al., 2004a; Strahler et al., 2010b), e incluso otros una respuesta incrementada (Pascualy et al., 1999; Uchino et al., 1999).

Como conclusión, el uso de procedimientos de laboratorio ha permitido un estudio más completo de la respuesta de estrés. Aun así, los resultados de los estudios que investigan el impacto del envejecimiento en la respuesta de estrés, tanto a nivel del eje HHA como del SNA, todavía no son concluyentes. Por ello, el primer estudio de esta tesis está dirigido a profundizar en este tema, a través de la comparación de la respuesta del eje HHA y del SNA al estrés, entre personas jóvenes y mayores de ambos sexos.

Tal y como se expuso anteriormente, los estudios de administración farmacológica de glucocorticoides han demostrado que éstos pueden modular de forma aguda la capacidad de la memoria. De la misma forma, se ha investigado si la respuesta de estrés a procedimientos de laboratorio también tiene un impacto agudo en la capacidad de memoria. La mayoría de estos estudios han investigado personas jóvenes, y se han centrado principalmente en la capacidad de la memoria declarativa. Los resultados son contradictorios, porque cuando el estrés fue provocado antes del aprendizaje, algunos estudios no encontraron ningún efecto en el recuerdo inmediato (Elzinga et al. 2005; Hidalgo et al., 2011), otros encontraron efectos perjudiciales (Kirschbaum et al., 1996; Jelicic et al. 2004; Payne et al. 2006; Smeets et al. 2006) y otros, incluso, una mejora en la capacidad de memoria (Smeets et al. 2007; Schwabe et al. 2008). Esta inconsistencia puede deberse, al menos en parte, a la existencia de una relación en forma de U invertida entre los niveles de cortisol y la capacidad de memoria (para revisiones ver: Lupien y McEwen, 1997; de Kloet et al., 1999; Lupien et al., 2007). Según esta teoría, efectos de mejora en la capacidad de memoria se pueden observar bajo concentraciones moderadas de cortisol, que es cuando los receptores MRs están saturados y los receptores GRs se encuentran parcialmente ocu-

pados. Sin embargo, cuando los niveles de cortisol son demasiado bajos o demasiado altos, la capacidad de memoria se vería perjudicada.

Si estos mismos efectos agudos del estrés sobre la memoria pueden observarse en hombres y mujeres mayores es desconocido, puesto que solo unos pocos estudios han sido publicados y los resultados no son concluyentes. Así, dos estudios realizados con una muestra formada solo por mujeres mayores encontraron que, la exposición aguda al estrés antes del aprendizaje, no tuvo ningún efecto en el rendimiento de la memoria declarativa (Bohnen et al., 1990; et al de Domos., 2002). Sin embargo, Lupien et al. (1997) encontraron que el estrés agudo perjudicó el rendimiento de la memoria declarativa en una muestra compuesta por hombres y mujeres. Desafortunadamente, la muestra de este último estudio era demasiado pequeña (solo 7 hombres y 7 mujeres) para poder investigar posibles efectos de sexo. Pero, estos resultados sugieren que el sexo podría ser un moderador del impacto del estrés agudo sobre el rendimiento de la memoria de las personas mayores. El segundo estudio de esta tesis está dirigido a investigar si la capacidad de la memoria de las personas mayores es vulnerable a los efectos agudos del estrés, y si hay diferencias de sexo en esta vulnerabilidad.

Finalmente, desde mi conocimiento, ningún estudio ha investigado si la magnitud de la respuesta de cortisol al estrés agudo está relacionada con la capacidad de memoria de las personas mayores, cuando la memoria es medida en condiciones no estresantes, es decir, a la capacidad de memoria basal. Sin embargo, sí existe cierta evidencia preliminar en un estudio que investigaba el impacto agudo del estrés sobre la memoria, y que encontró que los participantes que respondieron a la situación estresante con incrementos de cortisol tenían peor capacidad de memoria que los que no incrementaron su secreción de cortisol, pero no sólo después de la situación estresante sino también antes de haber sido expuestos a la misma (Lupien et al. 1997). Esto sugiere que la magnitud de la respuesta de cortisol inducida por estrés, podría ser una medida del estado y de la flexibilidad del eje HHA, que estaría relacionada

con las diferencias individuales observadas en la capacidad de memoria basal de las personas mayores. Este asunto va a ser abordado en el tercer estudio de esta tesis.

3.3 *Cambios asociados al envejecimiento en la respuesta matutina de cortisol y sus correlatos en la memoria*

El CAR es un periodo corto de incremento en la secreción de cortisol, que se inicia después de despertar por la mañana y que alcanza valores máximos, típicamente, entre 30 y 45 minutos después de despertar (Pruessner et al., 1997; para revisiones sobre el CAR ver: Fries et al., 2009; Clow et al., 2010a y b). Es un fenómeno complejo que todavía no está completamente entendido. El CAR parece estar controlado de forma independiente a la liberación de cortisol que se produce durante el resto del día, porque, a pesar de que la cantidad de cortisol total segregada después de despertar, está relacionada con la cantidad de cortisol total que se segrega durante el resto del día, la dinámica del CAR, es decir, el aumento total, no lo está (Edwards et al., 2001). Además, la función principal del CAR todavía es desconocida, pero se ha sugerido que podría estar relacionada con la transición del sueño a la vigilia y con el restablecimiento de la consciencia, así como que podría jugar un papel en la sincronización del cuerpo a los ciclos de sueño-vigilia y luz-oscuridad (Clow et al., 2010a). Consecuentemente, parece que el CAR está controlado por conexiones neuronales diferentes a la activación del eje HHA bajo otras circunstancias. Así, parece que el núcleo supraquiasmático del hipotálamo y el SNS jueguen un papel más importante en el control del CAR (Buijs et al., 2003; Clow et al., 2010). Además, parece que la influencia del hipocampo en el control del CAR es opuesta a la función que normalmente tiene en el control del eje HHA durante el resto del día. Así, es posible que la función del hipocampo en el CAR sea permisiva, en lugar de la función inhibitoria que ejerce durante el resto del día. Esta hipótesis ha sido derivada de estudios con pacientes que presentan lesiones en el hipocampo y no muestran CAR (Buchanan et al.,

2004; Wolf et al., 2005). Mucha investigación es todavía necesaria para poder entender completamente la función, el control y la regulación del CAR.

En la misma línea, la información disponible sobre el impacto del envejecimiento en el CAR es muy escasa y no concluyente. Mientras dos estudios no encontraron ningún efecto del envejecimiento sobre el CAR (Pruessner et al., 1997; Wust et al., 2000), un estudio encontró que el incremento de edad se asociaba con un CAR más bajo (Kudielka y Kirschbaum, 2003) y finalmente, un cuarto estudio encontró que, en las personas mayores, se podía distinguir entre dos perfiles de CAR: un grupo con un CAR normal (prevalencia del 73%) y un grupo con un CAR más elevado, que presentaba además, mayor cantidad total de secreción de cortisol durante el resto del día, pero con un patrón de secreción plano (27%) (Kumari et al., 2010). Es posible que, tal y como ocurre con el impacto del envejecimiento sobre los niveles de cortisol basales, el impacto del envejecimiento sobre el CAR no sea universal, sino que existan grandes diferencias individuales. Finalmente, parece que el sexo no tiene un impacto importante en el CAR (Fries et al., 2009).

Hasta la fecha, y según mi conocimiento, sólo dos estudios publicados han investigado si hay una relación entre la magnitud del CAR y la capacidad de memoria de personas mayores. Franz et al. (2011) sugirieron que el CAR no parecía contribuir de forma importante a la asociación entre la actividad del eje HHA y rendimiento de la memoria de personas mayores. Sin embargo, Evans et al. (2011) encontraron que un CAR más elevado estuvo asociado con un mejor rendimiento en pruebas de memoria dependientes, principalmente, del funcionamiento del córtex prefrontal (p. ej. memoria trabajo y fluencia verbal). Sin embargo, esta relación era pequeña y desaparecía después de controlar por la edad de los participantes. En mi opinión, el CAR es una faceta claramente diferenciada de la actividad del eje HHA, y es interesante seguir investigando si está relacionada con las diferencias individuales en la capacidad de memoria de las personas mayores. Por ello, el cuarto estudio de esta tesis está dirigido a investigar si el CAR está relacionado con la memoria declarativa y de trabajo de hombres y mujeres mayores.

4. Objetivos e Hipótesis

El resumen de la literatura disponible, que se ha presentado hasta este momento, ha demostrado que la actividad del eje HHA puede estar relacionada con las diferencias individuales en la capacidad de memoria de las personas mayores sanas. Pero, lo que también ha demostrado, es que hay escasez de estudios y que, en muchos casos, los resultados publicados son contradictorios. Para aportar más información, diseñé y realicé cuatro estudios. A continuación voy a detallar cuáles fueron los objetivos e hipótesis de cada uno de ellos.

Estudio 1: El objetivo de este estudio fue investigar el impacto del envejecimiento en la respuesta aguda del eje HHA y del SNS a una situación de estrés psicosocial. La actividad del eje HHA se evaluó a través de la medida de la respuesta de cortisol en saliva. La actividad del SNS se evaluó a través de la medida de la respuesta del alfa amilasa salival (sAA) y la frecuencia cardiaca (FC) al estrés. Siguiendo los resultados de estudios anteriores que también utilizaron un estresor psicosocial, nuestra hipótesis fue que las personas mayores mostrarían una respuesta de sAA y FC atenuada. Debido a los resultados contradictorios en la literatura sobre el efecto del envejecimiento en la respuesta de cortisol al estrés, exploré si ésta fue diferente entre el grupo joven y mayor.

Estudio 2: El objetivo de este estudio fue investigar, en personas mayores, si el sexo modera la respuesta del eje HHA a un estresor agudo de tipo psicosocial, y si esta respuesta tiene un impacto agudo sobre su capacidad de memoria. Las hipótesis fueron que la respuesta de cortisol de los hombres sería mayor que la de las mujeres, y que el impacto agudo del estrés sobre la memoria sería diferente entre hombres y mujeres.

Estudio 3: El objetivo de este estudio fue investigar si la magnitud de la respuesta del eje HHA al estrés agudo, está relacionada con la capacidad de memoria de las personas mayores, cuando la memoria es medida en condiciones no estresantes. Además, también investigué si la magnitud de la respuesta estaba relacionada con los niveles de autoestima, locus de control y autoeficacia de los participantes. La hipótesis fue que una respuesta de cortisol más elevada estaría relacionada con una peor capacidad de memoria.

Estudio 4: El objetivo de este estudio fue investigar si, en las personas mayores, las diferencias individuales en el CAR están relacionadas con las diferencias individuales en las capacidades de memoria. Dado que este tema has sido investigado muy poco y que los resultados son contradictorios, no elaboré una hipótesis con una dirección específica.

Spanish translation of Chapter 6

Capítulo 6

Discusión de los hallazgos principales

Los estudios presentados en los capítulos anteriores han demostrado que la actividad del eje HHA está relacionada con la capacidad de memoria de las personas mayores. Además, estos estudios demuestran que esta relación no se limita a una influencia aguda de los glucocorticoides sobre la memoria, sino que se trata de una relación más permanente. Estos resultados identifican al eje HHA como un factor crucial, implicado en las diferencias individuales que se observan en la capacidad de memoria de las personas mayores. Lo que ahora sigue es una corta descripción de los capítulos empíricos, de sus principales conclusiones, y una reflexión global de sus implicaciones.

1. Resumen de los hallazgos principales:

1.1 *Estudio 1*

Estudios anteriores han demostrado que el envejecimiento está asociado con cambios psicobiológicos que limitan nuestra capacidad para afrontar el estrés. El objetivo de este estudio fue clarificar qué cambios, asociados al envejecimiento, ocurren en los principales mecanismos fisiológicos que forman parte de la respuesta de estrés. Para ello, se investigó el impacto del envejecimiento en la respuesta de eje HHA y del SNA a un estresor agudo de tipo psicosocial. La actividad del eje HHA se midió a través del cortisol en saliva, y la actividad del SNA a través de la FC y de la liberación de sAA, que es un nuevo marcador de la actividad del SNA. Sesenta y dos participantes se dividieron en dos grupos de edad (grupo joven: N = 31, rango de edad: 18-35 años; grupo mayor: N = 31, rango de edad: 54-71 años) y fueron expuestos al TSST y a una condición control en un diseño intra-sujeto. El grupo de personas mayores tuvo una respuesta de cortisol al estrés más elevada, y un descenso de cortisol en la condición control menor, que el grupo de personas jóvenes. No se encontraron diferencias de edad en la reactividad de la FC o del sAA al estrés. Sin embargo, la

producción total de sAA fue más alta en el grupo mayor que en el joven. Además, en la condición estresante, la cantidad total de cortisol liberada, se relacionó positivamente con la cantidad total de sAA, mientras que el aumento de la FC se relacionó positivamente con el aumento de sAA. Estos resultados apoyan la existencia de un descenso en la capacidad de feedback negativo del eje HHA en las personas mayores. Sin embargo, no apoyan la existencia de una respuesta atenuada del SNA al estrés en edades avanzadas, sino la existencia de una elevación en la actividad simpática basal. Por otro lado, este estudio añade evidencia de la coordinación entre el eje HHA y el SNA en su respuesta al estrés agudo.

1.2 *Estudio 2*

El estrés ha sido identificado como un factor principal implicado en los cambios cognitivos que ocurren durante el proceso de envejecimiento. Este estudio investigó la existencia de diferencias de sexo, en la relación aguda entre la magnitud de la respuesta de cortisol al estrés y la capacidad de memoria de personas mayores sanas. Para ello, 16 hombres y 16 mujeres (54 a 72 años) fueron expuestos al TSST y a una condición control, en un diseño intra-sujeto. Después de la tarea estresante y de la tarea control, su memoria fue evaluada con una prueba estandarizada de memoria (RAVLT). El estrés afectó al rendimiento de la memoria sólo en las mujeres, así como, sólo en las mismas, hubo una relación significativa entre una mayor respuesta de cortisol al estrés y un peor rendimiento de la memoria, pero tanto en la condición estresante como en la condición control. Estos resultados confirman que el sexo es un factor importante en la relación aguda entre el aumento de cortisol y la memoria. Además, sugieren la existencia de una relación entre la magnitud de la respuesta de cortisol al estrés y la capacidad de memoria basal de las mujeres posmenopáusicas.

1.3 *Estudio 3*

El segundo estudio de esta tesis fue diseñado, originalmente, para estudiar los efectos agudos del estrés en la capacidad de memoria de las personas mayores. Sin embargo, los resultados sugirieron, que la magnitud de la respuesta de cortisol al estrés agudo, podría estar relacionada con la capacidad de memoria, también cuándo la memoria ha sido evaluada en condiciones no estresantes. Por otro lado, el sexo podría ser un factor moderador en esta relación. Este tercer estudio fue diseñado para profundizar en estos hallazgos. Para ello, se midió la capacidad de memoria declarativa y de trabajo, de 31 hombres y 35 mujeres de entre 55 y 77 años de edad, en una condición no estresante. En un día diferente, se midió la magnitud de su respuesta de cortisol a un estresor agudo de tipo psicosocial. La relación entre la respuesta de cortisol y la capacidad de memoria fue curvilínea: un incremento moderado de cortisol en respuesta al estrés, estuvo relacionado con una peor capacidad de memoria declarativa y de trabajo, mientras que un incremento alto o nulo se relacionó con un mejor rendimiento en ambos tipos de memoria. El sexo no moderó estas relaciones. Estos resultados sugieren que, una respuesta moderada de cortisol al estrés, podría reflejar un funcionamiento defectuoso del eje HHA en edades avanzadas, que iría acompañado por una peor capacidad de memoria. Por el contrario, una respuesta elevada parece reflejar un buen funcionamiento del eje HHA, y junto con la ausencia de respuesta de cortisol un estresor específico, podría ser un factor de protección contra déficits de memoria en las etapas más tardías de la vida humana.

1.4 *Estudio 4*

Tras haber encontrado que la respuesta aguda de cortisol al estrés está relacionada con la memoria de las personas mayores, en el cuarto estudio investigó si otra faceta de la actividad del eje HHA, el CAR, podría también estar relacionada con

la capacidad de memoria de las personas mayores. Para ello, se reclutó a 88 participantes (44 hombres y 44 mujeres) de 55 a 77 años, y se evaluó su memoria con dos pruebas para medir memoria declarativa (recuerdo de párrafos y lista de palabras) y dos pruebas para medir memoria de trabajo (amplitud de la memoria de trabajo y una prueba de memoria de trabajo espacial). Un CAR elevado estuvo relacionado con peor capacidad de memoria declarativa en hombres y mujeres, y con una mejor memoria de trabajo, pero sólo en los hombres. Estos resultados sugieren que la relación entre el CAR y la memoria es negativa, en hombres y en mujeres, cuando el rendimiento de la memoria depende en gran parte del funcionamiento del hipocampo (memoria declarativa), y es positiva, sólo en hombres, cuando el rendimiento de la memoria depende en gran parte de córtex prefrontal (i.e. memoria de trabajo).

2. Unas notas de cautela

Los hallazgos incluidos en esta tesis tienen que ser interpretados con cautela. A pesar de que en cada estudio se intentó controlar al máximo muchos factores que pudieran influir en los resultados, ningún control es perfecto y, con frecuencia, es posible encontrar explicaciones alternativas a los resultados. Cada capítulo empírico contiene las limitaciones principales de cada estudio. Aquí voy a describir las limitaciones generales que se pueden aplicar a los cuatro estudios, y que tienen que ser tenidas en cuenta a la hora de interpretar y poder generalizar los resultados adecuadamente. Todos los participantes de los estudios eran personas sanas, y muchos voluntarios fueron excluidos debido a que no cumplían con unos criterios de exclusión muy restrictivos. Estos criterios de exclusión se centraron, principalmente, en el estado de salud y la toma medicación, con el objetivo de evitar un gran número de enfermedades y medicaciones, que podían interferir con los objetivos y resultados de cada estudio. Sin embargo, la incidencia de enfermedades, agudas y crónicas, y la toma de medicaciones aumenta con el envejecimiento. Por otro lado, la muestra de

cada estudio estuvo compuesta por personas que acudían a clases universitarias para personas mayores de 50 años. Esto puede haber incluido un sesgo en las muestras, sobre todo, en relación con el nivel socioeconómico y educativo, y también, porque las muestras podrían incluir a más personas dentro del grupo denominado de "envejecimiento activo", que está formado por aquellas personas que optimizan sus oportunidades de salud, participación y seguridad para mejorar su calidad de vida. Por estas razones, los resultados de esta tesis no pueden ser generalizados a otros tipos de muestras, y es recomendable, replicar estos estudios para extender los resultados a otros tipos de poblaciones, incluyendo a personas que toman medicación y que tienen enfermedades relacionadas con el envejecimiento.

3. Conclusiones

Los resultados de los estudios de esta tesis subrayan el hecho de que el funcionamiento del eje HHA está relacionado con las diferencias individuales en la capacidad de memoria de las personas mayores. En mi opinión, los resultados encontrados han añadido nueva evidencia a la literatura existente. A continuación voy a detallar cuáles han sido los hallazgos más importantes de esta tesis:

- Las personas mayores reaccionan con mayores incrementos de cortisol en respuesta al estrés agudo que las personas más jóvenes. Además, el descenso en las concentraciones de cortisol, en condiciones no estresantes, es menor en las personas mayores que en las jóvenes. Estos resultados son compatibles con una alteración en la sensibilidad del feedback negativo del el eje HHA (Seeman y Robbins, 1994).
- El envejecimiento está asociado con una liberación de sAA más elevada. Sin embargo, la respuesta del sAA al estrés agudo no es diferente entre personas jóvenes y mayores. Estos resultados apoyan la existencia de una elevación en

el tono simpatoneural basal en las personas mayores, sin diferencias de edad en la respuesta al estrés.

- Los hombres, con independencia de la edad, reaccionan con mayores aumentos de cortisol al estrés que las mujeres, aunque este efecto del sexo puede estar moderado por las características del estresor.
- Las mujeres mayores presentan una mayor vulnerabilidad a los efectos agudos del estrés sobre su capacidad memoria que los hombres mayores. Así, sólo en las mujeres mayores, el estrés mejoró de forma aguda la atención pero perjudicó los procesos ejecutivos de la memoria de trabajo.
- En las personas mayores la capacidad de memoria declarativa y de trabajo basal está relacionada con la magnitud de su respuesta de cortisol al estrés. Esta relación es en forma de U: una respuesta moderada está relacionada con una peor capacidad de memoria, y una respuesta alta o nula está relacionada con una mejor capacidad de memoria.
- En las personas mayores la magnitud del CAR se asocia con su rendimiento en memoria. En ambos sexos, un CAR más elevado está relacionado con un peor rendimiento en tests de memoria declarativa, y, sólo en los hombres, con un mejor rendimiento en tests de memoria de trabajo.

En conjunto, estos hallazgos confirman que la relación entre el funcionamiento del eje HHA y la capacidad de memoria de las personas mayores es más compleja de lo esperado. No siempre es cierto que a más secreción de cortisol peor memoria. En cambio, la relación entre la actividad del eje HHA y la capacidad de memoria depende de qué indicador de la actividad del eje HHA está siendo evaluado.

4. Perspectivas de Futuro

Como la mayoría de estudios científicos, los estudios que componen esta tesis abren nuevas cuestiones y líneas de investigación. En general, estos estudios han medido la producción periférica de cortisol, y ésta ha sido relacionada con el funcionamiento de la memoria. Una cuestión importante que queda por resolver, es cómo esta relación se traduce a nivel del funcionamiento cerebral central, es decir, cuáles son los mecanismos específicos a través de los que se establecen las relaciones encontradas entre el eje HHA y la memoria de las personas mayores. Por otro lado, sería interesante conocer si estas relaciones, que han sido observadas en un momento determinado del ciclo vital de las personas, pueden estar relacionadas también con el deterioro cognitivo que se produce a través del tiempo. Para investigar esta cuestión serían necesarios estudios longitudinales.

Además, sería interesante encontrar nuevas variables relacionadas con la actividad del eje HHA. Por ejemplo, en esta tesis se ha encontrado que los niveles de autoeficacia general están relacionados con la respuesta aguda del cortisol al estrés. Sería interesante encontrar otras variables importantes, para poder identificar posibles dianas de intervención y así, en el futuro, poder trabajar para prevenir el deterioro cognitivo asociado al envejecimiento. Finalmente, los resultados de esta tesis recomiendan la exploración de relaciones curvilíneas en futuros estudios que investiguen la relación entre el eje HHA y la memoria.