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Psychophysiological stress response and coping styles to an acute laboratory stressor in healthy adults

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Presented by:
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Respuesta psicofisiológica y estilos de afrontamiento a un estresor agudo de laboratorio en adultos sanos

Carolina Villada Rodríguez

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Dissertation outline

The stress response is an essential phenomenon in life to maintain health and well-being. From an ecological point of view, all species have a survival action mechanism that involves psychophysiological activation to obtain enough energy to escape from a predator or hunt prey. This mechanism is characterized by the activation of physiological systems such as the autonomic nervous system (ANS), the hypothalamus-pituitary-adrenal axis (HPA-axis), and the immune system, among others. In humans, the stress concept involves the same mechanisms, but the main difference from other species is the time spent perceiving stressful life events. In other words, compared to other species that occasionally activate their physiological stress response when they perceive a threat (i.e. fleeing from predators), humans are exposed to psychosocial stressors every day, such as being evaluated by others, increasing or maintaining social status, job pressures, etc. These kinds of stressors do not disappear, and they even increase alertness and, consequently, the amount of time we are psychologically and physiologically activated. This situation has led to an increase in the time spent fighting for survival in today's societies, which would produce negative health consequences, increasing stress-related disorders.

However, it is well known that, faced with the same situation, people may perceive it and respond to it differently, due to individual psychophysiological differences. Among these differences, gender, sex hormones and age seem to play an important role. Therefore, in this thesis I am going to present the results of several studies focused on individual differences in the physiological and behavioral stress responses, focusing on age, sex and/or hormonal status, and personality traits.

The first chapter of this dissertation describes the evolutionary concept of the stress response and the main systems involved. The second part of this chapter encompasses the factors mentioned above, and their interactions are discussed. Hence, I will present a short overview of the previous studies that focus on how these factors may influence the stress response. Finally, the chapter ends with the main objectives and hypotheses that will be developed in the empirical chapters.

Chapters two to five describe the influence of some factors involved in the psychophysiological and behavioral stress response when individuals are presented with an acute psychosocial challenge, such as a public speaking task. Specifically, the second chapter presents an empirical study focused on the influence of sex and personality traits, such as coping styles and trait anxiety, attending to mood changes, cortisol and heart rate responses.

The third chapter includes a study of the role of psychological aspects such as perceived self-efficacy and trait anxiety in the autonomic response and the performance displayed during a stressful situation. The fourth chapter addresses the associations between the psychophysiological stress response and behavior displayed in two groups of women. These groups contain free cycling and oral contraceptive users; furthermore, the chapter describes the influence of coping styles and their interactions with behavior and the stress response. The fifth chapter, the last empirical study in this dissertation, expands on the information from the fourth chapter. It covers the stress response in four groups of women in different phases of the menstrual cycle phase and in menopause. This chapter not only focuses on the stress response, but it also tries to elucidate the factors involved in the capacity to recover from acute social stress.

The sixth chapter discusses the main findings of the empirical studies mentioned above. The strengths and limitations of these studies are also discussed, as well as future directions for the research on this topic.

Finally, the last chapter contains the main conclusions derived from the four studies included in this dissertation.

ABBREVIATIONS:

AUC_G = Area Under the Curve to Ground

AUC_I = Area Under the Curve to Increase

ACTH = Adrenocorticotropic Hormone

ANS = Autonomic Nervous System

BBT = Basal Body Temperature

BMI = Body Mass Index

BPS = Biopsychosocial Model

CRF = Corticotrophin Releasing Factor

ECSI = Ethological Coding System for Interviews

GAS = General Adaptation Syndrome

HR = Heart Rate

H-SE = High Self-efficacy

L-SE = Low Self-efficacy

HRV = Heart Rate Variability

HPA axis = Hypothalamic-Pituitary-Adrenal axis

ISRA = Situations and Responses Anxiety Inventory

NA = Negative Affect

OC users = Oral Contraceptive users

PANAS = Positive and Negative Affect Schedule

PA = Positive Affect

PNS = Parasympathetic Nervous System

PNV = Paraventricular Nucleus

S.D = Standard Deviation

SEM = Standard Error of Means

SNS = Sympathetic Nervous System

SMC = Method of the Smoothed Curve

STAI = State Anxiety Inventory

SES = Subjective Socioeconomic Status

TSST = Trier Social Stress Test

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CHAPTER I
GENERAL INTRODUCTION

1. The adaptive stress response

The stress response is a set of psychological, biological and behavioral mechanisms involved in a wide range of adaptive functions. The psychophysiological mechanisms are activated to maintain the internal balance after the perception of an acute stressful event, that is, a situation that is threatening to our organism. Therefore, the stress response is an adaptive process that involves the activation of cognitive, emotional, physiological and behavioral systems (Campbell and Ehlert, 2012). The main purpose of this activation is to maintain the internal balance, defined as "homeostasis" (Cannon, 1929). Subsequently, Hans Selye introduced this term to refer to the "nonspecific response of the body to any demand made upon it", resulting in a cascade of changes in the nervous, cardiovascular, endocrine, and immune systems. These changes constitute the stress response and are generally adaptive, at least in the short term (Selye 1956). At the same time, *homeostasis* has been directly linked to the General Adaptation Syndrome (GAS). Selye described this term as follows: "I call this syndrome general because it is produced only by agents which have a general effect upon large portions of the body. I call it adaptive because it stimulates defense.… I call it a syndrome because its individual manifestations are coordinated and even partly dependent upon each other." The GAS comprises three stages. The first one is the Alarm reaction, i.e. the immediate reaction to a stressor. This stage is characterized by preparing the body for the fight or flight response, but in return, the immune system decreases its effectiveness. The second one is the stage of resistance, which is also known as the stage of adaptation. During this stage, the body adapts to the stressors to which it is exposed as long as the stressor remains. Finally, the body's reduced resistance to stress characterizes the stage of exhaustion. In this stage, the immune system and the body's ability to resist disease decrease considerably. As the environment is changing continuously, the organism has the capacity to maintain homeostasis during changes (McEwen and Wingfield, 2003, 2010). However, the effort to maintain this balance has a cost, namely allostatic load (McEwen, 1998). It is true that excessive early life and chronic stress can lead to many diseases in the long term, such as depression, anxiety, or cognitive impairments (McEwen, 1999). However, the acute stress response in young healthy individuals is an evolutionary process with effective and clever homeostatic mechanisms to cope with acute stressors that do not entail a health burden (Schneiderman et al., 2005). From its initial definitions until now, stress has been related to several diseases. However, the stress response should be understood as a natural and necessary process that has great importance in promoting the best adaptation to the established environmental demands (Salvador, 2005). Hence, it can be concluded that, to deal with stressors, the physiological stress systems react with the purpose of providing enough resources to maintain the balance (Andrews and Pruessner, 2013).

With all of the above in mind, and in order to further examine the stress response in humans, we have to take into account the great complexity that characterizes humans compared to other species. In humans, the physiological mechanisms involved in the stress response are continuously interacting with environmental demands, but long-term behavioral, physiological, cognitive and social factors contribute to the adaptation to environmental challenges. Moreover, other factors, such as affective, cognitive and personality differences, as well as age, sex/gender and the reproductive hormones in the bloodstream (strongly related to age and gender), should be considered.

2. Mechanisms involved in the stress response

2.1. Psychological components

A number of theories in the scientific literature have emerged to explain how cognitive states may interact with the physiological and behavioral systems. As Cannon described several years ago, the adaptive function of negative emotion is to mobilize physiological resources to cope with threatening conditions in the environment (Cannon, 1932). Likewise, the individual's subjective appraisal of his/her own emotional reactions interacts with cognitive, physiological, and behavioral responses, that is, the way of coping with stress (Carver and Connor-Smith, 2010). Hence, the first step in initiating the stress response is the way we appraise the situation. Subsequently, differences in stress perceptions can affect the biological stress systems (Carver and Connor-Smith, 2010; Dickerson and Kemeny, 2004). In this sense, the concept of Self-efficacy emerges, referring to a persons' perceived ability to successfully execute specific tasks and cope with undesirable situations (Bandura, 1997). Empirical evidence suggests that higher self-efficacy increases one's ability to cope following exposure to stress (Schiaffino & Revenson, 1992).

Along these lines, the biopsychosocial model (BPS) of challenge and threat (Blacovich and Tomaka, 1996) is one of the most complete theories that highlights the relevance of cognitive states (appraisal) and their interactions with the physiological and behavioral stress responses. This model takes into account the way people appraise a stressful task before and after performing it, the physiological response, and the performance displayed. The BPS model differentiates two main appraisals, that is, whether the stressor is perceived as a threat or a challenge. It suggests that the challenge response would be characterized by a pattern of active coping because individuals consider that their personal resources surpass the evaluated demands. By contrast, the threat response would be characterized by a pattern of passive coping, where demands exceed resources. Both patterns (active vs. passive coping) would be associated with physiological activation. Therefore, if individuals perceive a situation as a challenge, they will show efficient autonomic reactivity and better performance, whereas if people perceive a situation as a threat, they will show a pattern of inefficient autonomic reactivity and poor performance.

Thus, it is possible that emotional changes to perceived threats and the associated physiological reactions are achieving the shared objective: coping efficiently with stressful situations. In this process, expectations of being able to cope with these threats play the most important role. Likewise, these expectations will be determined by psychosocial factors and previous experiences, but the main objective of coping is always to reduce the threat itself through action (Ursin and Eriksen, 2004).

2.2. Physiological components

The main stress response systems involve physiological changes in the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA). Alterations in these systems can modulate cognitive and affective processing (de Kloet et al. 2005; Roozendaal et al. 2006; Wolf 2006). Therefore, although the stress response also includes other neuroendocrine changes, we focused only on the ANS and HPA axis activity and on the parameters used to measure their functioning in normal conditions and under stress.

2.2.1. The Autonomic Nervous System (ANS)

The autonomic nervous system (ANS) is the first and one of the most important systems activated in the stress response. The ANS comprises two branches, the Sympathetic (SNS) and Parasympathetic (PNS) nervous systems. The stress signals are integrated by the paraventricular nucleus

(PVN). The PVN interacts with the nucleus of the solitary tract, the vagus nerve, the thoracolumbar spinal cord, and the locus coeruleus (Kyrou and Tsigos, 2009). As a result, an increase in heart rate through the secretion of catecholamines, such as adrenaline from the adrenal medulla and noradrenaline from the sympathetic nerves, is produced.

In conditions of stress, the SNS prepares the body for action (fight-or-flight response, Selye, 1936). Moreover, this autonomic branch has also been known as the catabolic nervous system because its activation leads to an increase in the utilization of many nutrients and hormones to energize the body. By contrast, the PNS is responsible for anabolic processes, digestion, elimination, and regulation of body repair. This branch also has the function of stimulating the immune system during sleep, and it has been related to the release of many key immune hormones and specialized immune messengers.

The most common indicators of these branches are heart rate (HR) and parameters of heart rate variability (HRV). In general, and under normal conditions, increases in HR and decreases in HRV reflect the predominance of sympathetic activity, whereas decreases in HR or HRV increases depict a predominance of parasympathetic activity. HRV parameters can be used as quantitative markers of ANS because they reflect the regulatory mechanisms originating from the sympathetic and parasympathetic nervous systems (Task Force, 1996). The most common time domain measures are those calculated with the means and standard deviations of the RR intervals (e.g., SDNN, RMSSD), which describe the time variations between consecutive heartbeats. More recently, it has been proposed that physiological measures such as HRV indices may provide an index of the degree of adjustment of the behavioral, endocrine, and affective systems involved in the stress response (Thayer, 2012).

2.2.2. The hypothalamus—pituitary—adrenal axis (HPA axis)

Following the SNS activation, the second stress system activated is the hypothalamus-pituitary-adrenal (HPA) axis. At first, the hypothalamus secretes a corticotrophin releasing factor (CRF), which elicits the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the bloodstream. Finally ACTH activates the release of glucocorticoids (cortisol in humans and corticosterone in rats) from the adrenal cortex. Cortisol is the major stress hormone, and its secretion exerts a catabolic effect on different target systems, increasing the availability of energy substrates (Sapolsky et al., 2000). This process facilitates fighting or fleeing, as well as an optimal adaptation to shifting demands from the environment. Moreover, the glucocorticoids distributed in a larger number of the body's cells allow an internal balance (homeostasis), where the HPA axis interacts with other systems involved in the stress response, such as the ANS or immune systems (McEween, 1998).

Compared to ANS, this system is slower, with the cortisol secretion peak appearing between fifteen and thirty minutes from the onset of the stress (Engert et al., 2011). In contrast to the faster SNS response, which finishes some minutes after the stressor onset, the cortisol levels return to baseline concentrations approximately one hour after stress cessation (Kirschbaum and Hellhammer, 1994). The HPA axis has a negative feedback cycle to recover baseline levels, where cortisol acts on the hypothalamus and anterior pituitary to suppress CRH and ACTH production. Therefore, in addition to responding to acute stress efficiently, the loss of the ability to return to basal levels is one of the most relevant indicators of properly functioning stress systems. Likewise, a link has been proposed between slower recovery after stress and negative health consequences (Sapolsky et al., 2000).

2.3. Behavioral components

Behavior is considered the last outcome of the stress response process. Until a few years ago, less attention was paid to the behavioral stress response in humans compared to physiological or emotional reactions. The best-known theory about the behavioral components of the stress response is the fight-or-flight response (Cannon, 1932). This response, as described above, is characterized physiologically by a sympathetic activation that then activates other hormonal secretions (e.g., cortisol in humans). This activation, depending on the nature of the stressor and other environmental and psychological factors, will lead to fight or to flight. Recently, this theory was complemented by the Tend-and-befriend theory, focused on the adaptive stress response in women (Taylor, 2000). Briefly, it tries to explain, based on observational studies in animals and humans, the benefits of affiliative behaviors and the neuroendocrine changes linked to these behaviors in women (e.g., increases in oxytocin), which might improve social interactions as an adaptive stress response in women. Combining both theories, we can develop a better explanation of the stress response in men and women.

Nevertheless, from a behavioral perspective, a new tool has been provided to investigate the human stress response more accurately. Following an ethological approach, Troisi (1999) developed the Ethological Coding System for Interviews (ECSI), taking into account the identification and description of motor patterns associated with the stress response that have a phylogenetic basis. The ECSI includes facial expressions and hand movements, which are grouped in several categories. Each of them reflects a different aspect of the subject's emotional and social attitude (Troisi, 1999), such as assertive, affiliative, submissive, displacement or escape behaviors, among others. Although the first goal of this coding system was to contribute to the understanding of psychiatric disorders, subsequent studies in healthy people corroborate the utility of ethograms to study more in depth the underlying mechanisms of the stress response. To illustrate, research in a young healthy population has shown a positive association between the emotional and physiological stress response and displacement behaviors (a set of behavioral patterns consisting of movements focused on one's own body, such as hand-face touching or scratching) (Mohiyeddini et al., 2013 a,b; Pico-Alfonso et al., 2007; Sgoifo et al., 2003). In addition, behaviors reflecting passive coping styles, such as submission or escape (flight behavior), have been related to greater cardiac response and poorer HPA axis activity (Pico-Alfonso et al., 2007; Sgoifo et al., 2003). Along these lines, previous research emphasized the negative implications of submissive behaviors on HR recovery after social stress (Pico-Alfonso et al., 2007). In conclusion, these studies shed light on behaviors theoretically involved in the stress processes and their link to psychophysiological changes in response to social stress.

3. Individual differences in the stress response

First of all, the literature on factors that interact with the mechanisms involved in the stress response, discussed in this part, focuses on acute stress in laboratory settings. Within the paradigms that include controlled social stressors, public speaking tasks and/or math tasks, the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) is considered the most commonly-used laboratory social stressor in the scientific literature in recent decades. This protocol includes a speaking task followed by an arithmetic task. These combined tasks are capable of provoking a higher stress response than other paradigms (e.g., physical stressors), due to their evaluation and uncontrollability properties (Dickerson & Kemeny, 2004).

3.1. Gender and sexual hormones

In young people, most of the research on acute stress was initially carried out in men, but it has been increasingly accepted that the presence or absence of certain sex hormones plays a role in the response to stress (Kajantie and Phillips, 2006). Being male or female seems to be one of the most important predictors of an individual's health, making the presence of sex differences in stress-related disorders meaningful. For example, many of these disorders have been related to the activation of the HPA axis, which has repeatedly shown sex differences in its functioning, leading to the consideration that the stress response is different in men and women (Kajantie & Phillips, 2006; Kudielka et al., 2009). Generally, men show higher cortisol responses than women (Almela et al., 2011; Childs, et al., 2010, 2014; Cornelisse et al., 2011; Huart et al., 2006; Kirschbaum et al., 1999; Kudielka, et al., 2004a, 2004b), although this sex effect depends on the phase of the menstrual cycle or contraceptive use (Espin et al., 2013; Kajantie & Phillips, 2006). By contrast, higher affective responses have been reported in women than in men (Childs et al., 2010; Kelly et al., 2008; Walder et al., 2012), although other authors failed to find sex differences (Cornelisse et al., 2011; Kirschbaum et al., 1999; Schoofs & Wolf, 2011).

When the cardiac response has been studied using different acute laboratory stressors, conflicting results have been found depending on sex. Some findings have shown greater HR increases in young women compared to young men faced with the Trier Social Stress Test (TSST) (Kudielka et al., 2004b) or a public exam (Fichera & Andreasi, 2000). Others have found no differences between men and women subjected to a mental arithmetic task (Earle, Linden & Weinberg, 1999), a public speaking task (Carrillo et al., 2001; Sgoifo et al., 2003), or the TSST (Kelly et al., 2008), or when groups of women were compared in different phases of the menstrual cycle (Kirschbaum et al, 1999). Moreover, the effects of the menstrual cycle phase on HR response are still controversial. Some studies revealed no significant effects of the menstrual cycle phase on HR and blood pressure reactivity (Gordon and Girdler, 2014; Tersman et al., 1991; Kirschbaum et al., 1999; Pico-Alfonso, et al., 2007) or on HRV to social stress (Pico-Alfonso et al., 2007). However, other studies focused on the menstrual cycle phase have found greater HR reactivity in women in the luteal phase compared to women in the follicular phase (Lustyck et al., 2010; Ossewarde et a., 2010).

Regarding the psychological changes in response to social stress, although it is commonly known that emotional reactions to stressful situations are different in men and women, mixed results have been found in controlled laboratory studies. While higher scores on perceived anxiety or higher negative mood states after stress have been described in women compared to men (Carrillo et al., 2001; Schmaus et al., 2008; Kelly et al., 2008; Childs et al., 2010), others failed to find these sex differences (Kudielka et al., 2004a; Preuss & Wolf, 2009). These heterogeneous results suggest that, as in the physiological response, the affective response could also be influenced by other factors, such as the menstrual cycle phase. For instance, Walder et al. (2012) showed greater state anxiety, anger and hostility in women in the follicular phase compared to men and women in the luteal phase. However, Ossewarde et al. (2010) found that women in the luteal phase had higher sensitivity to stress because they showed greater increases in negative affect compared to women in the late follicular phase. These discrepancies could be due to the moment in the follicular phase: in the first study follicular women were in an earlier stage than in the second study, where women were menstruating or very close to menstruation.

In recent years, studies have begun to investigate the influence of sex and hormonal status on behavior while individuals are performing a speaking task in front of a committee. Although previous studies did not find any effect of reproductive hormones on behavioral strategies to cope with stress (Pico-Alfonso et al., 2007; Sgoifo et al., 2003), more recently a cardiovascular function of displacement behaviors has been found in men, but not in women in the luteal phase (Mohiyeddini et al., 2013a). Additionally, it has been suggested that subjective perceptions of stress may modulate the relationships between behavior and the physiological stress response in women (Mohiyeddini et al., 2013b). In sum, this behavioral perspective of the stress response sheds light on the way the stress response components might interact (psychological, physiological and behavioral).

3.2. Age

The biological response to stress changes across the stages of the lifespan. This could possibly be due to structural and functional changes related to the aging process, such as a decreased muscle mass of the heart and contractibility (Lakatta, 1993) or a dysregulation of HPA axis negative feedback in the older population (Wilkinson et al., 2001). More specifically in women, the hormonal status may influence the capacity of the HPA axis to respond to stress, with this dysregulation being more prominent in women than in men (see meta-analysis: Otte et al., 2005).

Until now, literature focused on the effects of reproductive hormones on several physiological functions has not found important age differences in cortisol and HR capacity to respond to social stress (Kudielka et al., 1999; Hidalgo et al., 2014). When gender differences have been studied in the older population, no differences were found between men and women in the cardiac stress response (Traustadòttir et al., 2003; Kudielka et al., 2004), although a poorer return to baseline levels in women (menopausal) was described, compared to other groups of pre-menopausal women (Almela et al., 2011; Kudielka et al., 2004; Pattachioli et al., 2006). In addition, gender-dependent HRV decreases with aging have been described, with lower values in young women compared to young men, and higher HR decreases with aging in women; however, it seems that these gender differences disappear when individuals are over 50 years old (Umetani et al., 1998). In spite of these mixed results, it can be concluded that the stress response is preserved with age, but not the capacity to recover from stress, especially in women after menopause.

3.3. Trait anxiety and coping strategies

Regarding social stress, personality traits play an important role in the way people perceive it (Connor-Smith and Flachsbart, 2007). At the same time, several psychological disorders have been linked to maladaptive responses to acute stress (Bale 2005, 2006; Nemeroff, 1988). For this reason, personality could act as a risk or protective factor for some stress-related diseases. For instance, Chida and Hamer (2008) concluded in their meta-analysis that positive psychological states or traits seem to be associated with HPA hyporeactivity. Moreover, they indicated that greater cardiovascular stress response is related to hostility, aggression, or Type-A behavior, whereas poorer cardiovascular reactivity is associated with anxiety, neuroticism, or negative affect. Consequently, these differences in the stress perceptions are related to different biological stress responses (Carver & Connor-Smith, 2010).

On the one hand, trait anxiety emerges as an important mediator of the stress response, due to its relationship with the physiological stress response (Chida & Hamer, 2008), as well as its association with coping styles. Studies focused on the influence of trait anxiety on the stress response have found that people with higher anxiety reported more feelings of frustration and stress (Bagget et al., 1996). Whereas some studies described no relationships between the cardiac stress response and anxiety (Bagget et al., 1996; Calvo and Cano-Vindel, 1997), others have shown that higher social and cognitive anxiety is associated with greater cardiovascular activity during a speaking task (Feldman et al., 2004; Grammer et al., 2006). In addition, high trait anxiety may negatively influence the performance displayed in stressful situations such as speaking tasks (Bagget et al., 1996; Calvo and Cano-Vindel, 1997) or a job interview (Cook et al., 2000; McCarthy and Goffin, 2004).

On the other hand, the way people cope with stressful situations is closely related to trait anxiety and emotional and physiological responses. Understanding coping as the way we face stressful situations in an attempt to reduce distress (Carver & Connor-Smith, 2010), we can differentiate two extreme patterns, active or passive (Salvador, 2005, 2012; Salvador & Costa 2009). Passive coping can be defined as maladaptive strategies used when people face stressful situations, such as denial and mental disengagement, whereas active strategies include problem-focused coping (Carver et al., 1989). Indeed, active coping has been associated with an optimal activation of ANS and cortisol release, while passive coping is characterized by an inefficient autonomic and cortisol response after stress (Salvador, 2012). Along these lines, an adaptive coping style has been found to predict greater cortisol (Bhonen et al., 1991) and faster cardiac recovery (Faucheux et al., 1989) in individuals performing stressful cognitive tasks. By contrast, avoidance coping styles have been associated with greater blood pressure reactivity (Vitalino et al., 1993).

Previous literature has found a possible link between trait anxiety and coping styles, suggesting that highly trait-anxious individuals tend to respond to stress with maladaptive cognitive coping behavior (Houston, 1982). Similarly, several years later, Tuncay et al. (2008) showed a negative relationship between trait anxiety and active coping.

In conclusion, the way we appraise a stressful situation (challenge or threat) could determine our perceived abilities to deal with it. In social contexts, trait anxiety seems to play an important role and interacts with coping processes. This personality trait could have a key influence on the physiological stress response. Therefore, when studying social stress and its associated changes, we cannot ignore the implication of these factors.

4. Aims and hypothesis

Taking into account the literature mentioned above, the central aim of this dissertation is to clarify which factors are mediating in the stress response. To do so, in all the empirical studies in this dissertation we have used a standardized laboratory stressor, the Trier Social Stress Test (TSST). In addition, each study is focused on different factors (sex and sexual hormones, age, gender, personality) and/or different components of the stress response (cortisol, heart rate, behavior, and psychological changes).

Study 1.

The aim of this study was to examine the patterns of psychophysiological response (active vs. passive) in young men and women. First, we wanted to explore how gender can affect the subjective experience as well as the physiological stress response. Moreover, we decided to analyze the role of trait anxiety and coping styles as possible adaptive/maladaptive mechanisms. Finally, we aimed to study the relationship between psychological and physiological components of the stress response.

Although sex differences in the psychobiological response to social stress have not been completely established, we expected a greater cortisol response in men and greater affective response in women. Additionally, we hypothesized that the two stress response patterns (active/adaptive and passive/ maladaptive) would be associated differently with trait anxiety and dispositional coping. Finally, we would not expect to find significant relationships between anxiety, mood and cortisol response when people face the TSST (Campbell and Ehlert (2012).

Study 2.

The main objective was to analyze the importance of self-efficacy, as a substantial component of cognitive appraisal, in the stress response. First, we aimed to investigate how perceived self-efficacy might influence the psychological (state anxiety) and cardiac stress response (RR and r-MSSD) and the performance displayed during the TSST speaking task. Additionally, we explored the effects of state and trait anxiety and cardiac components (RR and r-MSSD) on the performance.

We expected that people with higher perceived self-efficacy would show greater cardiac reactivity along with smaller increases in state anxiety. Moreover, we also expected a negative relationship between self-efficacy and trait anxiety. Regarding performance, we hypothesized a positive influence of self-efficacy and sympathetic activation on the final outcome, while a negative effect of trait-state anxiety was expected.

Study 3.

The purpose of the present study was focused on behaviors displayed during the speaking task of the TSST in young women with different hormonal statuses, and their relationships with the psychophysiological stress response.

First, we wanted to assess whether the psychophysiological response to stress was different in the two groups of women (women in the follicular phase vs. women taking oral contraceptives). Second, we aimed to explore whether acute physiological stress reactivity is modulated by the behavioral patterns exhibited during the speech task.

According to the available evidence, we do not expect major differences between follicular women and those taking contraceptives in their psychophysiological stress response. We hypothesized that the intensity of cardiovascular and cortisol responses is associated with the number of submissive and escape behaviors, which are commonly associated with a passive coping style. Finally, we also wanted to confirm previous findings suggesting that displacement behavior is closely linked to the physiology response and anxiety.

Study 4.

The aim of this study was to expand the findings of the third study. Therefore, this study extends the information about coping styles and behavior when facing an acute social stressor in young women in different phases of the menstrual cycle (follicular and luteal) and in women after menopause. To do so, we first wanted to analyze the psychophysiological stress response, measured by means of cortisol and HR, and the mood experienced. Second, we aimed to investigate the role of age and hormonal status in the behavioral patterns displayed during the speaking task of the TSST. And finally, we aimed to explore how certain social behaviors and self-reported coping styles could influence the neuroendocrine capacity to react to and recover from social stress.

We expected to find a greater cortisol and cardiac response in young women, especially in women in the luteal phase, and a worse HPA regulation in post-menopausal women. Moreover, we hypothesized that acute physiological stress reactivity is modulated by the behavioral response patterns exhibited during the speech task of the TSST. More specifically, we anticipated that, in young women, the intensity of the cardiac and cortisol responses would be associated with the number of submissive and escape behaviors, which are commonly associated with a passive coping style. Given that post-menopausal women have never been studied from an observational, ethological perspective, we aimed to explore the relationships among behavior, physiological stress reactivity and the recovery processes in post-menopausal women compared to young women.

CHAPTER II

STUDY 1

STUDY 1

Individual differences in the psychobiological response to psychosocial stress (trier social stress test): the relevance of trait anxiety and coping styles

The main results of this study have been published in: Villada, C., Hidalgo, V., Almela, M., Salvador, A. (in press). Individual differences in the psychobiological response to psychosocial stress (trier social stress test): the relevance of trait anxiety and coping styles. Stress and health.

2.1. Introduction

Stress responsiveness involves the functioning of multiple response systems, with changes at cognitive, emotional, behavioral and physiological levels (Campbell & Ehlert, 2012) that could have negative repercussions on numerous disorders and diseases. In the past few decades, attention has been paid to studying individual differences in the stress response, due to the high incidence of disorders related to stressful experiences, many of them with a different prevalence in men and women. Many of these disorders have been related to activation of the hypothalamus-pituitary-adrenal axis (HPA), which has repeatedly shown sex differences, leading to the consideration that the stress response is different in men and women (Kajantie & Phillips, 2006; Kudielka et al., 2009).

In fact, most of the literature on sex differences in response to psychosocial stress shows that men and women differ in their psychological and physiological responses to acute stress, mainly assessed by parameters such as anxiety, mood, and cortisol. Generally, men show higher cortisol responses than women (Childs et al., 2010; Cornelisse et al., 2011; Huart et al., 2006; Kirschbaum et al., 1999; Kudielka et al., 2004a,b), although depending on the phase of the menstrual cycle or contraceptive use. In contrast, higher affective responses have been reported in women than in men (Carrillo et al., 2001; Childs et al., 2010; Earle, et al., 1999; Kelly et al., 2008), while other authors failed to find sex differences in this type of response to acute stress (Bouma et al., 2009; Cornelisse et al., 2011; Kirschbaum et al., 1999; Schofs & Wolf, 2011). These heterogeneous results suggest that, in addition to sex, other factors could moderate stress responsiveness, such as personality traits. However, even though it has been generally recognized that personality traits must play a very important role in how people confront daily stressful situations, their influence on the psychophysiological response to stress has been not sufficiently established.

In their meta-analysis on the relationships between the HPA axis and personality traits, Chida and Hamer (2008) indicated that reduced HPA axis reactivity was related to various positive psychological traits or states like positive mood and active coping. These authors suggested that some personality dimensions such as trait anxiety can moderate stress reactions; thus, it would be an important factor in understanding some stress-related diseases. Furthermore, studies focused on chronic stress indicate that anxiety is related to the way of coping with stressful situations; for example, people with higher levels of anxiety have obtained lower scores on problem-focused coping strategies (Tuncay, et al., 2008). Coping is one of the factors that may influence the response to social stressors, performance, outcome and possible future consequences. It has been defined as the way we face a threat or a challenge in an attempt to prevent or reduce associated distress (Carver & Connor-Smith 2010), and it shows two extreme patterns, active or passive (Salvador, 2005, 2012; Salvador & Costa, 2009).

Another relevant question is the relationship between mood or anxiety and cortisol changes in situations of social stress, which has not yet been completely established. Whereas a positive relationship between state anxiety and HPA response to psychosocial stressors has been reported (Oswald et al., 2004), other studies have found a negative relationship (Rimmele et al., 2007) or even failed to find significant relationships (Gaab et al., 2005, Kudielka et al., 1998; 2004a). These mixed results have recently been reviewed by Campbell and Ehlert (2012), who concluded that the subjective experience of stress and the physiological reactions are not always correlated. It seems that physiological measures like cortisol do not reflect anxiety, at least not at the same time (Campbell & Ehlert, 2012).

The purpose of the present study was (i) to examine the patterns of psychophysiological response to an acute psychosocial stressor (TSST) in young men and women, in order to verify the two patterns hypothesized, active and passive, using cluster analysis; (ii) to analyze the role of trait anxiety and coping style as possible adaptive mechanisms (iii) and the relationships among subjective and physiological components of the response. We hypothesized that the stress response would be influenced by the subject's sex; we expect that men will have higher cortisol responses than women. On the other hand, although sex differences in mood responses to social stressors have not been well established, we expect that the women will have a larger affective reactivity than men, especially when they are in the early follicular phase. Additionally, we consider that personality traits, such as trait anxiety and dispositional coping, may contribute to explaining differences in both psychological and physiological responsiveness to stress. Taking into account the previous literature, we expect that people characterized by an adaptive (active) coping style will have low trait anxiety and, consequently, confront the stressful situation (TSST) with less anxiety and negative mood. In contrast, individuals characterized by less active coping and higher trait anxiety will face the TSST with higher anxiety and negative mood. Finally, based on Campbell and Ehlert (2012), we do not expect to find significant relationships between anxiety and mood and cortisol response when presented with the TSST.

2.2. Method

2.2.1. Participants.

One hundred and seven volunteers were interviewed and completed a questionnaire to determine whether they met the study prerequisites. The final sample was composed of 35 subjects (17 women) between 18 and 35 years old (Total sample: *M* = 21.06, SEM = 0.732).

Most of them (97%) were college students from different academic areas. The criteria for exclusion were: smoking more than five 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 past 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. 17 were women in the menstrual phase (days 2-5 post menses), all of them were nulliparous with no gynecological problems, and they all had regular menstrual cycle lengths of between 24 and 36 days.

Participants who met 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 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 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.

2.2.2. Study protocol.

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 less than 10 days between sessions for men and 2-3 days between sessions for women. The sessions consisted of several phases of equal durations in both conditions. Overall, both sessions lasted approximately 1 hour, and they were always held between 16.00 and 19.00 hours. Each participant started his/her two sessions at the same time of day. In the first session, upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked whether they had followed the instructions given previously. In the last part of this first session, all the participants completed the personality questionnaires.

Stress Condition. To produce stress, we subjected the participants to the TSST. The stress task consisted of 5 min of free speech (job interview) and a 5 min arithmetic task. The participants remained standing at a distance of 1.5 meters from the committee. The committee was composed of a man and a woman who were professors at the University of Valencia. Interaction between participants and committee members was always with the opposite sex. 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, baseline measures were obtained for anxiety (STAI-S) and mood (PANAS). While subjects responded to these questionnaires, they provided the first saliva sample (-20 minutes pre-stress). After the habituation phase, the introductory phase began (duration 3 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. At that moment, they provided the second saliva sample (-5 minutes pre-stress).

Following the preparation phase, the stress task was carried out. Subjects had 20 minutes to recover after the stress task, and they answered two questionnaires (STAI-S and PANAS) and provided the third saliva sample (+15 minutes post-stress) during this recovery period. The room used for habituation, preparation and recovery was not the same one used for the introduction and stress task. The participants provided the last saliva sample 25 minutes later (+40 min post-stress).

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 was composed of 5 minutes of reading aloud and 5 minutes of counting without the committee. In the preparation phase, the participants read a book with neutral content. The times for collecting the saliva samples and the phase durations were the same for the two conditions, as well as the questionnaires used to evaluate mood and anxiety.

2.2.3. Biochemical analyses

2.2*.3.1. Salivary Cortisol*

Participants provided four 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 - 80° 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 C (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%.

2.2.4. State psychological assessment

2.2.4.1. Anxiety.

To assess state anxiety, the Spanish version (Seisdedos, 1988) of the State Anxiety Inventory was used (STAI form E, 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.

2.2.4.2. Mood.

Mood was evaluated by the Spanish version (Sandin 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). Sandin 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.

2.2.5. Trait Anxiety

We employed the Situations and Responses Anxiety Inventory (ISRA) (Miguel-Tobal & Cano-Vindel, 1994), a specialized questionnaire for evaluating trait anxiety that is frequently employed in Spanish studies. This questionnaire includes 24 anxiety items, following Lang's model (Lang, 1968) using the triple (Cognitive, Physiological, and Motor) response system. In addition, this inventory makes it possible to evaluate 22 situations grouped in four factors related to specific situational areas: Test Evaluation Anxiety (FI), Interpersonal Anxiety (F II), Phobic Anxiety (F III), and Anxiety in Daily Life (F IV). We used the first situational area, directly related to situations that involve evaluation or accepting responsibilities, and defined by situations such as public speaking tasks, receiving criticism and the possibility of being evaluated negatively. Cronbach's alpha ranges from $\alpha = 0.95$ to 0.98 for the anxiety responses system and $\alpha = 0.96$ for Test Evaluation Anxiety (FI).

2.2.6. Coping styles (COPE).

The dispositional version of the COPE Inventory is a theoretically-based self-report questionnaire that addresses different ways of coping (Carver et al., 1989). Subjects must indicate what they *generally* do and feel when experiencing stress. Items are rated on a 4-point scale, ranging from 1 (*I usually don't do this at all*) to 4 (*I usually do this a lot*). We employed the Spanish version of the long form, consisting of 60 items arranged in 15 factor scales within the following groups: problem-focused coping (active coping, planning, seeking instrumental support, suppression of competing activities, restraint coping), emotion-focused coping (seeking emotional support, positive reinterpretation, religion, acceptance, humor), and potentially maladaptive emotion-focused coping (venting of emotions, denial, mental disengagement, behavioral disengagement, and use of alcohol and drugs). The Spanish version of the scale had a Cronbach's alpha ranging from $\alpha = 0.78$ to 0.92 (Crespo & Cruzado, 1997).

2.2.7. Statistical analyses.

ANOVAs for repeated measures were used to assess the effects of acute stress on anxiety, mood and cortisol. We measured anxiety and negative mood before and after the stress and control tasks; for cortisol, we added Time (-20, -5, +15, +40 min) as a within-subject factor for both the control and stress conditions. Moreover, to take into account the individual differences in the cortisol, anxiety and mood responses in the stress condition compared to the control situation, reactivity to stress ("net reactivity") was defined as the difference between deltas in the stress condition and deltas in the control condition. For cortisol levels, deltas were calculated as the difference between the samples, (+15) and (-5), in the two conditions; for STAI and PANAS, deltas were calculated as the difference between scores obtained before and after both tasks. Cluster analyses were carried out in order to explore the patterns of psychological and physiological responses (see 3.2.).

We checked for order effects (whether the stress or control condition was first) by using an ANOVA for repeated measures, which did not reveal any effect of order (all p >0.168).

Spearman's rank correlation coefficients were calculated in order to assess whether the physiological (cortisol) and psychological changes (anxiety and negative mood) were related to each other and to personality traits (COPE and ISRA).

One-way analyses of variance (ANOVAs) were used to analyze individual differences on each trait questionnaire (ISRA, COPE) and on psychobiological reactivity to stress, "net reactivity" of cortisol, STAI and PANAS. To assess group differences, we included Group (Men vs. Women or Cluster 1 vs. Cluster 2) as a between-subject factor.

All p-values reported are two-tailed, and the level of significance was marked at <0.05. When not otherwise specified, results shown are means ± standard error of means (SEM). We used SPSS 15.0 to perform the statistical analyses.

2.3. Results.

2.3.1. Psychophysiological response

For anxiety, a repeated measures ANOVA revealed significant effects of Condition ($F(1, 34) = 19.810$, $p < 0.001$, $\eta^2_p = 0.368$), Time ($F(1, 34) = 13.603$, $p = 0.001$, $\eta^2_p = 0.286$) and the Condition × Time interaction ($F(1, 34) = 21.432$, $p < 0.001$, $\eta^2_p = 0.387$). No differences were found between the stress and control conditions before the task ($p = 0.574$), but we found higher anxiety in the stress condition, compared to the control condition, after the TSST ($p < 0.001$).

For negative mood, significant effects of Condition ($F(1, 34) = 22.771$, $p < 0.001$, $\eta^2_p = 0.401$), Time ($F(1, 34) = 6.121$, $p = 0.019$, $\eta^2_p = 0.153$) and the

Condition × Time interaction were found ($F(1, 34) = 34.225$, $p < 0.001$, $\eta^2_p = 0.502$). Negative mood was similar in both conditions before the tasks ($p = 0.173$), but the increases were higher after the tasks in the stress condition, compared to the control condition ($p < 0.001$).

For cortisol, a repeated measures ANOVA showed significant effects of Condition ($F(1, 34) = 8.506$, $p = 0.006$, $\eta^2_p = 0.2$), Time ($F(1.743, 59.260) = 8.724$, $p = 0.001$, $\eta^2_p = 0.204$) and the Condition × Time interaction ($F(1.530, 52.014) = 34.690$, $p < 0.001$, $\eta^2_p = 0.505$). Post hoc comparisons showed no differences between conditions before the task (all $p > 0.142$). However, the levels of cortisol were significantly higher after the TSST (all $p < 0.001$) in the stress condition, compared to the control condition.

Condition	Time	Cortisol	State Anxiety	Negative mood
Experimental	-20	6.10±0.64	15.71±1.04	13.52 ± 0.54
	-5	5.45±0.53		
	15	11.05±1.40	23.67 ± 1.63	18.2 ± 1.06
	40	7.38±0.71		
Control	-20	7.28±0.82	16.37 ± 1.55	14.43 ± 0.77
	-5	6.11±0.58		
	15	4.63±0.38	14.74 ± 1.23	12.45 ± 0.47
	40	3.91±0.27		

Table 1. Differences in Means±SEM, on cortisol (nmol/l), state anxiety, and negative mood in response to psychosocial stress (TSST) in the total sample (N = 35).

2.3.2. Cluster analysis

As we wanted to confirm the two response patterns, active vs passive (high and low response to stress, respectively), the "net reactivity" for cortisol, anxiety and negative mood was entered. The cluster solution resulted from a k-means analysis of the entire sample (n = 35). Two clusters were identified: Cluster 1 was characterized by low psychological reactivity (anxiety and negative mood) with moderate cortisol reactivity; and Cluster 2 was characterized by high psychological reactivity (anxiety and negative mood) with low cortisol reactivity. Cluster 1 was composed of fifteen men and five women, and Cluster 2 had three men and twelve women. ANOVA revealed that the differences between clusters on anxiety and negative mood were statistically significant ($p < 0.001$), but not for cortisol ($p = 0.384$). Table 2 shows the differences in "net reactivity" according to sex and cluster.

Mean scores ± SEM	Cluster 1 (N = 20)	Cluster 2 (N = 15)	ANOVA
State anxiety	2.16 ± 1.97	19.47 ± 2.24	$F (1, 33) = 30.437$, $p < 0.001$
Negative mood	3.10 ± 1.16	11.39 ± 1.43	$F (1, 33) = 22.293$, $p < 0.001$
Cortisol	7.95 ± 1.82	5.9 ± 1.14	$F (1, 33) = 0.779$, $p = 0.384$
Mean scores ± SEM	**Men (N =18)**	**Women (N =17)**	**ANOVA**
State anxiety	4.94 ± 3.12	14.49 ± 2.19	$F (1, 33) = 6.122$, $p = 0.019$
Negative mood	5.33 ± 1.64	8.05 ± 1.54	$F (1, 33) = 1.446$, $p = 0.238$
Cortisol	8.08 ± 1.72	6.00 ± 1.50	$F (1, 33) = 0.816$, $p = 0.373$

Table 2. Differences in Means (± SEM) on State anxiety, negative mood and cortisol "net reactivity" to psychosocial stress (TSST) in the subjects, grouped in two clusters and by sex.

2.3.3. Cluster differences in personality traits and coping styles.

Significant differences between the two clusters were found in the three components of anxiety studied: Cognitive ($F (1, 34) = 12.110$, $p = 0.001$, $\eta^2_p = 0.268$); Physiological ($F (1, 34) = 20.224$, $p < 0.001$, $\eta^2_p = 0.380$) and Motor ($F (1, 34) = 20.777$, $p < 0.001$, $\eta^2_p = 0.386$). Furthermore, significant differences appeared on Test Evaluation Anxiety ($F (1, 33) = 19.573$, $p < 0.001$, $\eta^2_p = 0.372$). Participants allocated to Cluster 1 were characterized by lower scores on all components of trait anxiety and the specific situation analyzed, compared to their counterparts in cluster 2 (Figure 1). Mean values (± SEM) for each anxiety component for Clusters 1 and 2 were the following, respectively: Cognitive (54.2 ± 4.73 vs 80.73 ± 6.14), Physiological (24.48 ± 2.63 vs 45.33 ± 4.05), Motor (27.1 ± 2.72 vs 47.6 ± 3.72) and Test Evaluation Anxiety (52.3 ± 4.83 vs 90.4 ±

7.58). All mean scores were in the normal range for Spanish data scales, between percentiles 25 and 75; therefore, the participants did not show subjective severe or extreme anxiety.

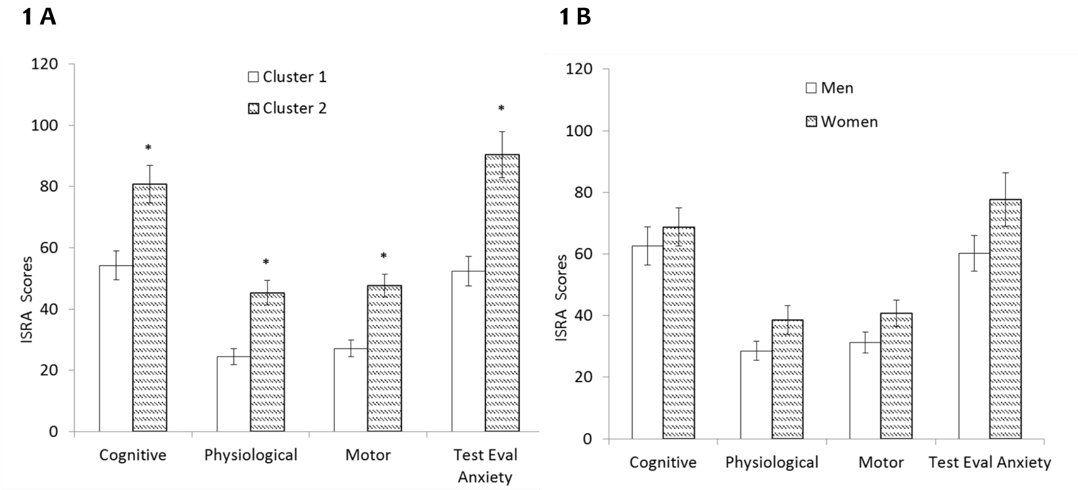

Figure 1A. Mean values ± SEM of the three components of anxiety studied and of Test Evaluation Anxiety from the ISRA inventory corresponding to subjects grouped by cluster. Figure 1B. Mean values ± SEM of the three components of anxiety studied and of Test Evaluation Anxiety from the ISRA inventory grouped by sex (*p ≤0.01).

Statistically significant differences in coping styles were only found for Active Coping ($F(1, 34) = 7.683$, $p = 0.009$, $\eta^2_p = 0.0.189$) and Planning ($F(1, 34) = 8.402$, $p = 0.007$, $\eta^2_p = 0.203$), but marginal differences appeared for Focusing on and Venting emotions ($F(1, 34) = 3.897$, $p = 0.057$, $\eta^2_p = 0.106$) and Mental disengagement ($F(1, 34) = 3.900$, $p = 0.057$, $\eta^2_p = 0.106$). Participants allocated to Cluster 1 obtained significantly higher scores on Active Coping and Planning, whereas, as a trend, participants allocated to Cluster 2 were characterized by higher scores on Focusing on and Venting Emotions and Mental Disengagement (Figure 2). Mean values (± SEM) of each scale for clusters 1 and 2, respectively, were: Active Coping (12.05 ± 0.38 vs 10.53 ± 0.37), Planning (12.45 ± 0.49 vs 10.13 ± 0.65), Focusing on and Venting emotions (9.3 ± 0.43 vs 10.73 ± 0.60) and Mental Disengagement (8.8 ± 0.41 vs 10.13 ± 0.55).

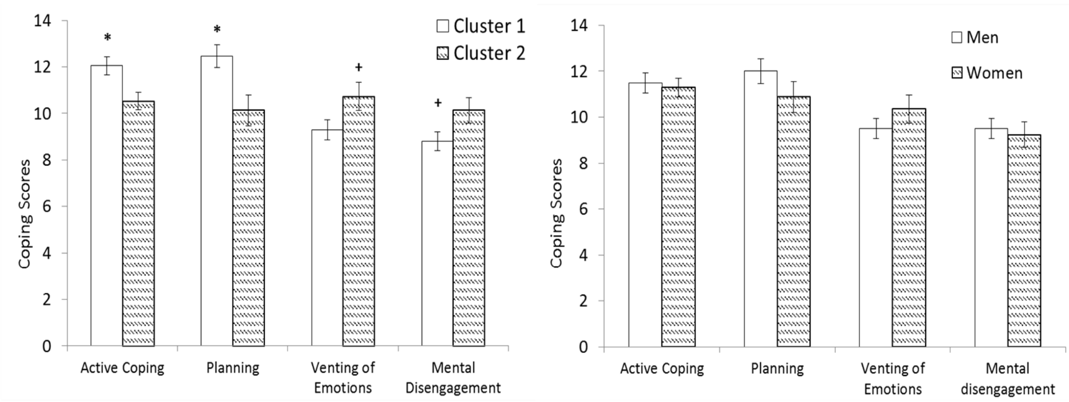

Figure 2A. Mean values ± SEM of the scales of the COPE inventory corresponding to subjects grouped by cluster. Figure 2B. Mean values ± SEM of the scales of the COPE inventory corresponding to subjects grouped by sex. ($^{*}p \leq 0.001$; $^{+}p = 0.057$).

2.3.4. Sex differences in personality traits.

Sex differences on the trait anxiety scales (ISRA) did not reach statistical significance for the three components of anxiety studied (all $p > 0.08$) and "test evaluation anxiety" (all $p > 0.10$) (Figure 1 and figure 2). Nor were significant effects of Sex found on the COPE Inventory scales (all $p > 0.132$).

2.3.5. Relationships among psychophysiological responses and psychological traits

Emotional (Anxiety and mood) reactivity did not correlate with cortisol reactivity. However, both anxiety and negative mood correlated significantly with all the ISRA and COPE scales that had shown significant differences between clusters (see table 3). On the one hand, anxiety reactivity correlated positively with the triple response system (cognitive, physiological and motor) and with the "test evaluation anxiety" situational factor (all $p \leq 0.001$). On the other hand, negative mood reactivity correlated positively with the triple response system (cognitive, physiological and motor) and with the "test evaluation anxiety" situational factor ($p \leq 0.001$). Furthermore, both anxiety and negative mood reactivity correlated negatively with "active coping" on the COPE ($p \leq 0.016$).

In addition, active coping was consistently and negatively correlated with all the trait anxiety scales, whereas the coping factors focused on emotions were positively correlated with the trait anxiety scales (see table 4).

	Active coping	Planning	Focus on emotions	Mental disengagement
Cognitive Anxiety	rho = -.368*	rho = -.167	rho = .638**	rho = .327+
Physiological Anxiety	rho = -.443**	rho = -.281	rho = .377*	rho = .325+
Motor Anxiety	rho = -.450**	rho = -.264	rho = .378*	rho = .269
Test Evaluation Anxiety	rho = -.415*	rho = -.314+	rho = .473**	rho = .331+

Table 4. Spearman rank correlations between ISRA factors and COPE scales for the total sample (N = 35). (**$p \leq .01$; *$p \leq .05$; $^{+}p < 0.1$).

2.4. Discussion

The present study investigated some factors involved in individual differences in the response to a standardized laboratory psychosocial stressor (TSST). Stress response was assessed by psychological (anxiety and mood) and physiological (cortisol) pre-post measures in an experimental and a control condition employing a crossover design.

First, our results confirmed that the psychosocial stress task (TSST) elicited significant changes, with perceived anxiety and negative mood increases and increases in cortisol levels. These results are in line with studies that have examined both the psychological and physiological responses to laboratory psychosocial stressors (Childs et al., 2010; Izawa, et al., 2008; Kelly et al., 2008; Kudielka et al., 2004a, b; Rimmele et al., 2007; Scholz, et al., 2009).

Faced with this psychosocial stressor, based on previous literature, we expected that men would show a higher cortisol response than women (Childs et al., 2010; Cornelisse et al., 2011; Kirschbaum et al., 1999), but contrary to our hypothesis, we did not find sex differences in cortisol reactivity. We also expected that women would show a greater emotional response, especially because they were in the earliest follicular phase, associated with the perimenstrual syndrome and with more psychological complaints compared to other menstrual cycle phases (Guillermo et al., 2010). Our results showed more anxiety increases in women than in men, but no differences in negative mood reactivity.

Moreover, no significant relationships between psychological variables and cortisol were found; this absence of relationships between cortisol and psychological reactivity to stress agrees with Campbell and Ehlert's conclusions (2012), mentioned above. Along these lines, Cohen et al. (2009) found that stress-induced anxiety changes were not associated with any of the biological responses studied, among them, cortisol response.

More interestingly, our results suggest that, apparently, psychological and physiological reactions could work in different ways. The cluster analysis revealed two patterns of response to stress. The first one was characterized by a low psychological reaction, whereas the second one presented a high psychological reaction; subjects displayed a mean net reactivity on anxiety (2.16 vs 19.47, respectively) and negative affect (3.10 vs 11.39, respectively) (see Table 1). In addition, each reaction seems to be related differently to the cortisol response, so that low psychological reactivity to acute stress was linked to moderate levels of cortisol, whereas high psychological reactivity appeared associated with less cortisol reactivity. These results agree with previous findings by Het and Wolf (2007), who reported that increases in cortisol levels were related to a reduction in stress-induced negative affect in women, using the TSST, and also to pretreatment with cortisol. Schlotz et al. (2008) also found negative relationships between cortisol and tense arousal in response to the TSST in men and women. Previously, in a study with stressed people, Boudarene et al. (2002) distinguished three types of psychophysiological responses to cognitive tasks; one of them was high emotional reaction without increases in cortisol, which they called biological silence, and they concluded that this response reveals psychological vulnerability. Taking this into account, we think that in this context cortisol has a positive function, reflecting the response of preparing to deal with stress.

Beyond being a strong psychosocial stressor, the TSST (Kirschbaum et al., 1999) is, in itself, a potent threat to self-concept, due to the personal information that subjects give to their interlocutors (Gonzalez-Bono et al., 2002), in this case, the committee that evaluates them. Moreover, due to evaluating the characteristic of being "the best applicant" for a job or position, the TSST can be considered an ecologically relevant stressor with an important competitive component (Salvador & Costa, 2009; Salvador, 2012). Therefore, from an evolutionary perspective, the response of the cluster 1 participants to this psychosocial stressor, a low anxiety and negative affect response with a moderate cortisol response, would allow them to deal with situations more effectively, and this adaptive response pattern throughout life would not to be harmful to health. Our results showed that these participants score higher on active coping, apart from obtaining lower scores on trait anxiety. Supporting these results, a recent study by van Santen et al. (2011) suggests that ineffective coping styles could be related to a higher cortisol awakening response (CAR), and it has even been reported that higher levels of CAR are related to some scales that indicate chronic stress, such as "worries" and "social stress" (Wust et al., 2000).

Moreover, this new classification facilitated by the clusters allowed us to examine how these differences are linked to personality traits such as trait anxiety and dispositional coping. Thus, we can see how subjects in cluster 2 (high anxiety and negative affect response with low cortisol response) scored higher on trait anxiety related to the triple response system (cognitive, physiological and motor) and the specific situational area related to the stress test employed (test evaluation anxiety). In addition, these participants also presented higher scores on the coping scales focused on emotions and mental disengagement. Moreover, the fact that all the trait anxiety factors studied correlate negatively with active coping and, although marginally, with planning, points to how some personality traits and coping styles, such as high anxiety or emotion-focused coping as a maladaptive stress response, could have physiological repercussions and, ultimately, negative consequences for health. Along these lines, Endler and Kocovsky (2001) proposed an interactive model of stress anxiety and coping styles, where both personal and situational variables, such as a challenge or a threat, are interconnected, leading to perceived stress. The result would be anxiety changes, which would lead to different coping reactions and biological and behavioral responses. These authors also argued that both perceived anxiety and final reactions respond to personal and situational variables. More recently, Costa and Salvador (2012) reported a passive coping pattern of an emotional nature involving increases in anxiety, negative mood, blood pressure and cortisol in a competitive situation in women; in this study, of the two patterns found using factorial analysis (active and passive coping), cortisol saturated in passive coping, but without reaching statistical significance.

Therefore, we conclude that negative emotions, such as anxiety and negative mood, provoke reactive coping, whereas a better psychological response under stressful situations, closer to a moderate activation of the HPA axis, seems to be better for pro-active coping. It must be taken into account that the physiological response provoked in this paradigm is a moderate response; other situations that are more stressful than the TSST and produce larger increases could have different effects. To the best of our knowledge, this is the first study to relate trait anxiety and coping styles using this paradigm in healthy young people. In our opinion, these results provide information about some components involved in the stress response. Although sex and/or hormonal status are able to modulate the psychophysiological response to stress, some personality traits play an important role, becoming potential modulators in the response to acute stress, beyond the role of sex.

The findings of the present study, although very interesting and suggestive, must be replicated in a larger sample with a more representative female sample, as we only included women in a special period of the menstrual cycle. It is necessary to increase the sample size, including women in the other phases and those using contraceptives. Both the emotional and HPA reactivity to stress are thought to be modulated by sex hormone status (Childs et al., 2010; Espin, et al., 2013; Kajantie & Phillips, 2006). Despite these limitations, we are able to show some very interesting relationships between personality traits and coping styles and psychophysiological response to an acute psychosocial stressor extensively employed in lab research. Understanding how the mechanisms involved in the stress response interact could have important benefits for future interventions, above all to understand and modify subjective perceptions and provide training in how to deal with stress. Further work on these issues, therefore, would be useful for the prevention and treatment of stress-related disorders.

CHAPTER III

STUDY 2

STUDY 2

Assessing performance on an evaluated speaking task: the role of self-efficacy, anxiety and cardiac autonomic reactivity

The main results of this study are under review: Villada, C., Hidalgo, V., Almela, M., Salvador, A. Assessing performance on an evaluated speaking task: the role of self-efficacy, anxiety and cardiac autonomic reactivity

3.1. Introduction

People are subjected to social stress throughout life, but young adulthood is one of the main periods when stressful evaluative conditions are experienced, such as certain exams and/or job interviews that could have important socio-economic and personal consequences. Self-perceived abilities to face a challenge (i.e., self-efficacy) and interpret the outcome obtained in these contexts (i.e., self-assessment) may influence the way of dealing with future social stressors (Salvador and Costa, 2009), and they could be considered important modulators of the stress response, especially its psychophysiological component. According to Bandura (1977), performance outcomes and past experiences are the most important sources of self-efficacy, creating a vicious cycle. Along these lines, self-efficacy arises as one of the most important factors in the stress response, particularly as a component of active coping (Gerin et al., 1995). Thus, self-efficacy would play a significant role in the response to social stressors, which presumably will affect the final outcome and future situations.

An outcome is a very broad concept, ranging from sports competitions to work situations, such as job interviews. In all of these contexts, there is a bad or good result, for instance, getting (or not) a job. Importantly, these stressful situations become a threat to the social-self and physiological systems (Dikerson and Kemeny, 2004). Along these lines, Blascovich and Tomaka (1996) proposed the Biopsychosocial model (BPS) of arousal regulation, a model that takes into account the cognitive stress appraisal before and after performing a stressful task, the physiological response to the stressful task, and the performance displayed. The authors suggest that cardiovascular responses differ depending on whether the situation is perceived as a threat or a challenge, and that the experience of success or failure will modulate the psychophysiological stress response. The challenge response would be characterized by a pattern of active coping. In individuals who appraise themselves as having the necessary personal resources to cope with the demands of the situation, along with an efficient autonomic reactivity that facilitates performance, the final perception will be one of a positive challenge. By contrast, the threat response is characterized by a pattern of passive coping, with an appraisal of not having enough resources to cope with the situation, along with a pattern of inefficient autonomic reactivity that inhibits performance.

Studies focusing on the effects of cognitive appraisal on cardiovascular reactivity to stress have shown that people who perceived a stressful situation (evaluated by an arithmetic task) as threatening showed lower cardiac reactivity than those who perceived the situation as a challenge (Tomaka et al., 1997; Schneider et al., 2008; but see Kelsey et al., 2000). Similarly, Hodgins et al. (2010) found that adults characterized by autonomous motivation (i.e., perceived the situation as a challenge) showed higher cardiac output compared to those characterized by controlled motivation (i.e., perceived the situation as a threat). However, the results are not consistent. Rith-Najarian et al. (2014), employing an oral speaking task as the stressful situation in adolescents, did not find significant relationships between pre-task stress appraisal (threatening appraisal of the speaking task) and cardiac reactivity. Recently, Jamieson et al. (2012) found that subjects exposed to the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993) who were instructed to reappraise their arousal as functional increased their perceptions of available resources and, consequently, improved their cardiovascular functioning. Therefore, although there are still some contradictory results, it seems that, in general, situations perceived as challenging are related to greater cardiac activity, confirming an active coping style to deal with social stressors. In addition, active coping has been associated with greater probabilities of obtaining a good outcome in social stress situations such as contests (Salvador, 2005), and the social context developed in the TSST involves a competition to the degree that the participant is told that he/she must better than the other participants (Salvador and Costa, 2009).

Another important issue related to the cognitive appraisal of stressful situations is anxiety. Earlier studies failed to find a relationship between trait anxiety (speech anxiety) and cardiovascular reactivity on a speaking task (Bagget et al., 1996; Calvo and Cano-Vindel, 1997). However, more recent studies showed that higher social and cognitive anxiety was associated with greater cardiovascular activity during the anticipation (Feldman et al., 2004; Gonzalez-Bono et al., 2002) and the speaking task (Feldman et al., 2004; Grammer et al., 2006). In fact, results suggest that low cardiovascular reactivity is related to high anxiety in response to acute social stress (see meta-analysis, Chida and Hamer, 2008). In general, these results highlight the relevance of studying cognitive appraisal together with state-trait anxiety, in order to provide a better understanding of the physiological stress response.

Moreover, there is some evidence about the relationships among personality traits related to social stress, the psychophysiological response, and the performance displayed during an acute challenge (oral speaking task). Thus, Saslow et al. (2014) found that physiological and emotional reactions, as well as higher trait stress reactivity, might modulate language complexity, resulting in poor performance. In addition, several studies have shown that high anxiety may be related to poor performance on a speaking task (Bagget et al., 1997; Calvo and Cano-Vindel, 1997) or in a job interview (Cook et al., 2000; McCarthy and Goffin, 2004), although not all the studies observed this relationship (Rith-Najarian et al., 2014).

With all this in mind, the main objective of the present study was to analyze the importance of self-efficacy, as a substantial component of cognitive appraisal, in the psychological (state anxiety) and physiological (RR and r-MSSD) stress response and the performance displayed during the TSST speaking task. The second objective was to explore the effects of the relationships between the psychological (state-trait anxiety) and physiological components (RR and r-MSSD) on the performance. We expect (i) to find higher cardiac reactivity along with minor increases in state anxiety in the high self-efficacy group. We also expect (ii) self-efficacy to be negatively related to trait anxiety, and (iii) that the final outcome will be positively influenced by self-efficacy and sympathetic activation, and negatively by trait/state anxiety.

3.2. Methods

3.2.1. Participants

The final sample was composed of 35 (men: 18; women: 17) college students from different areas (e.g., psychology, medicine, work sciences) of the University of Valencia, between 18 and 35 years old (M = 22.31, SEM = 0.715, p = 0.715). For subject recruitment, announcements were posted and informative talks were held in the various departments of the University campus. Volunteers were interviewed by trained psychologists and completed an extensive questionnaire to check whether they met the study prerequisites. The criteria for exclusion were: smoking more than five cigarettes a day, alcohol or other drug abuse, visual or hearing problems, presence of a 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 cardiac, emotional or cognitive function, or one that was able to influence hormonal levels, such as glucocorticoids or β-blockers. To control possible differences in cardiac activation due to the hormonal status derived from the menstrual cycle (see Kajantie and Phillips, 2006), all the women had been taking oral contraceptives (monophasic formulas) for at least 6 months.

Participants who met 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 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 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.

32.2. Study protocol

This study employed a within-subject design with two randomized and counterbalanced conditions in two separate sessions: a stress condition and a control condition. The interval between sessions was approximately one week. The sessions consisted of several phases with equal durations in both conditions. Overall, both sessions lasted approximately 1 hour, and they were always held between 16.00 and 19.00 hours. Each participant started his/her two sessions at the same time of day. Upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked whether they had followed the instructions given previously. In the last part of the first session, all the participants completed the Situations and Responses Anxiety Inventory (ISRA) questionnaire, regardless of whether this session was experimental or control.

Stress Condition. To produce stress, we subjected the participants to the TSST (Kirschbaum, et al, 1993). The stress task consisted of 5 min free speech (simulated job interview: the job was always related to each participant's career, and it was a position with a high degree of responsibility), and a 5 min mental arithmetic task. The participants remained standing at a distance of 1.5

meters from the committee. The committee was composed of a man and a woman who were professors at the University of Valencia. The interaction between participants and the committee was always with the committee member of the opposite sex. 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 this phase started, baseline measures were obtained for anxiety (STAI-S). After the habituation phase, the introductory phase began (duration 3 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 min to prepare for the task at hand. Prior to the preparation phase, participants were asked about their perceived self-efficacy to perform the speaking task. Following the preparation phase, the stress task was carried out. Subjects had 20 min to recover after the stress task, and they answered the anxiety questionnaire post-task (STAI-S). The room used for habituation, preparation and recovery was not the same one used for the introduction and stress task.

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 was composed of 5 min of reading aloud and 5 min of counting without the committee. In the preparation phase, the participants read a book with neutral content. The phase durations were the same for the two conditions, as were the questionnaires used to evaluate mood and anxiety.

3.2.3. Self-efficacy

Self-efficacy (Bandura, 1997) was operationalized with 2 items: (i) how capable are you of performing the speech successfully? (ii) how confident are you that you will perform the speech successfully? Subjects responded to each question on a 100-point Likert scale (none = 0, very much = 100); the average of the 2 items made it possible to obtain a total self-efficacy score (vand der Meij et al., 2010), with a Cronbach's alpha of 0.95.

3.2.4. State-trait Anxiety

To assess state anxiety, the Spanish version of the State Anxiety Inventory was used (STAI form E, Spielberger et al., 1970). It consists of 20 items (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).

To assess trait anxiety, we employed the Situations and Responses Anxiety Inventory (ISRA) (Miguel-Tobal and Cano-Vindel, 1994), a specialized questionnaire for evaluating trait anxiety that is frequently employed in Spanish studies. This questionnaire includes 24 anxiety items, following Lang's model (Lang, 1968) using the triple (Cognitive, Physiological, and Motor) response system. Due to the nature of the study, we selected the Cognitive and Physiological response system (Cronbach's alpha ranges from $\alpha = 0.95$ to 0.98). In addition, this inventory makes it possible to evaluate 22 situations grouped in four factors related to specific situational areas: test evaluation anxiety (F I), interpersonal anxiety (F II), phobic anxiety (F III) and anxiety in daily life (F IV). In this study we used the first dimension, directly related to situations that involve evaluation and defined by situations such as public speaking tasks, receiving criticism, and the possibility of being evaluated negatively. Cronbach's alpha for test evaluation anxiety (F I) was 0.96 (Miguel Tobal and Cano Vindel, 1994).

3.2.5. Heart Rate Variability (HRV)

Due to the nature of the experimental design, subjects had to be free to move around the lab and go to different rooms. Therefore, heart rate was continuously recorded in the experimental and control conditions using a heart rate monitor Suunto ® T6 (Suunto Oy, Vantaa, Finland). The moments when participants were walking from one room to another were removed, and only the 5 central minutes of each phase: Baseline (BL), Preparation, Speech and Recovery, were used to calculate the participants' average heart rate. Heart rate variability was analyzed with Kubios software for advanced HRV analyses (Biomedical Signal Analysis Group, University of Kuopio, Finland). The following parameters from time domain analysis were quantified: the mean R–R interval duration (R-R in ms), reflecting an 'instantaneous' measurement of heart rate; and following recommendations (Task Force, 1996), we selected the root mean square of successive differences (r-MSSD in ms), focused on high-frequency from the time domain analysis and short-term variations in the R–R interval, which are mainly due to parasympathetic nervous system activity.

3.2.6. Performance

After the TSST, participants were asked about their perceived performance (How well do you think you did the task?) Subjects responded to this question on a 5-point Likert scale (not at all = 1, to extremely = 5).

Furthermore, the speech was video-and audio-recorded with a camera adjusted so that the subject's face and trunk were in full view. Two trained observers examined the videotape and scored the subject on a 4-point Likert scale ranging from 1 (bad performance) to 4 (perfect performance) on each of these 7 items: Introduction (e.g., self-presentation, greeting the evaluators cordially), Development (coherence and logical thread of the speech), Closure (integration of the speech), Verbal fluency (no lengthy silences and/or crutches), Volume (tendency to decrease the volume), Speech Continuity (After lengthy silences, the participants are asked to continue, and they continue (or not) with the speaking task), and Content (whether the participants link their personal characteristics with the hypothetical job). We calculated an intraclass correlation coefficient (ICC) for each item; the repeatability of the rating ranged from 0.71 to 0.95 for each observer. Each observer evaluated the videos two times, and then we used the average of the first and second ratings on each item to assess the level of inter-observer reliability (see Table 1). Finally, a factorial analysis was performed with the main components method and Varimax rotation, using the 7 grand average items with the results of three main factors: Performance, Content and Structure of the speaking task (Table 1).

External Evaluation	Items	Inter-observer reliability	Saturation Factor
Factor I. Performance	Development (coherence and logical thread of the speech)	0.69	0.876
	Verbal fluency	0.91	0.934
	Volume	0.91	0.709
	Speech continuity	0.78	0.787
Factor II. Structure	Introduction	0.94	0.864
	Closure	0.92	0.907
Factor III. Content	Content	0.82	0.975

Table 1. Data on external evaluation, inter-observer reliability and saturation of each item. Variance explained 39.88 %, 23.58 % and 16.14 % for Factors 1, 2 and 3, respectively.

3.2.7. Data reduction and statistical analyses

Data were checked for normal distribution and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests before statistical procedures were applied. As the HRV parameters did not have a normal distribution, they were square-root-transformed, but raw data is shown in the figures and tables.

To take into account the individual differences in the stress response compared to the control situation, we calculated net values of psychological and physiological variables. Net values were calculated as the difference between stress minus the mean control values in each Period (Basal Line, Preparation, Speech and Recovery) for the HRV parameters; net values for perceived state-anxiety were calculated as the difference between pre- and post-task stress minus control scores. Finally, net reactivity was calculated for cardiac parameters as the difference between mean values for the speech period and baseline mean values for each condition; for state-anxiety, net reactivity was calculated as the difference between pre-task and post-task measures for each condition (Almela et al., 2012; Villada et al., 2014a).

To investigate the role of self-efficacy in the stress response, in agreement with other studies (Tomaka et al., 1994; Bègue, 2005), we divided subjects into two groups by the median split: the Low self-efficacy (L-SE) group included individuals scoring below 60 (n=15), and the High self-efficacy (H-SE) group included those scoring 60 or above (n= 20 because 4 subjects scored 60). ANOVAs for repeated measures with Period (Anxiety: pre and post; RR and rMSSD: Basal Line, Preparation, Speech, and Recovery) as within-subject factor were used to assess differences in the psychological and physiological stress response between Groups (High self-efficacy vs. Low self-efficacy).

One-way ANOVAs were used to analyze differences between groups (H-SE vs. L-SE) in the demographic/anthropometric variables, and in the psychophysiological reactivity indices, performance assessment, and ISRA dimensions.

Pearson correlations were calculated in order to assess relationships between self-efficacy and Net reactivity of R-R, r-MSSD, anxiety and performance during the speaking task.

We checked for order effects (whether the stress or control condition was first) by using one-way ANOVA for ISRA factors (all $p > 0.41$) and ANOVA for repeated measures, which did not reveal any effect of order for the HRV parameters or subjective measures (all $p > 0.420$).

For HRV analyses, 3 participants were removed (3 men) due to technical problems, and one woman due to missing data during the speech.

We used Greenhouse-Geisser when the requirement of sphericity in the ANOVA for repeated measures was violated. Post hoc planned comparisons were performed using 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 otherwise specified, results shown are means ± standard error of means (SEM). We used SPSS 22.0 to perform the statistical analyses.

3.3 Results

Preliminary analyses

First, we examined whether the stress protocol (TSST) produced a different effect on the psychophysiological response compared to the control situation. For the R-R interval, the ANOVA of repeated measures showed a main effect of Condition $F(1,31)=13.810$, $p=.001$, Period $F(1.48,67.785)=60.041$, $p\leq.001$, and the Condition x Period interaction $F(2.39,74.099)=2.579$, $p=.058$. For r-MSSD, the ANOVA revealed a main effect of Condition $F(1,31)=4.173$, $p=.050$, Period $F(3,93)=9.997$, $p\leq.001$, and the Condition x Period interaction $F(3,93)=3.159$, $p=.028$. For anxiety (STAI-S) (N= 35), the ANOVA showed a main effect of Condition $F(1,34)=16.422$, $p\leq.001$, Period $F(1,34)=5.648$, $p=.023$, and the Condition x Period interaction $F(1,34)=9.737$, $p=.004$. The total sample showed decreases in the RR and r-MSSD parameters and increases in state anxiety in response to stress (TSST) compared to the control condition (all $p \leq .05$) (see Table 2).

Condition	Period	RR	r-MSSD	STAI-S
Stress	Base Line	772,08±23,49	37,57±3,26	14,51±1,06
	Preparation	698,83±19,01	31,96±2,63	
	Speech	634,71±17,97	30,51±2,64	
	Recovery	795,35±20,39	41,26±3,35	20,06±1,63
Control	Base Line	767,32±17,77	37,94±3,52	14,34±1,44
	Preparation	784,49±19,26	39,89±3,27	
	Speech	700,98±16,70	35,01±2,57	
	Recovery	822,97±19,85	43,47±3,29	12,71±1,06

Table 2. Mean ± SEM of the physiological (RR and r-MSSD) and psychological changes (STAI-S) in the different periods of the protocol by condition for the total sample (N= 32).

After that, posterior analyses were performed with net values and based on the Self-efficacy groups, which were formed based on the median split of the Self-efficacy scores (Median=60; X=55.57, SEM=3.17). No significant differences were found (all $p > 0.1$) between the H-SE and L-SE groups in demographic or anthropometric variables (Table 3) and ISRA factors (Table 4).

Variable	Total Sample (N=35)	H-SE (N=20)	L-SE (N=15)
Age	22.31±0.71	22.53±1.1	22.15±0.9
BMI	22.92±0.53	23.25±0.62	22.67±0.82
Physical exercise	4.43±0.41	4.4±0.67	4.45±0.53
SES	6.43±0.16	6.73±0.28	6.2±0.21

Table 3. Mean ± SEM of characteristics of total sample and subgroups (age, Body Mass Index, BMI, physical exercise, and Subjective Socioeconomic Status: Subjective SES scale, Adler et al. (2000). Data are presented for the total sample (N= 35) and subgroups: High Self-Efficacy (H-SE) and Low Self-Efficacy (L-SE).

3.3.1. Self-efficacy and psychophysiological stress response

ANOVA of repeated measures with Net values for R-R showed significant effects of Period, $F(2.472,74.58) = 15.250$, $p \leq 0.001$, and the Period x Self-efficacy interaction, $F(2.472,74.58) = 4.834$, $p = .007$. Post-hoc analyses showed higher values at baseline in H-SE compared to L-SE ($p = .042$). Additionally, whereas H-SE showed decreases in all periods compared to baseline (all $p \leq .018$), L-SE did not significantly change their pattern over time (all $p \geq .098$). For r-MSSD, a significant effect of Period, $F(3,90) = 3.363$, $p = .022$ was found, but not of the Period x Self-efficacy interaction, $F(3,90) = 1.263$, $p = .29$.

Variable		Total Sample	H-SE	L-SE	ANOVA
ISRA	Cognitive anxiety	61.74±4.45	53.93±6.46	67.89±5.88	F(1,33)=2.50, p=0.12
	Physiological anxiety	30.62±3.71	27.03±3.94	33.45±5.89	F(1,33)=0.73, p=0.39
	Evaluation Anxiety	62.56±4.99	54.73±6.30	68.74±7.25	F(1,33)=1.99, p=0.17
External evaluation	Performance	3.09±0.09	3.35±0,10	2.9±0.14	F(1,34)=6.07, p=0.019
	Structure	1.61±0.10	1.72±0,17	1.52±0.12	F(1,34)=0.82, p=0.37
	Content	2.66±0.10	2.7±0,17	2.6±0.13	F(1,34)=0.11, p=0.74
Situational Appraisal	Perceived performance	2.44±0.15	2.69±0.21	2.25±0.2	F(1,34)= 2.24, p=0.14

Table 4. Mean ± SEM of the ISRA Factors, external evaluation performed by the experts, and the situational appraisal of total sample and subgroups (in bold are the significant between-group differences).

For state-anxiety we found a significant effect of Period, $F(1,33)= 8.599$, $p≤.001$, and a marginal effect of the Period x Self-efficacy interaction, $F(1,33)= 3.497$, $p=.07$; participants in the H-SE group showed no changes in anxiety ($p=.485$), whereas L-SE increased significantly ($p=.001$). No main effects of Self-efficacy were found on cardiac parameters or anxiety (all $p≥.526$). Raw data for R-R and r-MSSD by period and condition and subgroups are presented in Figure 1, and for state-anxiety in Figure 2.

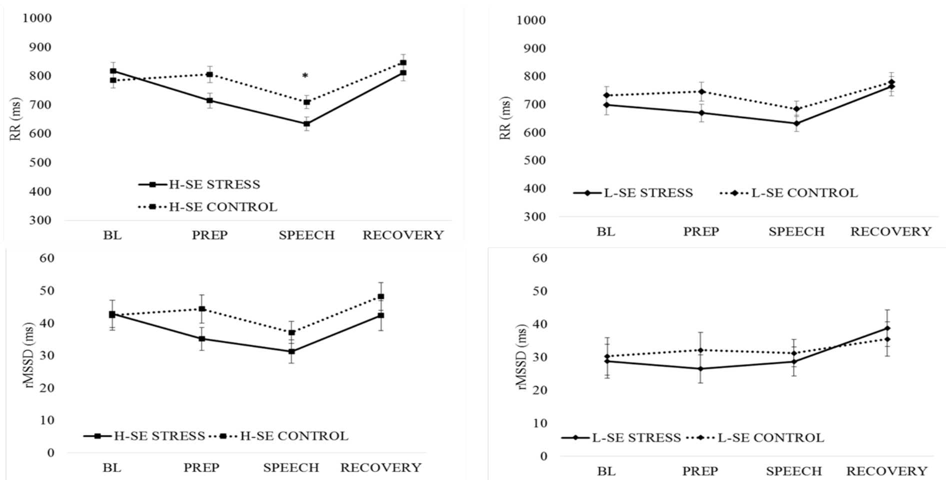

Figure 1. Time course of cardiac parameters (R–R interval and rMSSD) in the stress and control condition by group (High self-efficacy: H-SE) and (Low-self-efficacy: L-SE) (*p< 0.05). The values for both parameters were calculated using the central five minutes for each period. Lower values on both parameters indicate higher autonomic activation.

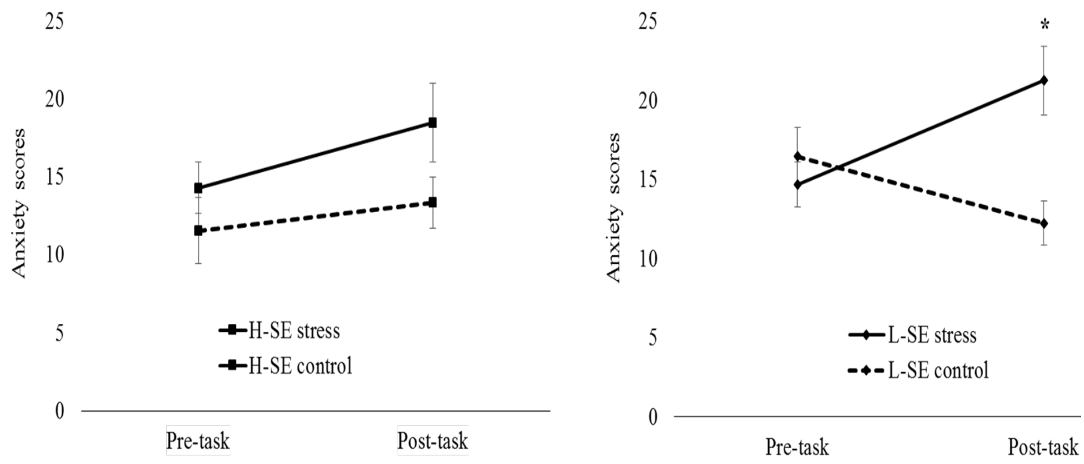

Figure 2. State anxiety (STAI-S) scores by group (High self-efficacy: H-SE) and (Low-self-efficacy: L-SE) before and after the stress and control tasks. (*p< 0.01).

Finally, one-way ANOVA with Net reactivity showed higher cardiac reactivity to stress, R-R, F(1,33)= 8.794, *p*=.006, and lower inhibition of the parasympathetic branch, r-MSSD, F(1,33)= 4.266, *p*=.047, in H-SE participants compared to the L-SE group. As a trend, H-SE participants showed higher increases in anxiety than participants in the L-SE group F(1,34)= 3.477, *p*=.07. In general, the H-SE group revealed a greater activation to social stress than the L-SE group.

3.3.2. Perceived and externally- evaluated performance

No significant differences between groups were found in perceived performance (p >0.1; see table 3), although there were significant differences in Performance evaluated by others, F(1,34)=6.07, *p*=.019. Participants in the H-SE group were rated higher than those in the L-SE group; no differences were found in either Structure, F(1,34)=0.82, *p*=.37, or Content, F(1,34)=0.11, *p*=.74.

3.3.3. Relationships among Performance and Self-efficacy, Trait anxiety and Psychophysiological stress response

We performed Pearson correlations to explore associations between performance and self-efficacy, ISRA factors and the psychological and physiological components of the stress response (Net reactivity values).

Externally-evaluated performance was positively related to perceived performance (r=.559, *p*=.001) and self-efficacy (r=.394, *p*=.02). Participants with higher self-efficacy also perceived better performance on the oral presentation, and they were rated higher by others on their presentation (higher scores on Performance).

Correlation analyses showed that the higher the self-efficacy, the lower the anxiety reactivity and the higher the cardiac activation to stress (r=-.370; r=-475; r=-.557, for state anxiety, R-R and r-MSSD, respectively; all *p*< .05). Moreover, those participants with higher increases in state-anxiety perceived better performance (r=.38, *p*=.025).

However, perceived performance correlated negatively with Test Evaluation Anxiety (r=-.383, *p*=.025) and marginally with Cognitive Anxiety (r=-.313, *p*=.071), and so those who scored higher in these dimensions of trait anxiety appraised a worse presentation. In the same direction, participants who scored higher on Cognitive and Test Evaluation Anxiety (ISRA factors) were rated lower on Performance (r=-.358, *p*=.04; r=-.312, *p*=.078, respectively), although only marginally. However, the other external evaluation factors (Structure and Content) were unrelated to the psychological and physiological elements studied (all p >.1).

Finally, as hypothesized, Self-efficacy correlated negatively with Cognitive, Physiological, and Test Evaluation anxiety (r=-.508; r=-.436, r=-.495, all *p*≤.01). Participants who scored higher on these trait anxiety factors showed lower scores on Self-efficacy.

3.4. Discussion

The main aim of the current study was to investigate the relevance of self-efficacy in the psychophysiological stress response and performance on the speaking task of the Trier Social Stress Test (TSST). To do so, we first examined how self-efficacy is related to the changes in R-R, r-MSSD and state-anxiety, as well as trait anxiety. Finally, we also explored the relationships between all these factors and the performance perceived by the person him/herself and by others.

To provoke stress, we used the TSST, and our results indicate that it was effective in triggering a stress response because the activation of the autonomic nervous system (RR and rMSSD) and changes in anxiety scores (STAI-S) were higher in the stress condition than in the control condition in the total sample (see Table 2).

We confirm the first hypothesis; participants with higher self-efficacy (H-SE) showed greater cardiac activation, reflected in lower R-R values, compared to the Low-Self-efficacy group (L-SE), although differences in parasympathetically mediated HRV (r-MSSD) were less clear. Only when we compared the groups on Net reactivity were we able to find decreased cardiac reactivity in the L-SE. In addition, the H-SE group showed less activation at baseline, reflected in higher R-R values. By contrast, although not significantly, the H-SE group showed less parasympathetic regulation compared to the L-SE group, showing lower r-MSSD values in the recovery phase. Additionally, state anxiety did not change significantly in the H-SE group, as it only increased in the L-SE group. In agreement with Salvador and Costa (2009), these results reflect an active coping style in more self-efficacious people, characterized by higher physiological activation along with lower levels of negative psychological states (anxiety). Our results also agree with Obrist's theory, which explains that in situations of active coping, the cardiac control is basically sympathetic (Obrist, 1981). Furthermore, these findings have been supported by the negative relationships between Self-efficacy and reactivity in anxiety, R-R and r-MSSD. Thus, higher self-efficacy might be assumed to imply active coping styles reflected in greater physiological arousal (less anxiety along with high cardiac activity) to deal with acute stressors satisfactorily. According to Salvador and Costa (2009), the TSST speaking task involves cognitions and perceptions related to a competitive situation because demonstrating that one is the best candidate for a job would motivate similar mechanisms to a competition, in this case, competing with the other potential candidates.

Regarding trait anxiety, we expected participants who scored higher on self-efficacy to be characterized by lower scores on trait anxiety, but our results did not show any differences between the two groups. However, we found that higher scores on Self-efficacy were related to the ISRA factors. Hence, the dimensions of trait anxiety analyzed in this study, all of them related to social evaluation, seem to be negatively related to perceptions of coping efficiently with social stress. In agreement with these findings, other studies have found negative associations between trait anxiety and self-efficacy (Calvo and Cano-Vindel, 1997; Muris, 2002) when participants were exposed to a social stress paradigm.

Regarding the quality of the performance evaluated by experts, we expected that higher self-efficacy would result in a better self-presentation. With this external assessment of the speech performance, we obtained a target performance value in order to correlate it with the cognitive perceptions of the participants. The results confirmed that participants with higher self-efficacy were rated higher by the raters on the speaking task. Only one external evaluation factor (Performance) was related to self-efficacy, but this factor explains 39.88 % of the variance, a large percentage compared to the other factors. The components of Performance (Development, Verbal fluency, Volume and Speech continuity) seem to be good indicators of the performance on an oral speech.

Coinciding with Gerin et al. (1996), we confirm self-efficacy as one of the components of active coping. Likewise, because less self-efficacy is directly related to perceiving the environment as threatening, our findings also agree with studies that have found associations between threat appraisal and poor performance in social evaluative situations (Hodgins et al., 2010; Schneider et al., 2008). In addition, we expected that people who respond with greater cardiac activity (R-R and r-MSSD) and less anxiety would perform better on the speech. Previous studies have pointed out the strong link between psychophysiological changes and the behavior displayed in acute psychosocial challenges, such as an oral speaking task in front of a committee (Troisi, 2002; Sgoifo et al., 2003; Pico-Alfonso et al., 2007; Mohiyeddini et al., 2013a, b; Villada et al., 2014b). These studies often identify behaviors that reflect passive coping styles (e.g. Flight) related to negative psychological states (anxiety and mood) (Villada et al., 2014b), or submissive behaviors with lower autonomic activation (Sgoifo et al., 2003). In these coping patterns, the way we perceive the tasks (as a challenge or as a threat) plays an important role. These cognitive perceptions and psychophysiological reactions to stress will consequently translate into a better or worse behavior outcome. However, we failed to find differences between groups allocated to high and low self-efficacy groups, or relationships between cardiac reactivity and performance. As in previous findings by Tomaka et al. (1997), our results revealed that higher self-efficacy was related to better cardiac reactivity, but this arousal activation did not translate into enhanced performance.

Furthermore, we found that increases in state anxiety only affected the perceived performance, but they did not have a significant influence on the performance evaluated by others. However, cognitive trait anxiety was negatively related to performance, suggesting that it may also influence the performance in situations that require demonstrating one’s capabilities. Thus, research focused on social anxiety has found that groups with high social anxiety generally showed lower levels of social competences or poor social performance (Gramer, 2006; Stevens et al., 2010). One interpretation of these results could be that higher trait anxiety plays a negative role in our competitive competencies and, therefore, may be followed by perceptions of defeat. Consequently, higher trait anxiety would influence our ability to deal with social stress, leading to a poor outcome, or in other words, feelings of defeat. Repeatedly experiencing this kind of situation will have negative health consequences (Griffiths et al., 2014). Therefore, our results partly confirm the hypothesis proposed, with trait anxiety being considered a more important factor to study than changes in perceived anxiety before and after a stress task.

In conclusion, perceiving a stressful situation as a threat or as a challenge usually leads to autonomic activation. This activation "per se" may not have anything to do with a good performance or outcome. Therefore, we can conclude that the psychological factors (cognitive appraisal and trait anxiety) will really be responsible for an adaptive performance, which is essential to success. But it is true that this conclusion can only be used to explain reactions and performance in contexts of acute psychosocial challenges. In agreement with this idea, Bagget et al. (1996) found that people in the low speech anxious group compared to the high speech anxious group did not differ in their cardiovascular responses; however, the high-speech-anxious group reported higher scores on anxiety and more negative appraisals of the speaking task, and their performance was judged to be worse than the low-speech-anxious group.

However, the present findings must be interpreted with caution. Some limitations should be mentioned, such as the small sample. Although we have used an intra-subject design, improving the power of the study, these results must be replicated in future studies with a larger sample. In addition, we selected a group of women taking oral contraceptives to homogenize the groups. Future research should take into account different groups of women, in order to study more in depth the coping reactions in women depending on their hormonal changes. Finally, dividing the groups by the median of the self-efficacy scores could yield misleading conclusions. For this reason, we support each analysis with Pearson correlations to corroborate or rule out the preliminary group analyses (H-SE vs. L-SE).

The present study adds new aspects to this field of research: the extension of results on heart rate variability parameters in public speaking tasks; and the examination of different psychological components, such as self-efficacy, state and trait anxiety, which are theoretically involved but not usually studied in this field, related to performance on the TSST speaking task.

CHAPTER IV

STUDY 3

STUDY 3

Coping with an Acute Psychosocial Challenge: Behavioral and Physiological Responses in Young Women

The main results of this study have been published in Plos One: Villada, C., Hidalgo, V., Almela, M., Mastorci, F., Sgoifo, A., Salvador, A. Coping with an acute psychosocial challenge: behavioral and physiological responses in young women. Plos One, 9(12): e114640.

4.1. Introduction

In recent years, growing recognition of the effect of stress on health has led to intense research on individual differences in coping with an environmental challenge, mainly employing controlled laboratory stressors. Among them, the Trier Social Stress Test (TSST) has been used extensively as a psychosocial stress paradigm (Kirschbaum et al., 1993). Due to its uncontrollability and evaluative-threat properties (Dickerson and Kemeny, 2004), it is able to provoke clear adrenocortical, autonomic and mood changes (Almela et al., 2011a,b; Het et al., 2009; Kirschbaum et al., 1999). A large amount of attention has been focused on these psychophysiological mediators of the "stress-health" relationship, while also trying to explain the considerable individual variations in the stress response and vulnerability (Foley and Kirschbaum 2010).

In young people, most of the research on acute stress was initially carried out in men, but it has been increasingly accepted that sex hormones, or their absence, play a role in the response to stress (Kajantie and Phillips, 2006). The first large impact study employing the TSST to determine the influence of sex steroids on the stress response found that men and luteal women showed a similar cortisol response, which was higher than that of women in the follicular phase and oral contraceptive users (OC users), with the latter two groups exhibiting similar cortisol, cardiovascular and mood responses (Kirschbaum et al., 1999). Since then, numerous studies have been carried out on women, but only a few studies have included women in menstrual cycle phases other than the luteal phase (Duchesne et al., 2012; Kelly et al., 2008; Preuβ et al., 2009) or women taking oral contraceptives (Kumsta et al., 2007; Rohleder et al., 2003). Overall, these studies have reported no sex differences in heart rate, although lower cortisol responses and larger increases in negative mood have been found in free-cycling women compared to men (Kelly et al., 2008; Preuβ et al., 2009). In addition, women taking oral contraceptives have generally shown a blunted cortisol response compared to free-cycling women (Cornelisse et al., 2011), specifically in the luteal phase (Rohleder et al., 2003). Most studies have shown that these differences are smaller when the follicular phase is compared to the use of oral contraceptives. In sum, there is some evidence suggesting that women's hormonal status affects the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS) responses to laboratory stressors.

Apart from their physiological responses and subjective perceptions, when people face a psychosocial stressor they adopt a set of behavioral strategies that play an important role in coping with the situation. The ethological approach is a valuable tool for studying individual differences in behavioral coping strategies, and it can provide further insight into individual responsivity to a psychosocial stressor (Pico-Alfonso et al., 2007; Troisi, 1999). Physiological and behavioral stress responses have been grouped in the so-called "active/proactive" or "passive/reactive" coping styles (Koolhas et al., 1999, 2010), whereas cognitive dimensions have been emphasized in humans (Ursin and Eriksen, 2004). Unlike the fight-or-flight response to stress (Cannon, 1932), the tend-and-befriend theory described by Taylor (2000) proposes that women reveal an adaptive reaction to stress that is more related to affiliative behaviors, which promote social interactions. Therefore, studying behavioral stress responses in women could help providing insight on the relationship between behavioral style of coping and physiological stress responsivity.

To the best of our knowledge, only a few studies have been carried out in healthy young people using an ethological approach to obtain additional information about the interrelationships among different psychophysiological markers of stress responsivity. Sgoifo et al. (2003) compared men and women (undifferentiated phase of the cycle) faced with a social stressor (interview) (Sgoifo et al., 2003). A few years later, using the same experimental approach, Pico-Alfonso et al. (2007) compared women in the follicular and ovulatory phases (Pico-Alfonso et al., 2007). These two studies found several interesting relationships between psychophysiological changes and a number of behavioral patterns displayed during the stressful task. For example, escape (flight) behavior (e.g. look away or look down: social contact is temporarily broken off by disengaging from any interaction) was negatively related to cortisol response (Sgoifo et al., 2003), whereas cardiac activation was positively associated with submissive behavior (e.g mouth corners back or lips in) and negatively with displacement behavior (Pico-Alfonso et al., 2007). Recently, it has been suggested that displacement behaviors (a set of behavioral patterns that consists of movements which are focused on one's own body such as hand-face or scratch) would regulate the cardiovascular stress response in men but not in luteal women (Mohiyeddini et al., 2013a), whereas the relationship between behavior and physiological stress response in women seems to be modulated mostly by the subjective perception of stress (Mohiyeddini et al., 2013b).

The purpose of the present study was to analyze the behaviors displayed during the TSST and their relationships with (i) the responses of the two most frequently employed biomarkers of acute stress response (cortisol and heart rate) and (ii) the mood experienced by women in the follicular phase or taking oral contraceptives. These two groups, which are both characterized by low oestrogen concentrations, were shown to have similar cortisol, heart rate, and mood responses to the TSST (Kirschbaum et al., 1999; Cornelisse et al., 2011).

At first we wanted to assess whether the psychophysiological response to stress was different between the two groups of women. Although, according to the available evidence, dramatic differences were not likely to be found among follicular women and those making use of contraceptives, we believe that such a comparison could provide an additional piece of information to the available literature. In addition, we hypothesized that acute physiological stress reactivity is modulated by the behavioral patterns of response exhibited during the speech task. More specifically, we anticipated that the intensity of cardiovascular and cortisol responses are associated with the amount of submissive and escape behaviors, which are commonly associated with a passive coping style (Pico-Alfonso et al., 2007; Sgoifo et al., 2003). We also expected to confirm previous findings suggesting that displacement behavior is tightly linked to individual physiology and anxiety (Mohiyeddini et al., 2013a,b); in other words, we anticipated a positive relationship between displacement behavior and pre-stress levels of anxiety, and negative relationships with baseline and stress response values of heart rate (Pico-Alfonso et al., 2007; Mohiyeddini et al., 2013a,b; Sgoifo et al., 2007). Moreover, given that these groups of women have never been studied from an ethological perspective, we aimed at exploring the relationships among behavioral and psychophysiological stress responses also split by group, to provide new information to the literature considering the menstrual cycle phase and the use of oral contraceptives.

Finally, we aimed at exploring possible associations between coping strategies (as detected via the evaluation of self-reports) and non-verbal behavior displayed during the speech test, which have never been reported in the literature so far.

4.2. Methods

Ethics Statement

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.

4.2.1. Participants

A total of 107 female volunteers, recruited through advertisements in the university campus, were interviewed and completed a questionnaire to find out whether they met the study prerequisites. The criteria for exclusion were: smoking more than five cigarettes a day, alcohol or other drug abuse, visual or hearing problems, presence of a 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 cardiac, emotional or cognitive function, or one that was able to influence hormonal levels, such as glucocorticoids or β-blockers.

Finally, 34 women (between 18 and 29 years old) participated in two sessions in a counterbalanced order. All of them were nulliparous with no gynecological problems. Half were free-cycling, with regular menstrual cycle lengths of between 24 and 36 days. The follicular phase was chosen because it is the most reliable in the absence of sex steroid analyses; in addition, we selected the early follicular phase (2-5 days after the beginning of the menstruation) because this period has been studied less in this research field. The other half of the women had been taking oral contraceptives (monophasic formulas) for at least 6 months. Socio-demographic data are presented in Table 1. Most of the participants (94%) were college students from different fields (psychology, medicine, occupational sciences, among others), and none of the participants received academic or economic compensation for their participation.

Participants meeting the criteria were contacted by telephone and asked to attend experimental sessions that took place in a laboratory at the Faculty of Psychology (University of Valencia, Spain). Before each session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to only drink water, and not eat or take any stimulants such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session.

	OC user (N =17)	Follicular (N =17)	Total Sample (N =34)
BMI (Body Mass Index) Kg/m2	21.54 ± 0.68	21.85 ± 0.69	21.70 ± 0.48
Age (years)	20 ± 0.707	22.59 ± 0.72	21.29 ± 0.545
SES (scores 1-10)	6.47 ± 0.273	6.12 ± 0.225	6.29 ± 0.177
Physical Exercise (scores 0-7)	3.41 ± 0.59	3.63 ± 0.54	3.52 ± 0.39

Table 1. Demographic and anthropometric characteristics of total sample and subgroups (Mean scores ± SEM) of age, Body Mass Index, Subjective Socioeconomic Status (Subjective SES scale: Adler et al., 2000) and physical exercise.

4.2.2 Study protocol

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.

In both conditions, each session lasted approximately 1 hour, and they were always held between 16.00 and 19.00 hours. Each participant started her two sessions at the same time of the day. In the first session, upon arrival at the laboratory, the participants' weight and height were measured, and the experimenter checked whether they had followed the instructions given previously. In the last part of this first session, all the participants completed the personality questionnaires.

Stress Condition. To produce a stress response, the participants were subjected 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 (see Kirschbaum et al. 1993, for details), and it was performed in front of a committee composed of a man and a woman (both university teachers in the psychology department). The participants remained standing at a distance of 1.5 meters from the committee. Additionally, a video camera and a microphone were clearly visible, and the speech task was video recorded.

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 this phase started, baseline measures were obtained for anxiety (Stai-S) and mood (PANAS). After the habituation phase, the introduction phase started (duration 3 min). In this phase, 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 in the first room. During this period, they provided a saliva sample (-5 min pre-stress). Following the preparation phase, the stress test was carried out. Subjects had 20 minutes to recover after the 10-min stress task; during this period, they again answered the two questionnaires (Stai-S and PANAS) and provided the second saliva sample (+15 min post-stress). The participants provided the last saliva sample 25 minutes later (+40 min post-stress).

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, but not in front of an audience. In the preparation phase, the participants did not prepare for their task; instead, they read a book with neutral content. The times for the saliva samples, the questionnaires, and the phase durations were the same as those described for the stress condition.

4.2.3. Biochemical analyses

4.2.3.1. Cortisol

Participants provided three saliva samples of 3 ml each in plastic vials. They took approximately 5 minutes to fill the vial. The samples were frozen at - 80° C until the analyses were performed. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Coat-A-Count C (DPC, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Assay sensitivity was 0.5 ng/mL. The findings are expressed in nanomolar units (nmol/L). For each participant, all the samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%.

4.2.4. Heart rate measurements

Heart Rate (HR). HR was continuously recorded in the experimental and control conditions using the heart rate monitor Suunto ® T6 (Suunto Oy, Vantaa, Finland), which consists of a chest belt for detection and transmission of the heartbeat and a “watch” for data collection and storage (Radespiel-Troger et al., 2003; Roy et al., 2009). Heartbeat detection is performed with an accuracy of 1 ms, every heartbeat is transmitted and stored in the flash memory of the watch. The recording periods when participants were walking from one room to another were removed, and only the 5 central minutes of each phase - namely (from -20 to -15 min for baseline, from -6 to -1 min for preparation, from 0 to +5 for speech and from +15 to +20 for recovery) - were used to calculate the participants’ average heart rate values. After eliminating the artifacts, the HR mean for each phase was computed. HR artifacts and HR analysis were performed with Kubios software (Biomedical Signal Analysis Group, University of Kuopio, Finland).

4.2.5. Coping Strategies

The dispositional version of the COPE Inventory is a theoretically driven self-report questionnaire that addresses different ways of coping (Carver et al., 1989). Subjects must indicate what they generally do and feel when experiencing stress. Items are rated on a 4-point scale, ranging from 1 (I don't usually do this at all) to 4 (I usually do this a lot). We employed the Spanish version of the long form, which consists of 60 items from 15 subscales (such as Planning, Seeking Instrumental Support, Suppression of Competing Activities, Restraint Coping, Venting of Emotions, among others). With a second-order factor analysis, they can be grouped in five basic coping domains: behavioral, cognitive and emotional coping measures (active coping), and behavioral and cognitive avoidance (passive coping). The Spanish version of the scale had Cronbach's alphas ranging from 0.78 to 0.92 (Crespo and Cruzado, 1997).

4.2.6. Psychological assessment

4.2.6.1. Anxiety

To assess state anxiety, the Spanish version of the State Anxiety Inventory was used (STAI form E) (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 Cronbach's alphas ranging from 0.90 to 0.93 (Seisdedos, 1988).

4.2.6.2. Mood

The mood was evaluated by the Spanish version (Sandin 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). Sandin et al. (1999) reported a high internal consistency for the Spanish version, with Cronbach's alphas for PA ranging from 0.87 to 0.89, and for NA from 0.89 to 0.91.

4.2.7. Ethological analysis

The participants' behavior during the speech task of the TSST was quantified by means of the Ethological Coding System for Interviews (ECSI) (Troisi, 1999). The interview was videotaped with a camera adjusted so that the subject's face and trunk were in full view. Subsequently, behavioral assessment was carried out according to Troisi and colleagues (Pico-Alfonso et al., 2007; Troisi, 1999; Sgoifo et al., 2007). This version of the ECSI includes 32 different patterns, mostly facial expressions and hand movements. The ECSI was specifically designed to measure non-verbal behavior during stress interviews by combining behavior patterns described in published human ethograms (Troisi, 1999). The 32 behavioral patterns were then grouped in seven behavioral categories, each reflecting a different aspect of the subject's emotional and social attitude, namely: (1) eye contact; (2) affiliation; (3) submission; (4) flight; (5) assertion; (6) gesture; (7) displacement. The score of a given behavioral category was expressed as the sum of the percentages of all the behavioral patterns belonging to it.

4.2.8. Statistical analyses

One-way ANOVAs were used to analyze differences between groups in the demographic/anthropometric variables and behavioral patterns. We employed Group (follicular women vs. OC users) as a between-subject factor and Condition (stress vs. control) as a within-subject factor. ANOVAs for repeated measures were used to assess the effects on mood, anxiety, cortisol and heart rate. For the mood and anxiety analyses, we added a within-subject factor: pre and post task. For the HR analyses, we added Time (-20, -5, +5, and +15 min) as a within-subject factor; for the cortisol analyses, we also added Time (-5, +15, +40 min) as a within-subject factor. Additionally, cortisol and stress-induced HR reactivity were also quantified as the area under the response time curve to ground (AUC_G) (Pruessner et al., 2003); finally, for anxiety and mood changes, the differences between post-task and pre-task scores (Delta) were calculated.

We checked for order effects (whether the stress or control condition was first) by using an ANOVA for repeated measures, which did not reveal any effect of order (all $p > 0.18$).

Pearson's correlations (two-tailed) were calculated in order to assess whether the physiological (basal levels and AUC_G) and psychological values (basal levels and Deltas) were related to the behavioral patterns. For the analysis of ethological data, six subjects were removed: 3 OC users due to technical problems with the images analyzed, and 3 women in the follicular phase on the basis of the $p > 0.001$ criteria for Mahalanobis distances for eye contact behavior. 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 otherwise specified, results shown are means

± standard error of means (SEM). We used SPSS 19.0 to perform the statistical analyses.

4.3. Results

4.3.1. Cortisol

ANOVAs for repeated measures showed significant effects of Condition, $F(1,32)=7.043$, $p = .012$, $\eta^2_p = .108$, Time, $F(1.362,43.569) = 5.387$, $p = .016$, $\eta^2_p = .144$, and the Condition×Time interaction, $F(1.34,42.893) = 21.867$, $p < .001$, $\eta^2_p = .406$. Post-hoc analyses revealed that there were no baseline differences between conditions, $p = .265$, whereas after the task (+15 min and +40 min time points), differences between conditions were found (both $p < .01$).

A marginal Condition×Time×Group interaction was found, $F(1.34, 42.893) = 3.65$, $p = .051$, $\eta^2_p = .101$. Figure 1 shows no statistically significant differences between groups (follicular and OC users) at any time point within each condition (stress and control) (all $p > .176$).

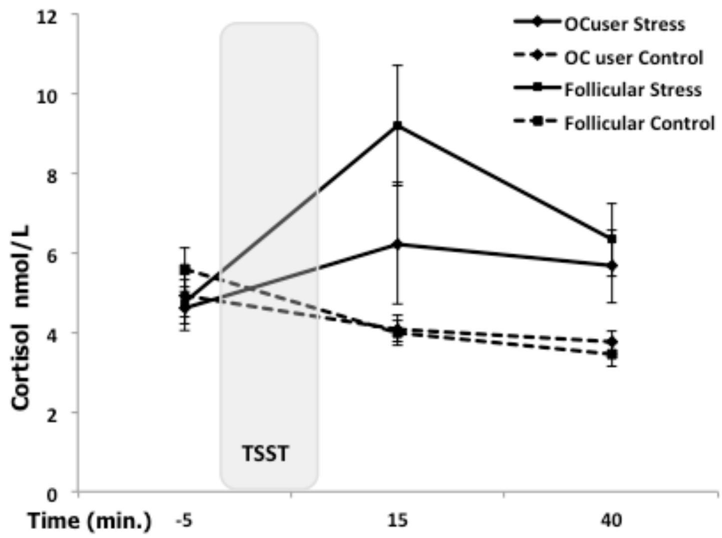

Figure 1. Mean values ±SEM salivary cortisol response (nmol/L) during the stress and control conditions by group (*p < 0.05).

Women in the early follicular phase showed significantly higher cortisol levels in the stress condition than in the control condition at +15 min and +40 min samples (all $p < .01$). OC users did show a similar difference in the time course of cortisol levels between the stress and control condition, although statistical significance was not reached (+15min, $p = .171$; +40 min, $p = .051$).

4.3.2. Heart Rate (HR)

ANOVA for repeated measures revealed a significant effect of the Condition×Time interaction, $F(2.236, 67.091) = 16.856$, $p < .001$, $\eta^2_p = .228$. No differences between conditions (stress vs. control) were found in the baseline [stress condition: (83.97±1.34) vs. control condition (85.43±1.69)] and recovery periods [stress condition: (78.94±1.62) vs. control condition (77.16±1.53)] (both $p > 0.1$), but there were higher HR values in the stress condition than in the control condition in the two key periods of the TSST: Preparation [stress condition: (90.31±1.89) vs. control condition (83.25±1.6)]and Speech [stress condition: (101.89±2.58) vs. control condition (93.29±1.77)] (both $p < .01$). No significant effects of Group or other interactions were found.

4.3.3. State Anxiety

A repeated-measures ANOVA of the STAI scores revealed significant effects of Condition, $F(1, 32) = 25.826$, $p < .001$, $\eta^2_p = .447$, Time, $F(1, 32) = 21.268$, $p < .001$, $\eta^2_p = .399$, and the Condition×Time interaction, $F(1, 32) = 31.511$, $p < .001$, $\eta^2_p = .526$. Post hoc analyses revealed that anxiety increased significantly after the stress condition ($p < .001$) and slightly decreased after the control condition ($p = .076$). A main effect of Group was also observed, $F(1, 32) = 5.233$, $p = .029$, $\eta^2_p = .141$: overall, women in their follicular phase obtained significantly higher scores on anxiety than the OC users (Mean±sem for follicular women: 20± 1.4; for OC users: 15.4± 1.41).

4.3.4. Mood

For positive mood, significant effects of Time, $F(1, 32) = 10.242$, $p=.003$, $\eta^2_p = .242$, Group, $F(1,32)=11.865$, $p=.002$, $\eta^2_p = .27$, and the Condition×Time×Group interaction were found, $F(1, 32)=4.774$, $p=.036$, $\eta^2_p=.13$. Women in their follicular phase showed a decrease in positive mood after the stress task, compared to their pre-stress scores ($p< .001$), whereas OC users did not show stress-related changes in this parameter ($p= .17$). Significant, between-group differences were observed after the TSST, with follicular women showing lower positive mood than OC users ($p= .002$) (see Figure 2).

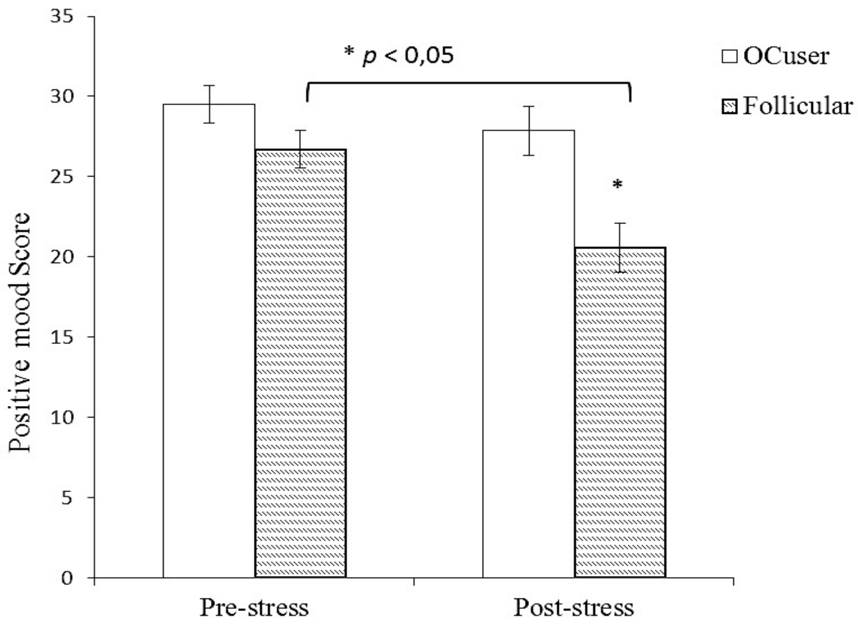

Figure 2. Mean values ± SEM of positive mood scores during the stress condition by group (*p <.01).

For negative mood, significant effects of Condition, $F(1, 32)= 24.308$, $p<.001$, $\eta^2_p = .432$), Time, $F(1, 32)=11.724$, $p=.002$, $\eta^2_p=.268$) and the Condition × Time interaction were found, $F(1, 32) = 40{,}431$, $p< .001$, $\eta^2_p=.558$. Overall, negative mood increased after the stress task ($p < .001$) and decreased after the control task ($p < .001$). No significant effects of Group or interactions were found ($p > .1$). [Stress condition: pre-task (12.9±0.4) vs. post-task (18.1±1.04); control condition: pre-task (13.8±0.6) vs. post-task (12.1±0.4)].

4.3.5. Ethological data (ECSI)

Table 2 summarizes the behavioral response exhibited by the two groups of women (follicular and OC users) during the speech task, each behavioral category being quantified as cumulative percentages. The overall sample was characterized by higher values of flight and affiliation behaviors compared to the other behavioral categories analyzed.

Ethological Data	OC users (n=14)	Follicular (n=14)	Total Sample (n=28)
EYE CONTACT	**83.21 ± 3.03***	**93.57 ± 1.84***	88.39 ± 2.01
FLIGHT	120 ± 8.25	134.64 ± 7.27	127.32 ± 5.57
SUBMISSION	17.85 ± 3.73	22.14 ± 4.53	20 ± 2.90
AFFILIATION	116.07 ± 10.99	122.14 ±14.63	119.11 ± 9.0
GESTURE	17.14 ± 7.99	2.85 ± 2.85	10 ± 4.38
DISPLACEMENT	76.42 ± 8.91	82.14 ±13.18	79.28 ± 7.82
ASSERTION	**9.64 ± 3.03***	**28.92 ± 5.86***	19.28 ± 3.73

Table 2. Total sum of the behavioral patterns' values of each behavioral category (% mean ± SEM) displayed during speech (ECSI) (In bold are the categories with significant between-group differences, both $p = 0.007$).

Women in their follicular phase displayed higher percentages compared to OC users, for all the behavioral categories, except for Gesture (Table 2). However, statistically significant differences between groups only appeared of them: Eye Contact, $F(1,27) = 8.482$, $p = .007$, and Assertion, $F(1,27) = 8.534$, $p = .007$, (Table 2).

4.3.6. Relationships among Behavioral and Psychobiological responses to the TSST

As the differences between groups in the stress response (cortisol, heart rate and subjective stress response) were rather sporadic, and in order to facilitate potential comparisons with results of previous studies (Pico-Alfonso et al., 2003; Sgoifo et al., 2007), correlations were performed considering the two female groups as a whole.

The amount of displacement behavior exhibited during the speech task was negatively related to the basal levels and AUC_G of the HR (r=-.545, *p*=.004; r=-.492, p=.013, respectively) and positively related to the degree of anxiety experienced before the TSST (r=.448, p=.017). Furthermore, the amount of eye contact correlated negatively with negative mood before the stress task (r= -.375, *p* = .05). The expression of behavioral patterns included in the assertion category (low-aggressiveness) was positively related to $_{Delta}$negative mood (r= .415, *p*=.028), and the anxiety reaction to stress (r=.378, *p*=.047), but inversely to $_{Delta}$positive mood (r= -.570, *p*=.002).

Finally, submissive behaviors during the speech were negatively related to basal cortisol levels (r= -.402, *p*=.034), but did not reach significance with the AUC_G values of this hormone (r= -.320, *p*=.09).

Subsequently, given that these groups did show some differences in cortisol, anxiety and mood, , and with the aim of providing new information on the relationship between neuroendocrine and behavioral stress responsivity, correlation analyses were also performed split by group.

We found out that the relationships previously found for the study group as a whole were confirmed for the group of women in the follicular phase. In addition, OC users showed: (i) positive associations of both Affiliation and Flight with the basal levels and the AUCg of cortisol; (ii) negative relationship between anxiety and negative mood (see table 3).

Follicular group		BL_Cort	AUCgCort	BL_HR	AUCgHR	STAIpre	Delta STAI	Delta mood (+)
	SUBMISSION	r=-0,559 *p*= .038	r=-0,479 *p*=.083	ns	ns	ns	ns	
	DISPLACEMENT	ns	ns	r=-0,692 *p*=.009	r=-0,585 *p*= .036	r=0,56 *p*=.037	ns	
	ASSERTION	r=0,532 *p*=.05	ns	ns	ns	ns	r=0,667 *p*= .009	r=-0,549 *p*=.042

OC users group		BL_Cort	AUCgCort	Delta STAI	Delta mood (-)
	FLIGHT	ns	ns	r=0,721 *p*= .004	r=0,662 *p*= .01
	AFFILIATION	r=0,718 *p* = .004	r=0,584 *p*= .028	ns	ns

Table 3. Pearson correlations split by group between psychophysiological basal levels, AUCg of cortisol and HR, anxiety and mood reactivity with the behavioral patterns performed during the speaking task of the TSST.

4.3.6. Coping styles (COPE)

A one-way ANOVA with a second-order factor analysis of the COPE questionnaire showed a main effect of Group for Factor $I_{(behavioral\ coping)}$, F(1, 32) = 7.036, *p*=.012, η^2_p = .180, Factor $II_{(cognitive\ coping)}$, F(1, 32) = 7.298, *p*=.011, η^2_p = .186, and Factor $IV_{(emotional\ coping)}$, F(1, 32) = 4.366, *p*=.045, η^2_p = .12. Follicular women had lower scores than their OC counterparts on all these factors (Figure 3), reflecting differences in the dimensions of active coping. No differences between groups were found in dimensions that indicate passive coping (all *p* > .397).

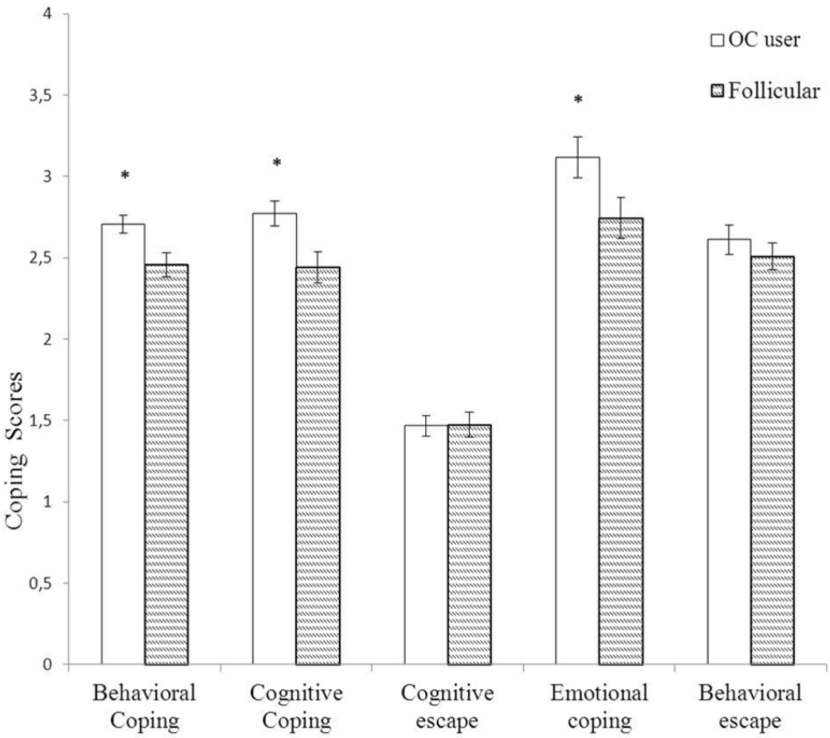

Figure 3. Mean values ± SEM of the five second-order factors of the COPE questionnaire that represents active coping (Behavioral coping, Cognitive coping, and Emotional coping) and passive coping (Cognitive escape and Behavioral escape) (*p <.05).

A few significant correlations were found between the basic coping styles and some behavioral categories. Specifically, active coping correlated negatively with assertion and eye contact (r= - .450, *p*=.016; r= - .461, *p*=.014, respectively), and marginally with the amount of affiliation exhibited (r= .362, *p*=.058).

4.4. Discussion

The present study investigated the subjects' physiological response, subjective anxiety and mood, as well as their nonverbal behavior, while undergoing the Trier Social Stress Test (TSST). This study provides new insights on the relationship between the patterns of behavior displayed during a public speaking task and a number of psychobiological stress-related changes. To this purpose, women in their follicular phase and women taking oral contraceptives were exposed to an experimental condition and a control condition in a within-subject design.

Our results confirmed that the TSST provoked significant changes in cortisol, heart rate, anxiety and mood, in accordance with other studies that have examined both psychological and physiological responses to this type of laboratory stressor (Kelly et al., 2008; Cornelisse et al., 2011; Childs et al., 2010; Kudielka et al., 2004a,b). As originally hypothesized, stress-induced cortisol and heart rate increases were modest but significant, and no major differences were found in the physiological stress response of oral contraceptive users compared to women in their follicular phase, these results largely resembling those reported by Kirschbaum et al. (1999). The blunted cortisol response of OC users is also in accordance with a previous study on adolescent women, where another psychosocial stress paradigm (Groningen Social Stress Test) was employed (Bouma et al., 2009).

We observed a marked decrease in positive mood following the stress episode only in women in their follicular phase, suggesting that they are more sensitive to stress-induced changes in mood. Interestingly no significant differences were found on this regard in two previous studies that compared free-cycling women with OC users (Cornelisse et al., 2011; Bouma et al., 2009). Recently, however, an association between psychological complaints and the follicular phase of the menstrual cycle has been reported (Guillermo et al., 2010). It is worthwhile to note that women in our study were tested in the earliest follicular phase, which means that their stress-induced, short-term psychological consequences could be due to the discomfort of menstruation symptoms themselves. Although this discomfort was not objectively measured in this study, we found that women in the early follicular phase also showed higher scores on overall anxiety, and also displayed a larger amount of eye contact and assertive behavior compared to OC users during the TSST. In this line, Troisi et al. (1999) found a positive relationship between anxiety and eye contact, which seems to agree with the association found in our study. In addition, women in the follicular phase also displayed a slightly larger amount of flight, submission and displacement behaviors. In summary, it appears that women in an early follicular phase are psychologically more sensitive to stress, and this is also reflected in their non-verbal response.

Regardless group differences, we confirmed the hypothesis that the most common patterns of behavior displayed by women in response to an acute social stressor were flight and affiliation (see Table 2). These results point, on one hand, to the pattern of flight response described by Cannon (1932) and, more importantly, to the tend-and-befriend strategy described by Taylor et al. (2000); in other words, women seem to adopt a gender specific strategy of behavioral coping, making a large use of patterns of affiliative behavior as an adaptive stress response.

The COPE scores provide information about the way an individual usually copes with stress situations. Although we did not expect to find dramatic differences between women groups, our study revealed that coping strategy was not fully the same between OC users and non-users. Indeed, follicular women obtained lower scores in the behavioral, cognitive and emotional dimensions of active coping compared to OC users. An interesting implication of this evidence is that the increasing use of oral contraceptives in young women and the associated differences in coping styles compared to non-users suggest that future studies should focus on this specific female population.

Although the available literature on acute physiological stress responsivity underscores the similarities between follicular women and OC users, our data point to undeniable differences in psychological and behavioral parameters. These differences could be explained by hypothesizing greater mood stability in OC users (Guillermo et al., 2010) and higher anxiety and negative feelings in follicular counterparts, as recently suggested by other authors (Oinonen et al., 2002; Walder and Mazmanian, 2012).

In our study we failed to find clear relationships between the most frequent categories of behavioral stress response (affiliation and flight) and concurrent psychophysiological changes for the group as a whole. However, in OC users group we found positive relationships between these categories and the basal levels and AUCg of cortisol, but negative ones with mood and anxiety reactivity. These results support the two common theories of stress from a biological perspective, that is, OC users showed the most typical stress reactions (flight and affiliation) when they have higher levels of cortisol. The hypothesized associations between heart rate values and cortisol levels with submissive behavior were confirmed, namely stress tachycardia and hypocortisolism were associated with high scores of submission. Moreover, baseline cortisol levels were negatively correlated with the amount of submission during the test: the lower the resting activity of the HPA axis the larger the use of submissive patterns of nonverbal behavior during the acute psychosocial challenge. In other words, a highly submissive strategy of coping with a stressor appears to be anticipated by a lower HPA axis activity. This evidence is in line with the general view that high cortisol levels prior to a challenge are functional to an active engagement with the stressful situation (physiological anticipation) (Salvador 2005; Salvador and Costa, 2009); in the present study, low cortisol levels allowed only a submissive/passing strategy of coping with the TSST.

Another interesting outcome of this study was the negative relationship between the overall heart rate response to the stressor (AUC_G) and displacement. This result agrees with a recent study in luteal women that found a negative relationship between the AUC_G of heart rate and displacement behavior (Mohiyeddini et al., 2013b). These behavioral-autonomic associations seem to confirm the view on the dearousing properties of displacement behaviors (Spruijt and Rousseau, 1992) and suggest that they likely represent a successful behavioral strategy for promoting a prompter return to baseline homeostatic conditions. In addition, we also found that assertion (consisting of facial expressions and head movements that signal low-level aggression and hostility) and eye contact are linked to negative mood states. This result resembles what is reported in Troisi (1999), who described a higher percentage of assertive behavior in depressed women compared to healthy counterparts.

When behavioral-physiological correlations where performed within each women group, findings were replicated only for women in the follicular phase. We can speculate that estrogens' concentrations may be regulating the behavioral stress response. These results highlight the importance of controlling for the menstrual cycle phase and the use of oral contraceptives when studying the behavioral stress response with the neuroendocrine regulation jointly.

The finding that the active coping dimension of the COPE questionnaire correlated negatively with assertion and eye contact supports the relationships found between changes in mood and assertion and eye contact behaviors. It seems that women who usually face stressful situations with a less active coping strategy do also decreased positive mood and exhibit low-level aggressive behavior.

To the best of our knowledge, this is the first study that analyzed the behaviors displayed during and the psychophysiological responses to the TSST in two different groups of young, healthy women. Recently, two studies analyzed displacement behaviors during the TSST, but only in luteal women, or compared to men (Mohiyeddini et al., 2013 a,b). This is the first time that OC users' behavior analyzed during the TSST, together with their psychophysiological stress response and compared to women during the follicular phase with ECSI and COPE questionnaire. One could speculate that the use of oral contraceptives is influencing several factors of the stress response, modulating, not only the physiological stress response (e.g. cortisol), but also the psychological (cognitive states and coping styles as a trait) as well as the behavioral stress reactions, i.e. the ability to face it.

In the present study, in spite of the relatively small sample size, differences in coping behavior between follicular women and oral contraceptives were found. We believe that these results, besides supporting previous findings in different groups of women, also provide relationships involving behavioral and physiological stress responsivity, such as the negative association between cortisol and submission. This finding looks important in view of a clearer definition of the biological substrates of active vs. passive coping strategies (Koolhaas et al., 2010). In addition, we wish to highlight the inverse relationships between the amount of displacement behavior during the TSST and heart rate responses on one hand, and anxiety levels (obtained via questionnaires) on the other hand. They underscore the importance of distinguishing - and possibly combining in the same study- self reports with objectively measured behavioral and physiological responses.

Limitations of this study

The results of this study are be interpreted with caution, due to the large number of correlations performed with a relatively small sample of subjects. The appropriateness of using multiple comparisons in such a situation is often debated, due to the increases of type I error. However, the choice of multiple comparisons corrections also increases the possibility of carrying type II error, thus hampering potentially important findings. Undoubtedly, future studies with larger samples are needed to further validate these results.

Another limitation of this study is that it did not include for comparison groups of women in other phases of the menstrual cycle. More so, future research should also control for the menstrual cycle phase of OC users when measuring coping styles, in order to perform a more reliable comparison with other groups of free cycling women.

CHAPTER V

STUDY 4

STUDY 4

The influence of coping strategies and behavior on the physiological response to social stress in women: the role of age and menstrual cycle phase

The main results of this study are under review: Villada, C., Espin, L., Hidalgo, V., Rubagotti, S., Sgoifo, A., Salvador, A. The influence of coping strategies and behavior on the physiological response to social stress in women: the role of age and menstrual cycle phase.

5.1. Introduction

The main stress response systems involve physiological responses of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA). Both systems have been studied exhaustively, although differences in the regulation of the physiological stress response due to changes in reproductive hormones are not yet clear. In women, changes in hormonal levels induced by the menstrual cycle and menopause may modulate the changes observed with aging (Kajantie and Phillips, 2006).

Regarding the effects of changes in reproductive hormones due to menopause, few studies have focused on age differences in women considering the physiological stress response, and contradictory results have been reported. After menopause, the physiological stress response to social stress does not seem to differ from that of younger women in terms of cortisol (Kudielka et al., 1999; Hidalgo et al., 2014) or heart rate (Kudielka et al., 1999). Although no age differences have been found in the response to stress, there appears to be a lower capacity to recover from stress in older women compared to young women (in the follicular phase) (Pattachioli et al., 2006; Kudielka et al., 2004a). These findings agree with the concept of an HPA negative feedback dysregulation in the older population (Wilkinson et al., 2001), which is more prominent in women (Otte et al., 2005). This diminished capacity to recover could be associated with lower levels of estrogen in women, which would lead to cardiovascular diseases due to the protective properties of sex steroids (Gordon et al., 1978).

So far, most of the research focused on young women shows a greater HPA axis reactivity (salivary cortisol) in response to social stress in the luteal phase than in the follicular phase or in women taking oral contraceptives (Espin et al., 2013; Kajantie and Phillips, 2006; Kirschbaum et al., 1999), although some studies failed to find these differences (Walder et al., 2012). Likewise, greater catecholaminergic reactivity to social stress has been found in the luteal phase than in the follicular phase (Childs et al., 2010; Gordon and Girdler, 2014); however, no effects of the menstrual cycle phase have been found on heart rate and blood pressure reactivity

(Gordon and Girdler, 2014; Kirschbaum et al., 1999) or heart rate variability changes in response to social stress (Pico-Alfonso et al., 2007). As a result, HPA axis activity may be more sensitive to changes in reproductive hormones than the autonomic nervous system. In addition, women's hormonal status seems to have an effect on psychological perceptions. For example, Guillermo et al. (2010), in a study of psychological, social, and behavioral aspects of steroid hormones, found higher negative feelings and anxiety during the follicular phase compared to the luteal and mid-cycle phases. Moreover, when psychological changes have been measured in reaction to an acute social stressor, higher scores on anxiety and anger-hostility in women in the early follicular phase compared to women in the middle luteal phase have been reported (Walder et al., 2012). A negative relationship has also been found between negative mood states and the cortisol response to stress in women in the follicular phase, while these associations were positive for women in the luteal phase (Duchesne et al., 2011; Walder et al., 2012). Taken together, these findings highlight the relevance of the menstrual cycle phase in the physiological stress response, as well as its interactions with psychological states.

Behavior could be considered an output of the stress response processes that is closely related to coping strategies, which in turn will interact with the stress response. Previous studies from our laboratory have already compared the behavior and the psychophysiological stress response in young women, comparing women taking oral contraceptives (OC users) with free-cycling women (during the follicular phase) (Villada et al., 2014). In this study, we found that women in their early follicular phase were more sensitive to stress, displaying a slightly larger amount of flight, submission and displacement behaviors, and a significantly larger percentage of assertive behaviors (low-aggressiveness) compared to OC users. In addition, we found that passive coping styles (e.g. flight) were related to negative psychological states (anxiety and mood), and submissive behaviors were related to lower basal cortisol levels. To our knowledge, few previous studies on this topic have been carried out. One study compared women in different phases of the menstrual cycle, and no differences were found in the behavioral patterns displayed based on their hormonal status (Pico-Alfonso et al., 2007); nor did any group differences emerge when men and women were compared (Sgoifo et al., 2003). However, these studies found some relationships between behaviors displayed during a speaking task and physiological changes, suggesting that submissive behaviors were related to lower autonomic activation (Sgoifo et al., 2003). Regarding the role of behavior in the capacity for cardiac recovery, in women, displacement behaviors during social stress have been found to reduce heart rate at recovery, whereas the patterns included in the submission category produced the opposite effect (Pico-Alfonso et al., 2007). However, behavior as a modulator of the self-regulation systems has not yet been studied in the older population.

In addition, in the study of social stress, the role of coping styles should also be taken into account because they contribute to a more comprehensive understanding of the psychological and neuroendocrine responses to this type of stress. In a previous study carried out with young men and women, we found greater cortisol response, along with lower psychological response, in individuals who scored higher on active coping styles. However, individuals who scored higher on coping focused on emotions and mental disengagement showed a stress response pattern characterized by lower cortisol response and higher affective increases (Villada et al., in press). Indeed, active coping has been associated with an optimal activation of the autonomic nervous system (ANS) and cortisol release, whereas passive coping is characterized by an inefficient autonomic and cortisol response (Salvador, 2012). Hence, a different psychobiological stress response pattern could be associated with distinct ways of coping with social stress, emphasizing the importance of individual differences.

Considering the aforementioned studies, we want to expand the findings about coping styles and behavior in young women dealing with an acute social stressor, taking into consideration different phases of the menstrual cycle (follicular and luteal), and comparing them to women in menopause. To do so, we aim to analyze the psychophysiological stress response, measured by cortisol and heart rate, and the mood experienced. The second aim was to investigate the role of age and hormonal status in the behavioral patterns displayed during the speaking task of the Trier Social Stress Test (TSST). And finally, we aim to explore how certain social behaviors and coping styles measured by self-reports may influence the neuroendocrine capacity to react to and recover from social stress.

Based on previous studies, we expect to find a greater cortisol response in young women, especially in women in the luteal phase (Espin et al., 2013; Kirschbaum et al., 1999; Kudielka et al., 1999; Patachioli et al., 2006), and a lower HPA regulation in menopause (Otte et al., 2005), that is, a worse capacity to recover in post-menopausal women. Although there are mixed results in the literature about the effect of hormonal status on cardiac reactivity to stress, we expect to find a greater heart rate response during the luteal phase (Kudielka and Kirschbaum, 2005; Kajantie and Phillips, 2006).

We hypothesized that acute physiological stress reactivity would be modulated by the behavioral response patterns exhibited during the speech task of the TSST. More specifically, we anticipated that, in young women, the intensity of the cardiac and cortisol responses would be associated with the number of submissive and escape behaviors, which are commonly associated with a passive coping style (Sgoifo et al., 2003; Villada et al., 2014). Moreover, given that post-menopausal women have not been studied from an ethological perspective, we aimed to explore the relationships between behavior and physiological stress, as well as their recovery processes, compared to young women. To the best of our knowledge, this is the first study to analyze and compare behavior and the psychophysiological stress response in groups of young women in different phases of the menstrual cycle and in post-menopausal women.

5.2. Methods

5.2.1. Participants

The sample was composed of 66 women: 36 post-menopausal women between 56 and 73 years old (means±sem: age = 63.86±0.69; Body Mass Index (BMI) = 25,27±.53) and 31 young women between 18 and 23 years old (mean±sem: age = 19.07±0.27; BMI = 21.35±0.72) divided into two groups: 17 in the luteal phase

(4th to 8th day before the onset of the new menstrual cycle) and 14 in the follicular phase (5th to 8th day after the onset of the new menstrual cycle). For women in the luteal phase, the mean age was 19.11±0.44, and the mean BMI was: 22.14±0.97. For women in the follicular phase, the mean of age was 18.23±0.17, and the mean BMI was 20.32±1.04. The menstrual cycle phase was calculated using two estimation procedures (Espin et al., 2010, 2013). First, in order to establish the date of each subject's appointment, all the cycles were converted to a standard 28-day cycle, taking as reference points the day of onset of the last menstruation and the real length of the studied cycle (Rossi and Rossi, 1980). Second, to confirm the previous estimation and estimate the ovulation point, Basal Body Temperature (BBT) was recorded daily during two complete menstrual cycles by means of sublingual temperature taken for 5 min before getting up. To analyze the BBT, the “smoothed curve” method (SMC) was used, as described by McCarthy and Rockette (1983, 1986). All the post-menopausal women had their last menstrual period more than 2 years before the testing time, and none of these women had received estrogen replacement therapy. Most of the young participants (94%) were college students from different fields (psychology, medicine, occupational sciences, among others). The post-menopausal participants belonged to a study program at the University of Valencia for people over 55 years of age, and none of the participants received academic or economic compensation for their participation.

The exclusion criteria were: smoking more than 5 cigarettes a day, alcohol or other drug abuse, visual or hearing problems, presence of cardiovascular, endocrine, neurological or psychiatric disease, presence of a stressful live event during the past year, and use of medication related to cognitive, emotional or endocrine function, such as glucocorticoids, β-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies or psychotropic substances. In addition, the young women had to be nulliparous, with no gynecological problems and regular menstrual cycles (24–36 days). Participants meeting the criteria were contacted by telephone and asked to attend experimental sessions that took place in a laboratory at the Faculty of Psychology (University of Valencia, Spain). 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 only drink water, and not eat or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session.

5.2.2. Study protocol

Participants arrived at the laboratory, and the experimenter checked whether they had followed the instructions given previously. All the participants received verbal and written information about the study and signed an informed consent form. Then, participants' weight and height were measured.

To produce a stress response, the participants were subjected 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 (see Kirschbaum et al., 1993, for more details), 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, and the speech task was video recorded.

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 this phase started, baseline measures were obtained for Cortisol (-20 min pre-stress), anxiety (Stai-S) and mood (PANAS). After the habituation phase, the introduction phase started (duration 3 min). In this phase, 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 in the first room. During this period, they provided a saliva sample (-5 min pre-stress).

Following the preparation phase, the stress test was carried out. Subjects had 20 minutes to recover after the 10-min stress task. During this period, they again answered the two questionnaires (Stai-S and PANAS) and provided the second saliva sample (+15 min post-stress). The participants provided the last saliva sample 25 minutes later (+40 min post-stress). In the last part of the session, all the participants completed the coping strategies questionnaire (COPE, Carver et al., 1989).

5.2.3. Biochemical analyses

5.2.3.1. Cortisol.

Participants provided four saliva samples of 3 ml each in plastic vials. They took approximately 5 minutes to fill the vial. The samples were frozen at - 80° C until the analyses were performed. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Coat-A-Count C (DPC, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Assay sensitivity was 0.5 ng/mL. The findings are expressed in nanomolar units (nmol/L). For each participant, all the samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%.

5.2.4. Heart Rate (HR)

Heart rate was continuously recorded in the experimental and control conditions using the heart rate monitor Suunto ® T6 (Suunto Oy, Vantaa, Finland), which consists of a chest belt for detection and transmission of the heartbeat and a "watch" for data collection and storage (Radespiel-Troger et al., 2003; Roy et al., 2009).. Heartbeat detection is performed with an accuracy of 1 ms, and every heartbeat is transmitted and stored in the flash memory of the watch. The recording periods when participants were walking from one room to another were removed, and only the 5 central minutes of each phase (namely from -20 to -15 min for baseline, from -6 to -1 min for preparation, from 0 to +5 for speech, from +5 to +10 for arithmetic, and from +15 to +20 for recovery) were used to calculate the participants' average heart rate values. After eliminating the artifacts, the HR mean was computed for each phase. HR artifacts and HR analysis were performed with Kubios software (Biomedical Signal Analysis Group, University of Kuopio, Finland).

5.2.5. Psychological assessment

5.2.5.1. Coping Strategies

The dispositional version of the COPE Inventory is a theoretically driven self-report questionnaire that addresses different ways of coping (Carver et al., 1989). Subjects must indicate what they *generally* do and feel when experiencing stress. Items are rated on a 4-point scale, ranging from 1 (*I don't usually do this at all*) to 4 (*I usually do this a lot*). We employed the Spanish version of the long form, which consists of 60 items from 15 subscales (such as Planning, Seeking Instrumental Support, Suppression of Competing Activities, Restraint Coping, and Venting of Emotions, among others). With a second-order factor analysis, they can be grouped in four basic coping domains: active coping, cognitive and emotional coping measures (active coping strategies), and Avoidance (passive coping strategies). The Spanish version of the scale had Cronbach's alphas ranging from 0.78 to 0.92 (Crespo and Cruzado 1997).

5.2.5.2. Anxiety.

To assess state anxiety, the Spanish version of the State Anxiety Inventory was used (STAI form E) (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 participants felt at the moment they gave their answers. The Spanish version of the scale had Cronbach's alphas ranging from 0.90 to 0.93 (Seisdedos, 1988).

5.2.5.3. Mood.

Mood was evaluated by the Spanish version (Sandin, 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). Sandin et al. (1999) reported a high internal consistency for the Spanish version, with Cronbach's alphas for PA ranging from 0.87 to 0.89, and for NA from 0.89 to 0.91.

5.2.6. Ethological analysis

ECSI. Participants' behavior during the speech task of the TSST was quantified by means of the Ethological Coding System for Interviews (ECSI) (Troisi, 1999). The interview was videotaped with a camera adjusted so that the subject's face and trunk were in full view. Subsequently, behavioral assessment was carried out according to Troisi and colleagues (Pico-Alfonso et al., 2007; Troisi, 1999; Sgoifo et al., 2003). This version of the ECSI includes 32 different patterns, mostly facial expressions and hand movements. The ECSI was specifically designed to measure non-verbal behavior during stress interviews by combining behavior patterns described in published human ethograms (Troisi, 1999). The 32 behavioral patterns were then grouped in seven behavioral categories, each reflecting a different aspect of the subject's emotional and social attitude (Troisi, 1999), namely: (1) eye contact; (2) affiliation; (3) submission; (4) flight; (5) assertion; (6) gesture; (7) displacement. The score for a given behavioral category was expressed as the sum of the percentages of all the behavioral patterns belonging to it (Troisi, 1999).

5.2.7. Statistical analyses

One-way ANOVAs were used to analyze differences between groups in the demographic/anthropometric variables, coping strategies and behavioral patterns. We employed Age Group (Post-Menopausal women vs. Young women) or Hormonal Group (Post-Menopausal women, Luteal women, and Follicular women) as a between-subject factor. ANOVAs for repeated measures were used to assess the effects of stress on mood, anxiety, heart rate and cortisol. We added Period (mood and anxiety: pre and post task; HR: -20, -5, +5, +10 and +15 min; Cortisol: -20, -5, +15, +40 min) as a within-subject factor.

Additionally, cortisol and stress-induced HR reactivity were also quantified as the area under the curve with respect to the increase (AUC_i) (Pruessner et al., 2003). Finally, the Recovery index for cortisol and heart rate was calculated through the difference between mean values of speech minus recovery phase values.

Pearson's correlations were calculated to assess whether the physiological changes (AUC_i and Recovery indices) were related to the behavioral patterns. Stepwise regression analyses were conducted to examine whether coping styles (COPE) predict changes (AUCi and recovery indices) in cortisol and heart rate in each group. In stepwise regression, the independent variables are entered into the regression one at a time until they have all been added, with the provision that each meets the specified criterion. For this study, the criterion used was a significance level of $p < .100$ (SAS Institute, 2011). To avoid the problem of multicollinearity of independent variables, in the stepwise approach all the variables are reexamined after the addition of the other variables, in order to verify that each is still a significant and independent predictor.

Five participants were excluded from the analyses: 1 post-menopausal and 2 luteal women for the cortisol data and 1 post-menopausal woman for the HR because their levels differed by more than 3 S.D. from the sample mean, and 1 woman in the follicular phase due to technical problems in the analysis of the videotape and missing data in the physiological measures.

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 the *p*-values reported are two-tailed, and the level of significance was set at <0.05. When not otherwise specified, results shown are means ± standard error of means (SEM). We used SPSS 22.0 to perform the statistical analyses.

5.3. Results

The main aim was to explore the effect of age and hormonal status on the psychophysiological stress response and the behavior strategies. Thus, we first analyzed the stress response in each age group (menopausal women vs. young women)[1]. However, focusing on the hormonal status of the women (luteal vs. follicular vs. post-menopausal women), we had a better overview; hence, we performed the analyses focusing on these three groups. Figures show the results by age and hormonal status group.

5.3.1. Cortisol

ANOVAs for repeated measures showed significant effects of Period, $F(2.276,136.561) = 29.371$, $p \leq .001$, $\eta^2_p = .329$, Group, $F(2,60) = 14.869$, $p \leq .001$, $\eta^2_p = .331$, and the Group×Period interaction, $F(4.552, 136.561) = 8.427$, $p \leq .001$, $\eta^2_p = .219$. Post-hoc analyses revealed that post-menopausal women show, in general, the

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1 ANOVA of repeated measures indicated that only post-menopausal women did not show a significant cortisol stress response ($p > .1$). They showed lower levels of cortisol and heart rate than young women in all the periods analyzed (all $p < .029$). All groups showed a significant cardiac response (all $p < .05$). Moreover, post-menopausal women were characterized by lower scores on anxiety and negative mood, and they scored higher on positive mood compared to young women (all $p < .05$). Finally, post-menopausal women displayed greater percentages of Gestures, whereas young women displayed greater percentages of submissive and displacement behaviors (all $p \leq .006$), reflecting a more passive coping style compared to menopausal women.

lowest cortisol levels; these differences were significant compared to the two groups of young women at Baseline (both $p \leq .021$) and in the Preparation Period (both $p \leq .015$); no differences in these phases were found between the young groups (all $p > .1$). After the stress period, the luteal group showed the highest cortisol response, compared to the follicular and post-menopausal groups ($p = .007$; $p \leq .001$, respectively); and in the recovery period, the post-menopausal group showed the lowest levels of cortisol, but these differences were significant only in comparison to the luteal group ($p = .004$) (see Figure 1). When comparisons were made within each group, although the three groups showed increases greater than 2.5 nmol/L, only the luteal group showed statistically significant increases from Baseline to the Speech Period ($p \leq .001$); the luteal and post-menopausal groups did not recover from baseline levels, but only reached significance in the luteal group ($p = .002$; $p = .09$, respectively).

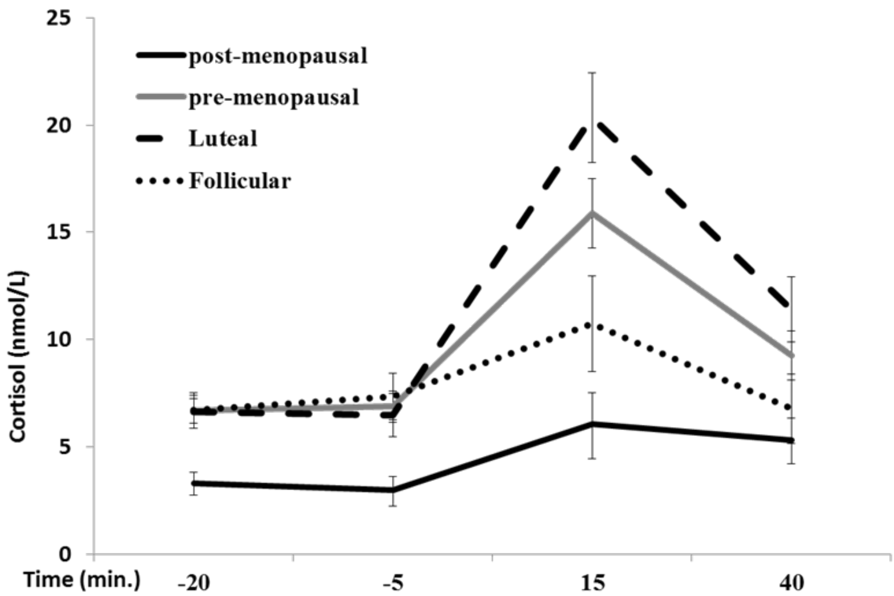

Figure 1. Mean values ± SEM salivary cortisol response (nmol/L) by age and hormonal status group (*p < .05).

5.3.2. Heart Rate (HR)

ANOVA for repeated measures revealed significant effects of the Period, $F(2.285, 141.697) = 110.87$, $p \leq .001$, $\eta^2_p = .641$, Group, $F(2,62) = 8.994$, $p \leq .001$, $\eta^2_p = .225$, and Group × Period interaction, $F(4.571, 141.697) = 4.147$, $p = .002$, $\eta^2_p = .118$. Post-menopausal women showed lower levels of HR than the two groups of young women at Baseline (both $p \leq .05$) and in the Anticipation period (with Luteal: $p = .003$; with Follicular: $p = .065$). In the Speech and Arithmetic periods, differences were significant only between the post-menopausal and luteal groups (both $p \leq .004$). No differences were found between the three groups in the Recovery period (all $p > .1$). No significant differences were observed between the young groups in any period analyzed (all $p \geq .41$). Post-hoc comparisons within each group revealed that all the groups increased their HR significantly from baseline to stress (Speech and Arithmetic) (all $p \leq .002$) (see Figure 2).

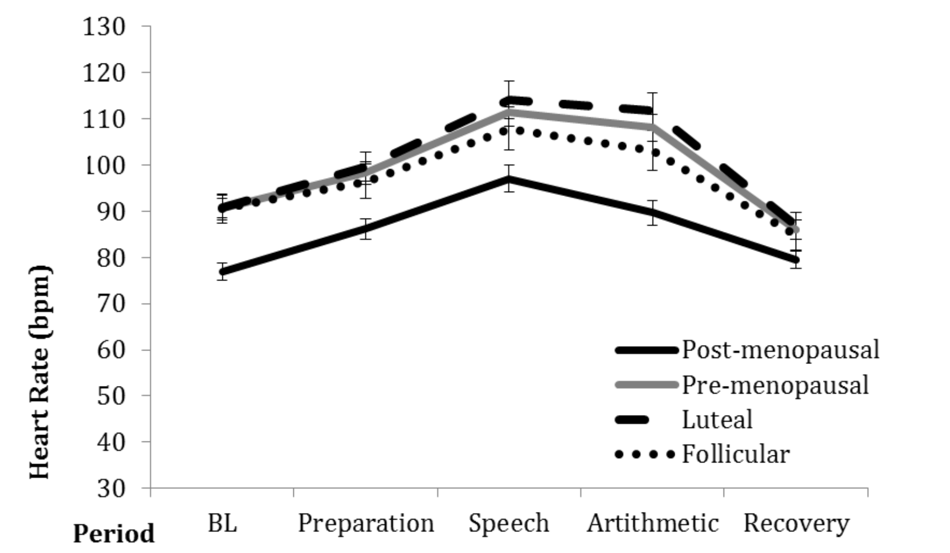

Figure 2. Means of heart rate (±SEM) response by age and hormonal status group.

5.3.3. State Anxiety

A repeated-measures ANOVA of the STAI scores revealed main effects of Period, $F(1, 63) = 64.551$, $p \leq .001$, $\eta^2_p = .506$, Group $F(2, 63) = 12.732$, $p \leq .001$, $\eta^2_p = .288$, and marginally, the Period × Group interaction, $F(2, 63) = 2.964$, $p = .059$, $\eta^2_p = .086$. The three groups increased their subjective anxiety scores after the stress (all $p \leq .001$). The post-menopausal group scored lower on anxiety before and after stress than the luteal group (all $p \leq .002$). No other significant differences were found between groups (all $p > .07$) (Mean±sem for post-menopausal women before task: 12.28±1.01, after task: 18.42±1.50; for luteal group before task: 18.65±1.47, after task: 30.71±2.19; for follicular group before task: 16.23±1.69, after task: 25.15±2.50) (see Figure 3).

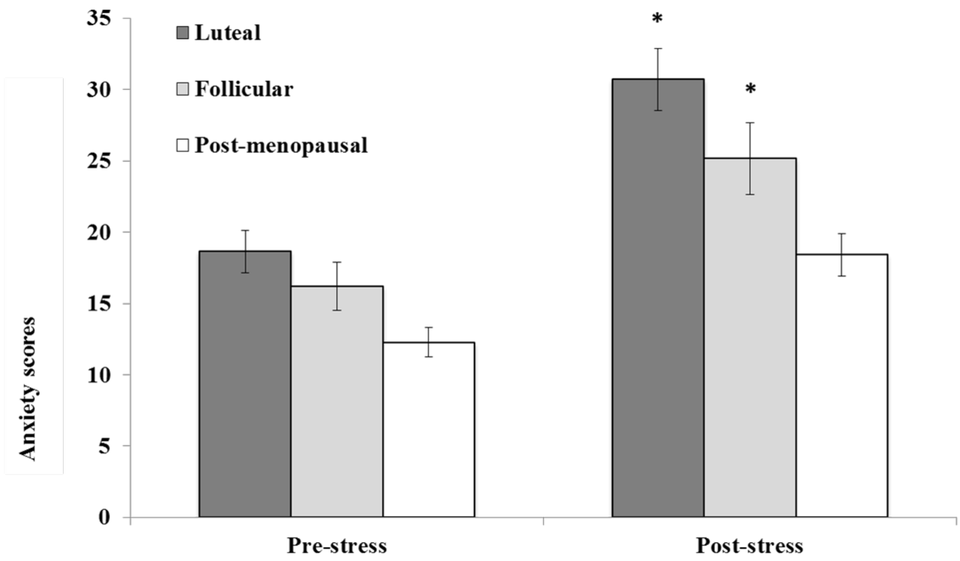

Figure 3. Means (±SEM) of anxiety scores (STAI-S) by hormonal status group.

5.3.4. Mood

For positive mood, the ANOVA revealed significant effects of Period, $F(1, 63) = 37.498$, $p \leq .001$, $\eta^2_p = .2373$, Group, $F(2, 63) = 9.984$, $p \leq .001$, $\eta^2_p = .241$, and the Period × Group interaction, $F(2, 63) = 5.400$, $p = .007$, $\eta^2_p = .156$. All the groups decreased their positive mood after the stress task (all *p*≤ .018). The post-menopausal group showed higher positive mood scores than luteal women before the task (*p*= .013). After the stress task, post-menopausal women scored significantly higher on positive mood only compared to luteal women (*p*≤ .001) and as a trend, follicular women (all *p*=.054). (Mean±sem for post-menopausal women before task: 31.167±0.90, after task: 29.46±0.99; for Luteal group before task: 26.47±1.31, after task: 20.71±1.44; for Follicular group before task: 27.69±1.50, after task: 24.77±1.66) (see Figure 4).

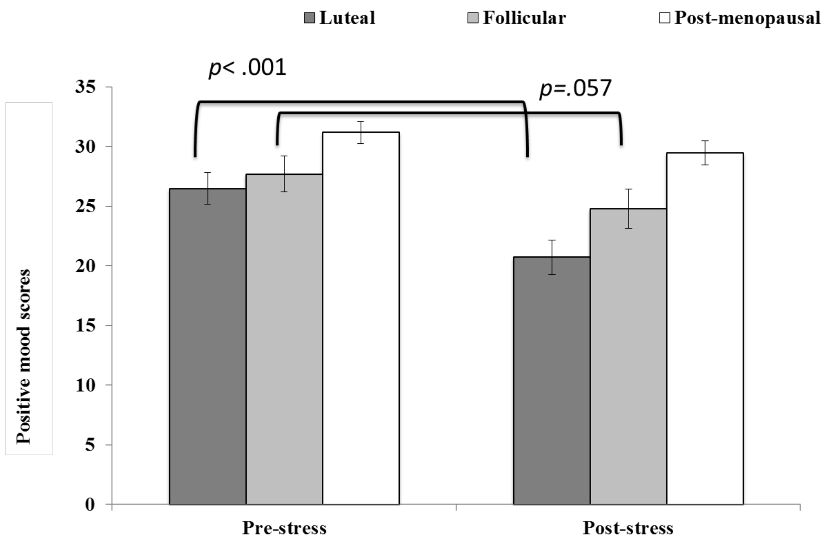

Figure 4. Means (±SEM) of positive affect scores (PANAS) by hormonal status group.

For negative mood, significant effects of Period, $F(1, 63) = 33.420$, $p \leq .001$, $\eta^2_p = .4347$) Group, $F(2, 63) = 8.683$, $p \leq .001$, $\eta^2_p = .216$) and the Period × Group interaction were found, $F(2, 63) = 3.507$, $p=0.036$, $\eta^2_p = .10$. All the groups increased their negative mood after the stress task, but these increases were only significant for the luteal and menopausal groups (both *p*= .001). The follicular group did not reach significance (*p*= .096). Before stress, post-menopausal women scored lower on negative mood than the young groups (both *p*=.032). In addition, negative mood scores after stress were lower in the post-menopausal group than the luteal group (*p*= .032). No other differences were found between groups (all *p*≥ .21) (Mean±sem for Menopausal group before task: 12.47±0.75, after task: 15.51±1.02; for Luteal group before task: 15.94±1.09, after task: 22.71±1.48; for Follicular group before task: 16.69±1.24, after task: 19.15±1.69) (Figure 5).

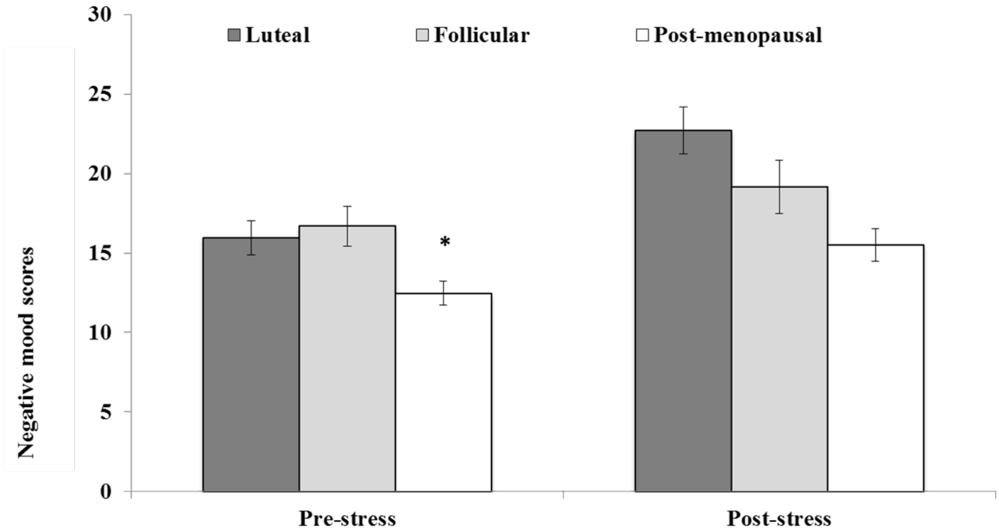

Figure 5. Means (±SEM) of negative affect scores (PANAS) by hormonal status group.

5.3.5. Ethological data (ECSI)

Table 1 summarizes the behavioral response exhibited by the three groups of women during the speech task, with each behavioral category being quantified as cumulative percentages. Statistically significant differences between groups were found in: Submission, $F(2,65) = 9.005$, $p \leq .001$; Gesture $F(2,65) = 4.760$, $p = .012$; and Displacement $F(2,65) = 8.634$, $p = .001$. Data revealed that post-menopausal women, compared to both groups of young women, showed lower percentages of Submissive behaviors (Luteal: $p = .039$, Follicular: $p = .001$) and Displacement behaviors (Luteal: $p = .012$, Follicular: $p = .002$). Finally, post-menopausal women showed significantly higher percentage of Gestures during the speaking task than follicular women ($p = .014$). No other differences were found between groups (all $p > .1$).

Ethological Data	Luteal (n=17)	Follicular (n=13)	Post-menopausal (n=36)
Eye contact	97,94 ±1,21	92,69±3,28	97,5±0,98
Affiliation	44,12±6,79	42,31±9,33	56,53±6,36
Submission	**12,35± 2,39**	**17,31±3,56**	**5,42±1,20**
Flight	129,11±3,84	131,54±4,36	124,31±4,36
Assertion	10,29±2,89	6,54±3,01	15,14±2,84
Gesture	**25±6,81**	**11,15±4,35**	**41,81±6,30**
Displacement	**63,23±7,04**	**73,46±12,31**	**33,05±5,43**

Table 1. Total sum of the behavioral pattern values for each behavioral category (% mean ± SEM) displayed during speech (ECSI). In bold are the categories with significant differences between groups (all $p < .05$).

5.3.6. Relationships between behavioral and physiological changes (AUCi and Recovery indices of Cortisol and HR)

Given that each of the three groups has a different hormonal status, correlation analyses were performed by group, in order to provide new insights on neuroendocrine and behavioral stress responsivity associations.

In the luteal group, Pearson correlations revealed that the number of submissive behaviors displayed during the speaking task was positively related to the Cortisol AUCi, and negatively related to the Cortisol Recovery index (r= .632, *p*= .011; r= -.607, *p*=.016, respectively). That is, these patterns of behaviors were related to a greater HPA axis regulation of stressful events. No relationships between HR indexes and behavior were found (all $p > .1$).

In the follicular group, the expression of behavioral patterns included in the assertion category (low-aggressiveness) was negatively related to the Cortisol

Recovery index (r= -.615, *p*= .025). No relationships between HR indexes and behavior were found (all $p > .1$).

In the post-menopausal group, the number of Affiliative behaviors displayed during the speaking task was negatively related to the HR Recovery index (r=-.355, *p*=.036), and as a trend, to the total amount of Eye Contact (r=-.302, *p*=.065). These behavioral patterns seem to be related to better cardiac recovery during menopause.

5.3.7. Coping Styles (COPE)

The analyses revealed significant differences between groups on two of the four basic coping styles, Active Coping: $F(2,65) = 13.399$, $p \leq .001$, and Emotional Coping $F(2,65) = 6.344$, $p = .003$. Post-menopausal women showed higher scores on Active coping than both groups of young women (both $p \leq .001$), and lower scores on Emotional coping, but these differences were statistically significant only in comparison to the luteal group (*p*= .005), and as a trend, the follicular group (*p*= .072). As a tendency, group differences were found in the Avoidance factor: $F(2,65) = 2.853$, $p = .065$, post-menopausal women showed higher scores than luteal women (*p*= .061), but similar scores to the follicular group (*p*=1). No differences were found in Cognitive coping between groups: $F(2,65) = 1.09$, $p = .342$, and no differences were found between the young groups in any COPE factor analyzed (all $p \geq .456$).

5.3.8. Relationships among coping strategies (COPE) and physiological changes (AUCi and Recovery indices of Cortisol and HR)

To test whether there were any associations between coping styles and changes in stress responsivity (reactivity and recovery of Cortisol and Heart rate), stepwise multiple regression analyses were conducted. In the first step of the regression analyses, we included BMI as a covariate. In the second step, the four coping styles from the COPE questionnaire were introduced (behavioral coping, cognitive coping, emotional coping, and avoidance) as possible predictors in four separate models, the first predicting the cortisol AUCi, the second predicting the heart rate AUCi, the third predicting the cortisol Recovery index, and the fourth predicting the heart rate Recovery index. We performed separate analyses for each Hormone Group.

In the Luteal and Follicular groups, the four coping strategies did not predict physiological increases (Cortisol and HR AUCi) or cardiac and HPA axis recovery capacity (all $p > .1$).[2]

Higher scores on the Avoidance factor predicted poorer recovery of Cortisol levels in women in menopause: $F(1,32)= 9.769$, $p= .004$, $\beta=.485$, accounted for 23.3% of its variance. A significant predictive model was also found for the HR AUCi, as higher scores on Active coping and lower scores on Avoidance factors predicted greater increases in heart rate ($\beta=.704$, $p\leq.001$; $\beta=-.392$, $p=.011$). Together, they accounted for 12.8% of its variance, $F(1,31)= 7.259$, $p= .011$. These associations reveal the positive influence of active coping, rather than avoidance strategies, in the physiological regulatory functions in situations of stress. No other associations were found among the coping styles, Cortisol AUCi, and heart rate Recovery index (all $p >.1$).

5.4. Discussion

The current study examined the psychophysiological and behavioral stress response in women while performing the speaking task of the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993). This study provides new information about how behavior and coping styles measured by self-report may affect the stress regulatory system in different ways depending on age and hormonal status.

First, our results confirmed that the speaking task of the TSST provokes a significant stress response in heart rate, anxiety and mood in all women. However, the cortisol stress response did not reach significance in post-menopausal and follicular women, although all the groups showed increases above 2.5 nmol/l. Therefore, we consider that there has been a significant response to the stressor in all the groups analyzed (Schommer et al., 2003).

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[2] When stepwise regression analyses were performed for the young groups as a whole, none of the four coping styles predicted physiological changes (all $p > .1$).

Examining the cortisol profile more in-depth, we observed a different response pattern across the three groups. As expected, women in the luteal phase showed the highest levels of cortisol after the stressor, compared to women in the follicular phase and post-menopausal women. The blunted cortisol response in post-menopausal and follicular women compared to luteal women agrees with previous findings focused on the effect of sex hormones on the stress response in young women (Espin et al., 2013; Kirschbaum et al., 1993; Lustyk et al., 2012) and compared to women in menopause (Lindheim et al., 1992; Patacchioli et al., 2006). As documented in a recent study by Gordon and Girdler (2014), these results are in accordance with the quantity of estradiol and progesterone in each group, where progesterone seems to increase HPA stress responsivity. Therefore, the assumed lower levels of estradiol and progesterone in women in menopause and in the follicular phase coincide with the lower cortisol response in these groups. However, focusing on the recovery phase, we found that only women in the follicular phase returned to baseline levels after the stress period. The fact that women in the luteal phase did not recover after stress could be due to their higher increases; hence, they would need more time (more than forty minutes after the stressor onset) to recover. However, in post-menopausal women, this poor recovery could be due to HPA axis negative feedback dysregulation, due to their loss of reproductive hormones (Otte et al., 2005; Wilkinson et al., 2001).

Regarding cardiac activity, based on previous literature suggesting a higher adrenocortical response in the luteal phase (Childs et al., 2010; Kudielka and Kirschbaum, 2005; Kajantie and Phillips, 2006), we hypothesized greater heart rate increases in response to stress in luteal women. Our results confirmed this hypothesis. In addition, we found that post-menopausal women showed the lowest heart rate increases, but this difference was only significant compared to women in the luteal phase. In general, post-menopausal women showed lower cardiac activity during all the phases, except the recovery phase, but no significant age or hormonal status differences were found. Moreover, we observed that although the three groups (young and post-menopausal) recovered until reaching basal levels, in post-menopausal women the heart rate remained high (but not statistically significant) (see Figure 2). These findings coincide with the aforementioned literature; however, other authors did not find associations between the menstrual cycle phase and cardiac stress responses (Pico-Alfonso et al., 2007; Stoney et al., 1990). Research focused on age-related differences has showed contradictory results. While some studies have found no significant age-related differences in heart rate reactivity to social stressors (Almela et al., 2011; Kudielka et al., 1999, 2004b), others have reported lower heart rate responses in older individuals than in younger people (Gotthardt et al., 1995; Traustadóttir et al., 2005). The different stress paradigms used in each study (TSST, speaking tasks or cognitive tasks) could explain these discrepancies in the literature.

In addition, with age, people's perceptions of stressful social situations could be attenuated due to their greater range of experiences and repertoire of coping resources (Aldwin et al., 1996). For example, older people have been found to report fewer negative emotions than younger people (Almela et al., 2011; Labouvie-Vief et al., 1987; Lawton, et al., 1993), and to perceive less frustration than younger adults when facing an acute laboratory challenge (Hidalgo et al., 2015). Following this line of reasoning, the psychological response (perceived anxiety and mood) to social stressors would be lower in older people than in younger ones. Our results confirmed that older women generally showed less change in anxiety and mood when facing an acute social stressor. In addition, we found a menstrual cycle phase effect. Thus, women in the luteal phase showed higher anxiety and mood changes than post-menopausal women, whereas the scores of women in the follicular phase were similar to those of post-menopausal women. These results, combined with the aforementioned physiological response, agree with previous studies that also found higher sensitivity to stress in women in the luteal phase, with greater increases in heart rate and negative affect (Childs et al., 2010; Ossewarde et al., 2010), but without finding differences in cortisol response. Therefore, we can confirm that age and hormonal status influence both the psychological and biological stress response, with post-menopausal women showing a buffered stress response, and women in the luteal phase being more reactive to stress.

Likewise, the behavioral strategies displayed during the stressor were different depending on age and hormonal status, with young women exhibiting a higher percentage of behaviors that reflect passive and reactive coping styles (Submission and Displacement), whereas post-menopausal women displayed higher percentages of behaviors that reflect an active coping style (Gestures), although the difference was only significant in comparison to the follicular group.

Based on previous studies in young women, we expected that the neuroendocrine response to stress would be positively influenced by displacement behaviors and negatively by submissive behavioral patterns in young women (Pico-Alfonso et al., 2007; Villada et al., 2014). However, in this study, we failed to find these associations. However, our results revealed that in young women (follicular and luteal groups), the behaviors that reflect passive coping (submission and assertion) are associated with an improved recovery of the HPA axis, whereas in post-menopausal women, affiliative behaviors seem to have a cardiac regulatory function. These latter results agree with the tend-and-befriend theory (Taylor, 2000), which explains the adaptive function of affiliative behaviors as a physiological regulation tool in women.

Finally, the COPE scores provide information about the way an individual usually copes with stressful situations. The main objective of using this questionnaire was to explore the possible differences in coping strategies and the influence of the coping styles on the physiological stress response. Some authors have suggested that older adults are more flexible in their problem-solving strategies than younger adults (Blanchard-Fields et al., 1995; Blanchard-Fields et al., 1997). At first, our results showed age–related differences, with the group of post-menopausal women scoring higher on the Active coping subscale than both groups of young women; and we also found that women in their luteal phase scored higher on Emotional coping styles than the post-menopausal and follicular groups. Second, with the regressions performed, we observed that, only in post-menopausal women, avoidance strategies were associated with a greater cortisol recovery index, whereas higher scores on Active coping and lower scores on Avoidance predicted greater heart rate reactivity. However, we failed to find any associations between the coping styles and neuroendocrine adjustment in young women. Previous research in adult women (41 to 69 years old) reported that an adaptive coping style predicted greater cortisol (Bhonen et al., 1991) and faster cardiac recovery in a group of women with a broad age range (18 to 79 years old) (Faucheux et al., 1989) who were undergoing stressful cognitive tasks. By contrast, avoidance coping styles have been associated with greater blood pressure reactivity in adult men and women (Vitalino et al., 1993).

In conclusion, in the present study, important differences in the psychophysiological responses and coping behavior among the three groups of women have been found. We believe that these results, in addition to supporting previous findings in different groups of women, also provide new relationships involving age and hormonal status in behavioral and physiological stress responsivity, as well as the influence of coping strategies on the neuroendocrine self-regulatory processes (cortisol and heart rate). In addition, the consideration of the stress response as the interaction of psychological perceptions and physiological systems highlights the need to better understand the stress response in the adult population. Specifically, it increases the interest in studying women in the post-menopausal period, as this group is characterized by dramatic decreases in reproductive hormones (Gordon et al., 2014), while displaying a social background that has the potential to be one of the most important protective factors in the study of the social stress response.

Limitations of this study

The associations found in this study must be interpreted with caution, due to the large number of correlations performed, increasing the possibility of a type I error. However, the choice of multiple comparison corrections also increases the possibility of a type II error, thus hampering potentially important findings. Undoubtedly, future studies with larger samples are needed to further validate these results. Finally, in this study we did not include a group of oral contraceptive users. This is an important limitation due to the social importance of the effect of contraceptives on the psychophysiological stress response.

In sum, these results provide new insights into the importance of considering age and the menstrual cycle phase and their interaction with coping processes, as well as the reactivity and recovery capacity, in situations of social stress.

CHAPTER VI

GENERAL DISCUSSION

The previous empirical studies included in this dissertation described the main results regarding individual differences (i.e. age, gender, sexual hormones, personality) in the psychophysiological (i.e. anxiety, mood, cortisol, heart rate) and behavioral stress response in people undergoing an acute laboratory social stressor: TSST.

A summary of the main findings of the studies described in the previous chapters is presented in this final chapter.

6.1. MAIN FINDINGS

6.1.1. Study 1

This study investigated some factors involved in individual differences in the response to a standardized laboratory psychosocial stressor (TSST) in healthy young men and women. Psychological (anxiety and mood) and physiological (cortisol) changes in response to stress were assessed in an experimental and a control condition, employing a crossover design. Moreover, coping styles (COPE) and trait anxiety (ISRA) were measured by self-report. Regardless of sex, two different patterns of response to stress were found. The first one (Cluster 1) was characterized by a lower psychological reaction along with a higher cortisol response, whereas the second one (Cluster 2) presented a greater psychological reaction and lower cortisol response. From an evolutionary perspective, the individuals in Cluster 1 would present an adaptive stress response in dealing with stressful situations. In addition, these participants also obtained higher scores on active coping and lower scores on trait anxiety, whereas individuals allocated to Cluster 2 scored higher on maladapted coping strategies (focused on emotions, mental disengagement) and higher on trait anxiety, specifically in the specific situational area related to the stress test employed (test evaluation anxiety). In conclusion, these results highlight the importance of personality characteristics beyond sex in the adaptive stress response to social stress.

6.1.2. Study 2

The purpose of this study was to investigate the relevance of self-efficacy in the psychological and cardiac stress response by means of anxiety, R-R and r-MSSD parameters, respectively. We also explored the influence of these variables on the performance displayed during the speaking task of the Trier Social Stress Test (TSST), and we examined the influence of trait anxiety on the psychological and cardiac aspects of the stress response. Finally, we explored the relationships between self-perceived performance and assessment by external evaluators through the psychophysiological stress response. The TSST provoked significant autonomic (R-R and r-MSSD) and psychological activation (anxiety increases) in the stress condition compared to the control condition in the total sample (men and women). We found that individuals with higher self-efficacy showed greater cardiac activation and better speech performance than their counterparts with lower self-efficacy. Nonetheless, no relationships between autonomic activation and performance were found. We did not observe any differences in trait anxiety between participants with higher and lower self-efficacy either, but we confirmed negative relationships between cognitive and social components of trait anxiety and self-efficacy. In addition, cognitive trait anxiety also correlated negatively with the performance displayed. We can conclude that regardless of the physiological activation, the psychological factors (cognitive appraisal and trait anxiety) would really be responsible for an adaptive performance, which is essential to success.

6.1.3. Study 3

The aim of this study was to investigate the subjects' physiological response, subjective anxiety and mood, as well as their nonverbal behavior, in two different groups of women, while undergoing the Trier Social Stress Test (TSST). To this end, women in their follicular phase and women taking oral contraceptives were exposed to an experimental condition and a control condition in a within-subject design. Both groups increased their levels of cortisol, heart rate and state anxiety and decreased their positive mood after the stressor. Women in their follicular phase were psychologically more sensitive to stress (higher state anxiety and greater decreases in positive mood), and this was also reflected in their non-verbal response, as they displayed more eye contact and assertive behavior compared to OC users during the TSST. In addition, follicular women obtained lower scores on the behavioral, cognitive and emotional dimensions of active coping (COPE questionnaire), compared to OC users. Furthermore, the associations between psychophysiological indices of stress and behavior were different in each group. In follicular women, submissive behaviors were associated with an enhanced HPA axis activity, displacement behaviors were related to de-arousing properties (lower heart rate), and behavioral patterns of assertion were related worse mood. By contrast, in OC users, the common stress response patterns (Flight and Affiliation) were related to worse mood states and greater HPA axis activity, respectively.

In sum, these results, in addition to supporting previous findings in different groups of women, also provide new insights into the relationship between the behavior patterns displayed during a public speaking task and a number of psychobiological stress-related changes.

6.1.4. Study 4

This study, following along the lines of study 3, examined the psychophysiological and behavioral stress response in young and older women performing the speaking task of the Trier Social Stress Test (TSST). We investigated how coping strategies may affect the stress regulatory system in different ways depending on age and hormonal status.

At first, our results confirmed that the TSST speaking task provoked a significant stress response in heart rate, anxiety and mood in all groups. We observed a greater psychophysiological response in women in their luteal phase, whereas follicular and post-menopausal women, characterized by comparable levels of reproductive hormones, displayed a similar physiological (cortisol and heart rate) and psychological stress response (anxiety and mood), although psychological changes were significantly lower in post-menopausal women. Hence, we can suggest that age and hormonal status exert an influence on the stress response, with post-menopausal women showing a buffered stress response, and women in the luteal phase being more reactive to stress. Moreover, significant differences emerged in behavioral strategies displayed during the stressor depending on age and hormonal status. Young women displayed a higher percentage of behaviors that reflect passive and reactive coping styles (Submission and Displacement), whereas post-menopausal women displayed higher percentages of behaviors reflecting an active coping style (Gesture). We also observed that in young women (follicular and luteal), the behaviors that reflect passive coping (submission and assertion) were associated with an improved recovery of the HPA axis, while in menopausal women, affiliative behaviors seem to have a cardiac regulatory function. Finally, only in post-menopausal women, we found that avoidance strategies (by self-report) were associated with a greater index of cortisol recovery, whereas higher scores on Active coping and lower scores on Avoidance predicted greater heart rate reactivity. In conclusion, this study describes new relationships involving age and hormonal status in behavioral and physiological stress responsivity, as well as the influence of coping strategies on the neuroendocrine self-regulatory processes.

6.2. LIMITATIONS AND STRENGTHS

The findings reported in each study of this dissertation must be interpreted with caution. Although in the first three studies we used an intra-subject design, improving the power of the study, the sample size was small. By contrast, in the fourth study we increased the sample size, but with no control session for comparison purposes. Undoubtedly, future studies with larger samples are needed to further validate these results. A general limitation in the four studies consists of the multiple comparisons performed, increasing the type I error, which may lead to misleading conclusions. However, the choice of multiple comparison corrections also increases the possibility of type II error, thus hampering potentially important findings. In addition, we have to take into consideration the characteristics of the sample used. The selection criteria were very rigorous, as all the participants included in these studies were healthy with no presence of diseases; in addition, they could not be taking any medication directly related to cardiac, emotional or cognitive function, or one that was able to influence hormonal levels.

Furthermore, young and old participants were recruited from the university; thus, their educational and socioeconomic status were medium-high. For these reasons, these findings cannot be extrapolated to the general population. Finally, we do not have objective information about the concentrations of reproductive hormones (e.g., progesterone, estradiol) in women; these measures should be added in future investigations.

However, some of these limitations are turning into strong points. For example, the rigor in selecting the sample in each study allowed us to exclude several confounding factors and obtain a homogeneous sample instead of a larger sample. Moreover, in the fourth study, selecting a group of women from a university program allowed us to study healthy women in menopause with an active psychological and physical life. Investigating this population is undoubtedly of interest to the scientific community, as it is possible to delve into the protective factors in the aging process. Finally, every empirical study in this dissertation has yielded clear information about the more relevant factors involved in the stress response in one of the most real life situations people are exposed to regularly: social stress.

6.3. FUTURE DIRECTIONS

Understanding how the mechanisms involved in the stress response interact with mediating factors could have important benefits for future interventions, above all to understand and modify subjective perceptions and provide training in how to deal with stress. Further work on these issues, therefore, would be useful for the prevention and treatment of stress-related disorders.

These findings seem important in more clearly defining biological substrates of strategies for coping with stress. At this point, we think future research should include measures of cortical activity while people are facing stressful events. With the new advances in electrophysiology, it should be essential to complement the neuroendocrine activity and subjective perceptions with brain processes. This approach would allow us to have an integrative and more comprehensive perspective of the stress response. Indeed, it is necessary to investigate these processes in more ecological conditions. Thus, a possible future scenario would be to register the cortical activity and the neuroendocrine response during real job interviews or academic exams.

We also think the findings obtained in healthy women open up a wide range of questions, not only about the influence of reproductive hormone levels on the stress response, but also about changes in personality throughout life, and how these psychological and behavioral experiences can regulate the physiological stress response.

Finally, focusing on trait anxiety and coping styles, we found that all of these measures together may explain a large proportion of the adaptive or maladaptive stress response. To extend this topic, we think it could be useful to investigate all the components analyzed in this dissertation in individuals with anxiety disorders (i.e. social anxiety). This population is characterized by poor strategies to deal with social stress, reinforcing negative perceptions about social situations and increasing feelings of defeat and frustration. It would be very interesting to analyze possible changes in all the components of the stress response (psychological, physiological and behavioral) before and after cognitive therapy. Furthermore, it would be necessary to study not only the stress response but also the physiological activity (HPA axis and ANS activity) in non-stressful situations.

CHAPTER VII

CONCLUSIONS

7. CONCLUSIONS

Taken together, the findings of each empirical study in this doctoral dissertation reveal the following:

- The findings confirm the two different stress response patterns (active vs. passive/reactive coping). These patterns highlight the positive role of cortisol in preparing to deal with stress, and the negative impact of greater anxiety and negative mood.
- In spite of the well-known sex differences in the physiological response to stress, the findings suggest that trait anxiety and coping styles are potential modulators that help to better understand the individual differences, beyond the role of sex.
- The results show that autonomic activation due to appraising a situation as stressful does not affect performance. Psychological factors such as cognitive appraisal and trait anxiety seem to be more responsible for an adaptive performance.
- The findings indicate the role of reproductive hormones in coping with acute challenges.
- They show how several behavioral patterns displayed during an acute stressor are related to the regulation (reactivity and recovery) of the main stress response systems (ANS and HPA axis).
- They point out the relevance of studying the behavioral stress response from an ethological perspective.
- They reveal age differences in all components of the stress response, and the fact that the associations between physiological, psychological (trait or state) and behavioral components also depend on the aging process.

As a general conclusion, in this doctoral dissertation we have covered the main components involved in the acute stress response (psychological, physiological and behavioral), and we have outlined the impact of moderating factors, such as age, gender, reproductive hormones and some personality traits, on them. We can highlight that these factors play an important role and should be considered in the study of protective and damaging factors in the stress response.

CHAPTER VIII

GENERAL SUMMARY IN SPANISH

La respuesta de estrés es un fenómeno vital en la vida animal cuyo objetivo es mantener la salud y el bienestar. Desde una perspectiva ecológica, cada especie posee un mecanismo de acción para sobrevivir, el cual implica una activación psicofisiológica para luchar o huir, es decir, para obtener los recursos suficientes para escapar de un predador o cazar una presa. Esta respuesta está caracterizada por la activación de sistemas fisiológicos, como por ejemplo, el sistema nervioso autónomo (SNA), o el eje hipotálamo-hipofisario-adrenal (HHA).

En humanos, la respuesta de estrés implica la activación de los mismos mecanismos, pero podríamos decir que la principal diferencia con otras especies es el tiempo que gastamos percibiendo estímulos estresantes. Los estresores sociales, como sentirse evaluado por otros, mantener o elevar el eestatus social, entre otros, caracteriza la lucha por la supervivencia en la sociedad actual, lo cual lleva a un incremento del tiempo empleado en luchar por ella. Estos incrementos en tiempo podrían llevarnos a sufrir consecuencias negativas en nuestra salud, aumentando la probabilidad de sufrir enfermedades relacionadas con el estrés.

Sin embargo, es bien sabido que ante una misma situación, la percepción de ésta y su respuesta asociada puede variar dependiendo de diferentes factores psicofisiológicos, es decir, de las diferencias individuales. Entre estas diferencias, juegan un papel importante el género, las hormonas sexuales, la edad y los rasgos de personalidad. Por tanto, en esta tesis voy a presentar los resultados de varios estudios centrados en las diferencias individuales en la respuesta psicofisiológica y conductual al estrés, atendiendo a la edad, sexo y/o eestatus hormonal y a rasgos de personalidad, como la ansiedad rasgo y las estrategias de afrontamiento utilizadas frente a situaciones de estrés.

8.1. Introducción

La respuesta de estrés es un conjunto de mecanismos psicológicos, biológicos y conductuales involucrados en un amplio rango de funciones adaptativas tanto en humanos como en otras especies animales. Como consecuencia a la percepción de un evento estresante, es decir, a una situación amenazante para nuestro organismo, los mecanismos psicofisiológicos se activan para mantener el equilibrio interno.

Por tanto, la respuesta de estrés es un proceso adaptativo que implica la activación de los sistemas cognitivo, emocional, fisiológico y conductual (Campbell and Ehlert, 2012). La finalidad de esta activación es mantener el equilibrio interno. Cannon (1929) llamó a este proceso "homeostasis". Hans Selye, introdujo este término para referirse a "la respuesta no específica del cuerpo para las demandas que ello implica". Esta respuesta, como resultado, conlleva una serie de cambios en cascada en los sistemas nervioso, cardiovascular, endocrino e inmune. Estos cambios constituyen las respuesta de estrés y son generalmente adaptativos, al menos a corto plazo (Selye, 1956). Este término fue directamente asociado con el "*Síndrome General de Adaptación*" *(GAS)*. El GAS está dividido en tres etapas. La primera es denominada Reacción de Alarma, y supone la inmediata reacción al estresor, siendo caracterizada para preparar al cuerpo para la respuesta de lucha y huida, sin embargo, el sistema inmune desciende su efectividad. La segunda etapa es la de Resistencia, también llamada etapa de adaptación. Durante esta etapa, el organismo se adapta al estresor si éste continúa en el tiempo. Finalmente, la etapa de Agotamiento está caracterizada por una reducción de la resistencia del organismo. En esta etapa, la capacidad el sistema inmune y del organismo para resistir a enfermedades son prácticamente nulas.

Desde entonces, y hasta nuestros tiempos, el concepto de estrés ha sido relacionado con patologías y enfermedades. Sin embargo, sabemos que la respuesta de estrés es un proceso natural y necesario, no sólo para la supervivencia de las especies, también para promover la mejor adaptación a las demandas establecidas por el entorno (Salvador, 2005).

Por consiguiente, cuando percibimos un evento como amenazante, los sistemas fisiológicos reaccionan con la finalidad de proveer suficientes recursos o mantener el equilibrio psicológico (Andrews and Pruessner, 2013). Aunque sabemos que un excesivo estrés en la edad temprana puede llevar a la aparición de enfermedades como depresión, trastornos de ansiedad y deterioro cognitivo en la edad adulta (McEween, 1999); la respuesta aguda de estrés en personas adultas y sanas es un proceso evolutivo con unos mecanismos homeostáticos inteligentes y efectivos para afrontar estresores agudos, lo cual no debe suponer una carga para la salud (Schneiderman et al., 2005).

Para ahondar en la respuesta de estrés en humanos, tenemos que tener en cuenta la gran complejidad que nos caracteriza en comparación a otras especies. En humanos, los mecanismos fisiológicos involucrados en la respuesta de estrés están continuamente interactuando con las demandas del entorno, pero los factores que contribuyen a largo plazo en la adaptación de los desafíos del ambiente que nos rodea, son los factores sociales, conductuales y cognitivos. Además, deben ser consideradas las diferencias en la respuesta afectiva, cognitiva y en personalidad, así como, la edad, el sexo/género, o las hormonas sexuales.

8.1.2. Diferencias individuales en la respuesta de estrés

8.1.2.1. Género y Hormonas sexuales

La mayoría de los estudios realizados en gente joven han sido llevados a cabo en hombres, pero se ha ido aceptando la idea de que la presencia o ausencia de ciertas hormonas sexuales juegan un importante papel en la respuesta de estrés (Kajantie and Phillips, 2006). El hecho de ser hombre o mujer parece ser uno de los más importantes predictores de salud, lo que hace significativa la presencia de las diferencias de sexo en los trastornos relacionados con el estrés. Por ejemplo, algunos trastornos han sido relacionados con la activación del eje hipotálamo-hipofisario-adrenal (HHA), mostrando de forma repetida diferencias de sexo en su funcionamiento (Kajantie & Phillips, 2006; Kudielka et al., 2009). Generalmente, se ha encontrado una mayor respuesta de cortisol en hombres que en mujeres antes situaciones de estrés agudo (Childs, et al., 2010, 2014; Cornelisse et al., 2011; Huart et al., 2006; Kirschbaum et al., 1999; Kudielka, et al., 2004a, 2004b), aunque también se ha visto que estas diferencias dependen de la fase del ciclo menstrual o del uso de anticonceptivos (Espin et al., 2013; Kajantie & Phillips, 2006).

Por el contrario, se ha mostrado una mayor respuesta emocional en mujeres que en hombres (Childs et al., 2010; Kelly et al., 2008; Walder et al., 2012), aunque varios autores han fallado en encontrar estas diferencias de género (Cornelisse et al., 2011; Kirschbaum et al., 1999; Schoofs & Wolf, 2011).

Cuando la respuesta cardiaca ante estresores de laboratorio ha sido estudiada, se han hallado resultados dispares dependiendo del género. Algunos hallazgos muestran una mayor respuesta de Frecuencia Cardiaca (FC) en mujeres jóvenes en comparación a hombres durante el Trier Social Stress Test (TSST) (Kudielka et al., 2004b), o ante un examen en público (Fichera & Andreasi, 2000). Otros, sin embargo, no encuentran estas diferencias cuando el estresor es una tarea mental aritmética, (Earle, Linden & Weinberg, 1999), o ante una tarea de hablar en público (Carrillo et al., 2001; Sgoifo et al., 2003), o frente al TSST (Kelly et al., 2008), ni tampoco cuando se atiende a las diferentes fases del ciclo menstrual (Kirschbaum et al, 1999).

Además, los efectos del ciclo menstrual sobre la respuesta de FC son todavía controvertidos. Algunos estudios revelan que no hay efectos significativos de la fase del ciclo sobre la reactividad en FC y Presión arterial (Gordon and Girdler, 2014; Tersman et al., 1991; Kirschbaum et al., 1999; Pico-

Alfonso, et al., 2007), tampoco en variabilidad de la frecuencia cardiaca (VFC) ante estresores sociales (Pico-Alfonso et al., 2007). Sin embargo, otros estudios centrados en la fase del ciclo menstrual han encontrado una mayor reactividad de FC en mujeres durante la fase lútea en comparación a mujeres en la fase folicular (Lustyck et al., 2010; Ossewarde et a., 2010).

En cuanto a los cambios psicológicos, aunque es comúnmente conocido que las reacciones emocionales a situaciones estresantes son diferentes entre hombres y mujeres, antes situaciones controladas de laboratorio se han encontrado resultados dispares. Por una lado se han visto, después de haber sido sometidos a un estresor, mayores puntuaciones en ansiedad o estado de ánimo negativo en mujeres en comparación a hombres (Carrillo et al., 2001; Schmaus et al., 2008; Kelly et al., 2008; Childs et al., 2010), otros, sin embargo, no han encontrado esas diferencias (Kudielka et al., 2004a; Preuss & Wolf, 2009). Estos resultados tan heterogéneos sugieren que, al igual que en la respuesta fisiológica, la respuesta afectiva podría estar influenciada por otros factores, como la fase del ciclo menstrual. Por ejemplo, Walder et al. (2012) mostraron que las mujeres durante la fase folicular del ciclo manifestaban mayor ansiedad estado, enfado y hostilidad en comparación a mujeres durante la fase lútea. Sin embargo, Ossewarde et al (2010) encontraron que las mujeres durante la fase lútea revelaban mayor sensibilidad al estrés, mostrando mayores incrementos en afecto negativo en comparación a mujeres en fase folicular. Estas discrepancias podrían ser debidas al momento seleccionado de la fase folicular: en el primer estudio estaban en una fase más temprana que en el segundo estudio; en esta fase tan temprana las mujeres podrían estar durante la menstruación o muy cerca de ella, con el malestar que ello conlleva.

En estos últimos años, se ha comenzado a investigar la influencia del género y del estatus hormonal sobre la conducta durante la ejecución de una tarea de hablar en público en frente de un comité. Aunque algunos estudios no han hallado un efecto de las hormonas sexuales sobre las estrategias de afrontamiento (Pico-Alfonso et al., 2007; Sgoifo et al., 2003), recientemente, se ha encontrado una función reguladora de las conductas de desplazamiento a nivel cardiovascular en hombres, aunque este efecto no se encontró en mujeres durante la fase lútea (Mohiyeddini et al., 2013a). Además, se ha sugerido que en mujeres, las percepciones subjetivas de estrés podrían estar modulando la relación entre conducta y respuesta fisiológica (Mohiyeddini et al., 2013b). En conclusión, esta perspectiva conductual de la respuesta de estrés está arrojando luz sobre cómo pueden interactuar entre si los componentes de la respuesta de estrés (psicológicos, fisiológicos y conductuales).

8.1.2.2. Edad

La respuesta biológica al estrés cambia a lo largo de las etapas de la vida. Esto podría ser explicado debido tanto a cambios estructurales y funcionales relacionados con el proceso de envejecimiento, como por ejemplo, el descenso de la masa muscular del corazón y de su contractilidad (Lakatta, 1993), o la desregulación del feedback negativo del eje Hipotálamo-Hipofisario-Adrenal (HHA) en población mayor (Wilkinson et al., 2001). Particularmente, en mujeres, se ha visto que el eestatus hormonal puede influir la capacidad de respuesta al estrés, siendo esta desregulación más prominente en mujeres que en hombres mayores (ver meta-análisis: Otte et al., 2005).

Hasta ahora, la literatura centrada en los efectos de las hormonas reproductivas en varias funciones fisiológicas no ha encontrado diferencias de edad en cortisol y FC en la capacidad respuesta a un estresor social (Kudielka et al., 1999; Hidalgo et al., 2014). Cuando estas diferencias de género se han estudiado en población mayor, tampoco se han apreciado diferencias entre hombres y mujeres en respuesta al estrés (Traustadòttir et al., 2003; Kudielka et al., 2004). Sin embargo sí se ha enfatizado la peor capacidad de recuperación al estrés durante la menopausia en comparación a otros grupos de mujeres pre-menopáusicas (Kudielka et al., 2004; Pattachioli et al., 2006). Asimismo, se han descrito descensos en VFC dependientes del género con la edad. Parece que las mujeres jóvenes muestran menores valores que hombres jóvenes, además de mayores descensos de FC con la edad. Sin embargo, parece que estas diferencias de género desaparecen cuando se alcanzan los 50 años aproximadamente (Umetani et al., 1988). A pesar de estos resultados tan contradictorios, se podría concluir que la respuesta de estrés está preservada con la edad, pero no tanto la capacidad de recuperar ante situaciones de estrés, especialmente en mujeres post-menopáusicas.

8.1.2.3. Ansiedad rasgo y estilos de afrontamiento

En el estudios del estrés social los rasgos de personalidad juegan un papel muy importante en cómo las personas perciben el estrés (Connor-Smith and Flachsbart, 2007). Al mismo tiempo, se ha relacionado varios trastornos psicológicos con respuestas desadaptativas a estresores agudos (Bale 2005, 2006; Nemeroff, 1988). Por esta razón, los rasgos de personalidad podrían actuar como factores de riesgo o factores protectores de algunas enfermedades relacionadas con el estrés. Por ejemplo, Chida and Hamer (2008) concluyeron en su meta-análisis que tanto rasgos como estados positivos parecen estar asociados con una hiporeactividad del eje HHA. Además indicaron que una mayor responsividad a nivel cardiovascular está relacionada con hostilidad, agresión o conducta tipo A, mientras que una menor reactividad cardiovascular está asociada a aspectos de ansiedad, neuroticismo o afecto negativo. Por consiguiente, estas diferencias en la percepción de estrés están relacionadas con las diferentes respuestas de estrés a nivel biológico (Carver & Connor-Smith, 2010).

Por un lado, la ansiedad rasgo emerge como un importante mediador de la respuesta de estrés, debido a su relación con la respuesta fisiológica al estrés (Chida & Hamer, 2008), así como su asociación con los estilos de afrontamiento. Estudios centrados en la influencia de la ansiedad rasgo en la respuesta de estrés han hallado que las personas con mayor ansiedad también mostraban mayores sentimientos de frustración y estrés (Bagget et al., 1996). Mientras que algunos estudios describen ausencia de relaciones entre la respuesta cardiaca al estrés y ansiedad (Bagget et al., 1996; Calvo and Cano-Vindel, 1997), otros han mostrado que mayores puntuaciones en ansiedad cognitiva y social está asociada con una mayor actividad cardiovascular durante una tarea de hablar en público (Feldman et al., 2004; Grammer et al., 2006). Además, diversos autores han mostrado que una alta ansiedad rasgo puede influir negativamente la ejecución durante una situación estresante, como es la de hablar en público (Bagget et al., 1996; Calvo and Cano-Vindel, 1997) o durante una entrevista de trabajo (Cook et al., 2000; McCarthy and Goffin, 2004).

Por otro lado, tanto la ansiedad rasgo como la respuesta emocional y fisiológica están estrechamente relacionados con la manera de afrontar situaciones estresantes. Por tanto, podemos entender el afrontamiento como el modo en el que afrontamos situaciones de estrés para reducir el llamado distrés o estrés negativo (Carver & Connor-Smith, 2010). Podemos diferenciar dos patrones extremos, activo o pasivo (Salvador, 2005, 2012; Salvador & Costa 2009). El afrontamiento pasivo se puede definir como las estrategias desadaptativas utilizadas para afrontar una situación estresante, como podrían ser la negación o la desconexión mental, mientras que las estrategias de afrontamiento activo incluirían centrarse en el problema (Carver et al., 1989). De hecho, las estrategias de afrontamiento activas han sido asociadas con una óptima activación del SNA y de la secreción de cortisol, mientras que las estrategias pasivas están caracterizadas por una ineficiente respuesta autónoma y de cortisol ante el estrés (Salvador, 2012). En esta línea, se ha encontrado que las estrategias de afrontamiento activo predicen una mayor respuesta de cortisol (Bhonen et al., 1991) y una recuperación cardiaca más rápida (Faucheux et al., 1989) en personas que se están enfrentado a tareas cognitivas estresantes. Por el contrario, estilos de afrontamiento de evitación han sido asociados con una mayor reactividad de presión arterial (Vitalino et al., 1993).

También se ha propuesto una posible asociación entre ansiedad rasgo y estilos de afrontamiento, sugiriendo que personas con mayor ansiedad rasgo tienden a responder con conductas de afrontamiento desadaptativas al estrés (Houston, 1982). En este sentido, varios años después, Tuncay et al. (2008) mostraron una asociación negativa entre ansiedad rasgo y afrontamiento activo.

En conclusión, el modo en el que percibimos una situación estresante (como amenaza o desafío) podría determinar la percepción de nuestras habilidades para afrontarla con éxito. En particular, en contextos sociales, la ansiedad rasgo parece jugar un importante papel además de interactuar con los procesos de afrontamiento. Este rasgo de personalidad puede ejercer una influencia clave en la respuesta fisiológica al estrés. Por tanto, en el estudio de la respuesta al estrés social y de sus cambios asociados, no se debe obviar la implicación de todos estos factores.

8.2. Objetivos e hipótesis

Teniendo en cuenta la literatura mencionada anteriormente, el objetivo central de esta tesis es clarificar qué factores están mediando en la respuesta de estrés. Para ello, en cada estudio empírico de esta tesis hemos usado un estresor de laboratorio estandarizado, el Trier Social Stress Test (TSST). Además, en cada estudio se han tenido en cuenta diferentes factores (género y hormonas sexuales, edad y personalidad), y/o diferentes componentes de la respuesta de estrés (cortisol, frecuencia cardiaca, conducta y respuesta psicológica).

8.2.1. Estudio 1

Aunque las diferencias de sexo en la respuesta psicobiológica al estrés social no están todavía completamente establecidas, esperamos encontrar una mayor respuesta de cortisol en hombres y una mayor respuesta afectiva en mujeres. Adicionalmente, hipotetizamos que los dos patrones de respuesta (activo/adaptativo y pasivo/desadaptativo) estarán asociados de forma diferente con ansiedad rasgo y estilos de afrontamiento.

Finalmente, no esperamos encontrar relaciones significativas entre ansiedad, estado de ánimo y respuesta de cortisol ante el TSST (Campbell and Ehlert (2012).

8.2.2. Estudio 2

El principal objetivo de este estudio fue analizar la importancia de la autoeficacia percibida, entendiéndola como un componente sustancial de la apreciación cognitiva, en la respuesta de estrés.

En primer lugar, investigamos la influencia de la autoeficacia percibida sobre la respuesta psicológica (ansiedad estado) y la respuesta cardiaca mediante los parámetros RR y r-MSSD, así como la ejecución realizada durante la tarea de hablar en público del TSST. Además, exploramos los efectos de la ansiedad, tanto estado como rasgo, y de los componentes de activación cardiaca (RR y r-MSSD) sobre la ejecución de la tarea.

En este estudio esperamos encontrar que las personas con mayores puntuaciones en autoeficacia percibida muestren una mayor reactividad cardiaca junto con menores aumentos en ansiedad estado. También esperamos encontrar una relación negativa entre autoeficacia y ansiedad rasgo. Con respecto a la ejecución de la tarea, pensamos que la autoeficacia percibida y la activación simpática ejercerán una influencia positiva en el resultado final, mientras esperamos un efecto negativo de la ansiedad rasgo y estado sobre el mismo.

8.2.3. Estudio 3

El objetivo de este estudio estuvo focalizado en la conducta realizada durante la tarea de hablar en público del TSST en mujeres jóvenes con un estatus hormonal diferente, y su relación con la respuesta psicofisiológica al estrés.

En primer lugar, el objetivo fue medir si la respuesta psicofisiológica al estrés era diferente entre dos grupos de mujeres (durante la fase folicular vs. toma de anticonceptivos). En segundo lugar, exploramos si la respuesta fisiológica al estrés está modulada por los patrones conductuales exhibidos durante la tarea de hablar en público.

En consonancia con la literatura previa, no esperamos encontrar grandes diferencias entre los dos grupos de mujeres en la respuesta psicofisiológica al estrés. Hipotetizamos que la intensidad de la respuesta cardiaca y de cortisol estará asociada con un estilo de afrontamiento pasivo. Finalmente, quisimos confirmar hallazgos previos que sugieren una relación entre las conductas de desplazamiento y la respuesta de ansiedad y fisiológicas (cortisol y frecuencia cardiaca).

8.2.4. Estudio 4

El objetivo de este estudio fue ampliar los hallazgos del tercer estudio. Por tanto, este estudio es una extensión de la información sobre los estilos de afrontamiento y la conducta cuando se afronta un estresor agudo y social en mujeres, atendiendo a diferentes fases del ciclo menstrual (folicular y lútea) y en mujeres durante la menopausia.

Para ello, primeramente analizamos la respuesta psicofisiológica al estrés mediante medidas de cortisol, frecuencia cardiaca, y estado de ánimo percibido. En segundo lugar, el objetivo fue investigar el papel de la edad y el eestatus hormonal en los patrones conductuales durante la tarea de hablar en público del TSST. Y finalmente, otro objetivo fue explorar la influencia de ciertas conductas sociales y estilos de afrontamiento, medidos mediante auto informes, sobre la capacidad de reacción y recuperación fisiológica.

Esperamos encontrar una mayor respuesta de cortisol y cardiaca en mujeres jóvenes, especialmente en aquéllas durante la fase lútea, y una peor regulación del eje HHA en mujeres post-menopáusicas.

Además, hipotetizamos que la reactividad fisiológica está modulada por patrones de conducta exhibidos durante la tarea de hablar en público. Específicamente, anticipamos que, en mujeres la intensidad de la respuesta cardiaca y de cortisol estará asociadas con el total de las conductas de huida y de sumisión, las cuales son comúnmente asociadas con estilos de afrontamiento pasivo. Dado que las mujeres post-menopáusicas nunca han sido estudiadas desde una perspectiva etológica, pretendimos explorar las relaciones entre conducta, los procesos de reactividad y recuperación fisiológica en comparación con mujeres jóvenes.

8.3. Resultados principales y conclusiones

8.3.1. Estudio 1

En este estudio investigamos algunos factores involucrados en las diferencias individuales en la respuesta de estrés a un estresor de laboratorio estandarizado (TSST) en mujeres y hombres jóvenes y sanos.

La respuesta psicológica (ansiedad y estado de ánimo) y fisiológica (cortisol) al estrés fue medida mediante una condición experimental y una control en un diseño cruzado. Además, medimos los estilos de afrontamiento (COPE) y ansiedad rasgo (ISRA), mediante auto informes.

Independientemente del sexo, encontramos dos patrones de respuesta claramente diferenciados. El primero (Cluster 1) se caracterizó por una reacción psicológica baja junto a una mayor respuesta de cortisol, mientras que el segundo (Cluster 2) presentó una mayor respuesta psicológica junto a menores incrementos de cortisol.

Desde una perspectiva evolutiva, los participantes del Cluster 1 presentaron una respuesta adaptativa de afrontamiento del estrés. Asimismo, estos participantes también obtuvieron mayores puntuaciones en afrontamiento activo y menores en ansiedad rasgo, mientras que los participantes del Cluster 2 obtuvieron mayores puntuaciones en estrategias de afrontamiento desadaptativas (centradas en la emoción, desconexión mental) y mayores puntuaciones en ansiedad rasgo, específicamente en las áreas situacionales relacionadas con el test de estrés empleado (ansiedad ante la evaluación).

En conclusión, estos resultados subrayan la importancia de las características de personalidad más allá del sexo/género en la respuesta de estrés adaptativa al estrés social.

8.3.2. Estudio 2

La finalidad de este estudio fue investigar la relevancia de la auto-eficacia en la respuesta de estrés psicológica y cardiaca mediante los parámetros de ansiedad, R-R and r-MSSD, respectivamente. También exploramos la influencia de estas variables sobre la ejecución realizada durante la tarea de hablar en público del TSST. Además, profundizamos en la influencia de la ansiedad rasgo sobre los aspectos psicológicos y cardiacos de la respuesta de estrés. Finalmente, exploramos las relaciones entre la propia percepción de la ejecución y el análisis realizado por evaluadores externos y la respuesta psicofisiológica.

El TSST provocó una respuesta autónoma (R-R and r-MSSD) y una activación psicológica (aumentos de ansiedad) significativas en la condición de estrés en comparación con la control en toda la muestra (hombres y mujeres). Encontramos que aquellos con mayores puntuaciones en auto-eficacia reflejaron una mayor activación cardiaca y una mejor ejecución de la tarea de hablar en público que sus homólogos con menores puntuaciones en auto-eficacia. No obstante, no encontramos relaciones entre activación autónoma y ejecución de la tarea. Tampoco observamos diferencias en ansiedad rasgo entre los participantes altos y bajos en autoeficacia, pero confirmamos relaciones negativas entre los componentes cognitivos y sociales de ansiedad rasgo y autoeficacia. Además, el componente cognitivo de ansiedad rasgo correlacionó negativamente con la ejecución realizada. Por tanto, podemos concluir que a pesar de la activación fisiológica, los factores psicológicos (apreciación cognitiva y ansiedad rasgo) podrían ser responsables de una ejecución adaptativa, esencial para el éxito.

8.3.3. Estudio 3

El propósito de este estudio fue investigar la respuesta fisiológica, la ansiedad y estados de ánimo subjetivos, así como la conducta no verbal, en dos grupos de mujeres durante la tarea de hablar en público del TSST. Para ello, contamos con un grupo de mujeres en la fase folicular y otro grupo que tomaban anticonceptivos orales (OCs), amos grupos fueron sometidos a una condición experimental y a una condición control en un diseño intra-sujeto.

Ambos grupos aumentaron sus niveles de cortisol, frecuencia cardiaca y ansiedad estado, y además disminuyeron su estado de ánimo positivo después del estresor. Las mujeres en la fase folicular se mostraron más sensibles al estrés (mayor ansiedad estado y mayores descensos de estado de ánimo positivo), y esto también se vio reflejado en la conducta no verbal durante el estresor, mostrando un mayor porcentaje de conductas de aserción y contacto visual en comparación a las usuarias de anticonceptivos. Además, las mujeres en fase folicular obtuvieron menores puntuaciones en las dimensiones cognitiva y emocional de afrontamiento activo (cuestionario COPE) en comparación a las OCs. También observamos que las asociaciones entre los índices de respuesta psicofisiológica al estrés y la conducta fueron diferentes en cada grupo. En las mujeres en fase folicular, las conductas de sumisión se asociaron a una mayor actividad del eje HHA, las conductas de desplazamiento se relacionaron con una menor frecuencia cardiaca, y los patrones conductuales de aserción se relacionaron con un empeoramiento del estado de ánimo. En cambio, en las usuarias de anticonceptivos, observamos que los patrones de respuesta más comunes (Huida y Afiliación) estaban relacionados con un peor estado de ánimo y una mayor actividad del eje HHA, respectivamente.

En resumen, estos resultados, además de apoyar hallazgos previos en diferentes grupos de mujeres, proporciona nuevas perspectivas sobre la relación entre patrones de conducta durante una tarea de hablar en público y los cambios psicobiológicos relacionados con el estrés.

8.3.4. Estudio 4

Este estudio, en línea con el estudio 3, examina la respuesta psicofisiológica y conductual al estrés en mujeres, esta vez mayores y jóvenes, mientras son sometidas a la tarea de hablar en público del Trier Social Stress Test (TSST). Investigamos como las estrategias de afrontamiento pueden afectar los sistemas regulatorios del estrés de distinto modo dependiendo de la edad y del estatus hormonal.

En primer lugar, nuestros resultados confirmaron que la tarea de hablar en público provocó una respuesta significativa en frecuencia cardiaca, ansiedad y estado de ánimo en todos los grupos. Observamos una mayor respuesta psicofisiológica en mujeres durante la fase lútea, mientras que las mujeres en la fase folicular y post-menopáusicas, caracterizadas por niveles similares de hormonas reproductivas, mostraron una respuesta fisiológica (cortisol y frecuencia cardiaca) y psicológica (ansiedad y estado de ánimo) similar; aunque los cambios psicológicos fueron significativamente menores en post-menopáusicas. Por tanto, podemos sugerir que tanto la edad como el estatus hormonal ejercen una influencia en la respuesta de estrés, siendo las mujeres post-menopáusicas quienes muestran una respuesta más aplanada, y las mujeres durante la fase lútea las más reactivas al estrés. También emergieron diferencias significativas en las estrategias conductuales realizadas durante el estresor dependiendo de la edad y del estatus hormonal.

Las mujeres jóvenes mostraron un mayor porcentaje de conductas que reflejan afrontamiento pasivo y reactivo (Sumisión y Desplazamiento), mientras que las mujeres mayores realizaron un mayor porcentaje de conductas que reflejan afrontamiento activo (Gestos). Además observamos que en mujeres jóvenes (foliculares y lúteas), las conductas que reflejan afrontamiento pasivo (sumisión y aserción) estuvieron asociadas con una mejor recuperación del eje HHA; mientras tanto, en post-menopáusicas, fueron las conductas de afiliación las que ejercieron un papel regulador de la función cardiaca. Finalmente, sólo en post-menopáusicas, encontramos que las estrategias de evitación (mediante auto-informe) estuvieron asociadas con un mayor índice de recuperación de cortisol, mientras que mayores puntuaciones en afrontamiento activo y menor en evitación predijeron una mayor reactividad cardiaca.

En conclusión, este estudio proporciona nuevas relaciones que involucran factores como la edad y el estatus hormonal en la capacidad de respuesta y sensibilidad conductual y fisiológica, así como en la influencia de las estrategias de afrontamiento sobre los procesos de auto-regulación neuroendocrinos.

8.3.5. Conclusiones principales

Tomados de manera conjunta los hallazgos de cada estudio empírico de esta tesis doctoral podemos concluir que:

- Hemos confirmado dos patrones distintos de respuesta al estrés (activo vs. Pasivo). Estos patrones destacan el papel positivo del cortisol para preparar al cuerpo para afrontar el estrés, y el impacto negativo de una mayor ansiedad y estado de ánimo negativo.
- A pesar de las reconocidas diferencias de sexo en la respuesta fisiológica al estrés, sugerimos a la ansiedad como rasgo y a los estilos de afrontamiento como potenciales moduladores que ayudan a revelar un mayor conocimiento en las diferencias individuales, más allá del rol de ser hombre o mujer.
- Demostramos que la activación autonómica debida a la apreciación de la situación estresante no afecta a la ejecución. Parece que los factores psicológicos como la apreciación cognitiva y la ansiedad rasgo son más responsables de una ejecución adaptativa.
- Constatamos el papel de las hormonas reproductivas en el afrontamiento a situaciones que suponen un reto o amenaza.
- Mostramos cómo varios patrones conductuales realizados durante un estresor agudo está relacionado con la regulación (reactividad y recuperación) de los principales sistemas de respuesta al estrés (SNA y el eje HHA).
- Señalamos la relevancia de estudiar la respuesta conductual al estrés desde una perspectiva etológica.
- Revelamos las diferencias de edad en todos los componentes de la respuesta de estrés, y cómo las asociaciones entre los componentes fisiológicos, psicológicos (estado o rasgo) y conductuales también dependen del proceso de envejecimiento.

Como conclusión general, en esta tesis doctoral hemos abarcado los principales componentes involucrados en la respuesta del estrés agudo (psicológicos, fisiológicos y conductuales). Y hemos esbozado el impacto de los factores moderadores, como la edad, el género, las hormonas reproductivas y varios rasgos de personalidad. Podemos subrayar que estos factores juegan un importante papel y deberían ser considerados en el estudio de los factores tanto protectores como perjudiciales de la respuesta de estrés.

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