University of Valencia

Department of Social Psychology

Ph.D. in HUMAN RESOURCES PSYCHOLOGY

Effect of psychosocial stress on cognitive processing and non-verbal behavior: the role of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis

Antecedents and consequences of psychological and physiological stress in a group of healthy young adults

PhD Thesis

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Valencia, May 2017

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Efecto del estrés psicosocial sobre el procesamiento cognitivo y el comportamiento no verbal: rol del sistema nervioso autónomo y del eje hipotalámico-pituitario-adrenal

Antecedentes y consecuencias del estrés psicológico y fisiológico en un grupo de jóvenes adultos sanos

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AKNOWLEDGMENTS

Esta tesis doctoral es el producto de un largo viaje hecho de aprendizaje y superación personal. Un viaje que me ha llevado por senderos difíciles que me han permitido fortalecerme y paisajes hermosos que han hecho que mereciese la pena. Durante este viaje, muchas son las personas que me han brindado su apoyo y cariño.

En primer lugar, quiero agradecer a mi directora de tesis Alicia Salvador por haberme acompañado en este proceso y por darme la posibilidad de aprender y desarrollarme tanto a nivel profesional como personal. Ha sido una gran oportunidad.

Quiero también agradecer a mis compañeros Vanesa, Carol y Matias por haberme ayudado tanto en el proceso experimental como en la redacción de los artículos de investigación. De cada uno de vosotros he aprendido muchas cosas que me llevaré siempre conmigo.

También quiero agradecer a Sara y Teresa por el apoyo durante la fase experimental.

Un agradecimiento especial para Marta, sin su apoyo y cariño no hubiese podido llegar hasta aquí. Sin ti el proceso experimental y las muchas horas de trabajo hubiesen sido infinitas. He encontrado una gran amiga.

Thank you to Professor Mare Teichman for allowing me to do the research stay at her department in Tallinn University of Technology. It has allowed me to meet interesting people and know a fascinating new culture.

No puedo no agradecer a los pilares de mi vida, los amigos de la SGI España: Simo, Eugenia, Cecilia, Bruno, Chiara, solo por mencionar algunos. Muchas gracias por el apoyo y por recordarme cada vez que lo necesitaba el principio fundamental de todo.

También quiero agradecer a mis amigas Cinzia, Deborah y Maria por soportar los momentos en que necesitaba desahogarme y por ayudarme a despejarme después de muchas horas de trabajo.

Per ultimo voglio ringraziare le persone più importanti della mia vita.

Moltissime grazie al presidente Ikeda per essere la mia guida e il mio faro nell'oscurità. Durante tutto questo periodo è stato fondamentale leggerti e cercarti in ogni azione che ho messo in atto. La tua presenza è stata fondamentale per diventare quello che sono.

Moltissime grazie alla mia famiglia: mio padre, mia madre e mio fratello per l'amore, l'appoggio, il coraggio e l'esempio che mi danno ogni giorno. Senza di voi tutto questo non sarebbe stato possibile.

A Veronia che non ho parole per descrivere tutto quello che mi regala ogni giorno da molti anni. Sei e sarai sempre la parte migliore di me. Questa è una vittoria anche tua.

Por último, quiero agradecer muy especialmente a Mario. Muchas gracias por todo el amor que me brindas cada día y por estar a mi lado SIEMPRE sin dudarlo ni un segundo. Eres el beneficio más grande de este doctorado.

Muchas gracias!

THESIS OUTLINE

In the current society stress is highly increasing among young adult people. Indeed, they can be constantly subjected to social evaluative situations (job interviews/exams/performance evaluations) due to the increased competitiveness in the job market. Generally, individuals feel stress when external situations exceed their ability to cope with it. Anything that might be a challenge or a threat to well-being is a stressor. Some stressors are good for people's life. Indeed, short term stress response permits the organism to mobilise energy to fight or flee from a threat enhancing its chances of survival (Cannon, 1932). On the other hand, some stressors are maladaptive and if prolonged over time they might have negative health consequences (McEwen & Gianaros, 2011).

Face a stressful situation triggers the activation of a wide range of physiological responses such as the autonomic nervous system (ANS), the hypothalamus-pituitary-adrenal axis (HPA-axis), and the immune system, among others (Foley & Kirschbaum, 2010). The subjective appraisal of stress and the subsequent physiological responses differ from person to person according to some psychobiological factors. Among these differences, major stressful situation (i.e. to be unemployed) (Ockenfels, Porter, Smyth, J., Kirschbaum, Hellhammer, & Stone, 1995), some personality traits (i.e. optimism) (Creswell, Welch, Taylor, Sherman, Gruenewald, & Mann, 2005), or psychological state (i.e. appraisal of stress) (Tomaka, & Blascovich, 1994) play an important role in stress responses. Moreover, the dissimilar psychophysiological way to react to a stressful stimuli lead people to different outcomes (i.e. cognitive and behavioral performance) (Sandi & Pinelo-Nava,

2007). Therefore, in this thesis I am going to present the results of three studies focused on the antecedents and consequences of a psychophysiological responses to stress in a group of young adults individuals.

The first chapter of this dissertation describes the evolutionary concept of the stress response and the main systems involved. Thus, I am going to 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 that will be developed in the empirical chapters.

The second chapter presents the methodology used to perform the three empirical investigations. Particularly, it includes a description of the sample and the sampling process, the experimental design, the variables and measures used, as well as statistical analyses used to evaluate the hypothesis of the studies.

Chapters third, fourth and fifth describe throughout three main empirical studies the influence of some antecedents and consequents factors involved in the psychophysiological stress response when individuals are subjected to an acute social evaluative challenge, such as a mock job interview. Particularly, chapter third describes the impact of to be a young adult unemployed looking for a job on the psychophysiological responses to stress. The study takes into consideration threat appraisal and cardiovascular responses to stress.

The fourth chapter describes the role of a personality trait on the psychophysiological responses to stress and its impact on behavioral performance. It mainly discusses the role of threat appraisal on the

relationship between optimism and displacement behaviors, as well as the impact of cardiovascular and cortisol responses to this relationship.

The fifth chapter presents a study about the role of sex on the psychophysiological responses to stress and its effect on working memory. Mainly, it discusses the different impact of threat appraisal, cardiovascular responses and cortisol release between males and females' young people and their effect on working memory.

The sixth chapter includes a general discussion, strengths and limitations of these studies. Future directions for research on this topic are also included.

The seventh chapter contains the main conclusions derived from the three studies included in this dissertation.

Finally, the eighth chapter presents a general summary of the studies and main findings in Spanish.

ABBREVIATIONS:

Δ = Delta

ACTH = Adrenocorticotropin hormone

ANCOVA = Analysis of Covariance

ANOVA = Analyses of Variance

ANS = Autonomic Nervous System

AUCG = Area Under the Curve to Ground

BMI = Body mass index

BPS = Biopsychosocial Model

CIs = confidence intervals

CRH = corticotropin-releasing hormone

DS = Digit Span

ECSI = Ethological Coding System for Interviews

fMRI = Functional magnetic resonance imaging

GAS = General adaptation syndrome

GCs = Glucocorticoids

HPA = hypothalamic-pituitary-adrenocortical

HR = Heart Rate

HRV = Heart Rate Variability

ICC = Correlation coefficient

LOT-R = Life Orientation Test Revised

MANOVA = Multivariate Analysis

MR/GR = Mineralocorticoid receptors/glucocorticoid receptors

OC = Oral contraceptive

OFC = Orbitofrontal cortex

OPAL = Labour Insertion Observatory of the University

PASA = Primary Appraisal Secondary Appraisal scale

PFC = Prefrontal cortex

PNS = Parasympathetic Nervous System

S.D. = Standard Deviation

SAM = Sympathetic-adrenomedullary

SEM = Standard Error of Means

SERVEF = Occupational and Labour Agency

SES = Subjective Socioeconomic Status

SET = Social evaluative threat

SNS = Sympathetic Nervous System

STAI-T = State-Trait Anxiety Inventory

TSST = Trier Social Stress Test

WM = Working Memory

CHAPTER I

GENERAL INTRODUCTION

1. The homeostatic stress responses

Stress refers to physical and environmental challenges as well as emotional and psychological ones that can have an effect on an organism's body (Fink, 2010). Stress response triggers a series of physiological, psychological and behavioral changes that allow to maintaining internal homeostasis (state of balance within the bodies of all organisms) and permit the individuals to adapt to stress (Johnson, Kamilaris, Chrousos, & Gold, 1992). Half a century ago, Selye (1946) described the complex responses elicited in an organism when its homeostasis is threatened. Selye coined the term "General Adaptation Syndrome" (GAS) to describe the combined adaptive modifications in nervous, cardiovascular, endocrine and immune system. The author describes the GAS as which “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”. He considers the GAS as composed by three stages. First, an initial “alarm reaction” associated with the activation of the peripheral sympathetic-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenocortical (HPA) axis. During this phase the body is prepared for the fight or flight responses. However, the immune system decreases its effectiveness making individuals more vulnerable to developing illnesses. Second, it is composed by a "stage of resistance" also known as the stage of adaptation. During this phase the adaptive responses triggered by the neuroendocrine signals help to reduce stress. Thus, the body

adapts to the stressor even if the stressor remains. Third, it is presented a "stage of exhaustion" characterized by a gradual decline of stress resistance. During this phase, the body has run out its energy and immune reserves. The body experiences exhaustion. However, after exhaustion the body recovers fully over time. On the contrary, prolonged or too strong stress may compromise the return to homeostasis causing permanent disorders in individuals' body or mind (McEwen, 1998). The compromise of the homeostasis has been defined from McEwen (1998) as "Allostatic load". The author describes three types of Allostatic load: the first is the continuous or frequent activation of the systems implicated in maintaining homeostasis or allostasis, as defined by McEwen. The second is the failure of these systems to shut off after stress. The third is an inadequate response of the allostatic systems to stress. Allostatic load block the dynamic responses to acute stress, leading to an impaired reactivity and recovery capability. All these Allostatic load types have been related to physical and mental diseases such as depression, anxiety, or cognitive impairment (McEwen, 1998). However, even if chronic stress is dangerous for human health, short term stress is beneficial and permits people to grow and adapt themselves according to the environment demands (Salvador, 2005). Particularly, the stress response in young adult people must be considered as a natural process of adaptation and useful to face daily challenges. Moreover, in healthy people, the psychophysiological responses to stress provide all the necessary reactions to maintain homeostasis (Folkman, 2013).

1.2. Mechanisms involved in the stress response

1.2.1. Physiological components

The main systems involved in the responses to stress are the autonomic nervous system (ANS) and the HPA axis. Their alteration as a response to stress may affect cognitive, emotional and behavioral responses (Blascovich & Tomaka, 1996). Even though the stress responses include other neuroendocrine changes (i.e. alpha-amylase, Nater & Rohleder, 2009) in this dissertation we focalized the attention on the ANS and HPA axis responses and on the parameters used to measure their function under stress.

The Autonomic Nervous System (ANS)

The ANS is the first and one of most important system activated in the stress responses. In fact, when someone faces a challenge situation the brain activation, mainly the amygdala (an area of the brain that contributes to emotional processing) permits interpret the situation and as soon as it is perceived as dangerous, it sends a distress signal to the hypothalamus.The hypothalamus, that has been considered as a command center, communicates with the rest of the body through the ANS which controls several involuntary body functions such as breathing, blood pressure and heartbeat. The ANS is composed by two components: the sympathetic nervous system (SNS), or the "fight or flight" response that prepare our body for action. All the organs involved in getting ready for a physical challenge ("fight") or preparing for a retreat ("flight") are activated through this system. The parasympathetic nervous system (PNS) or the "rest and digest"response that helps produce a

state of equilibrium calming the body down after the danger has finished (McCorry, 2007).

After the amygdala sends a distress signal, the hypothalamus activates the sympathetic nervous system by sending signals through the autonomic nerves to the adrenal glands. These glands respond by pumping the hormone epinephrine (also known as adrenaline) into the bloodstream. As epinephrine circulates through the body, it brings on a number of physiological changes (i.e. the heart beats faster than normal, pulse rate and blood pressure go up, person starts to breathe more rapidly). All of these changes happen quickly. In fact, the amygdala and hypothalamus start this cascade even before the brain has had a chance to fully process what is happening (McCorry, 2007).

The most common indicators used to assess ANS and the relationship between SNS and PNS and stress are heart rate (HR) and heart rate variability (HRV) (Task force, 1996). HR is measured in beats per minute. In normal conditions HR increment indicates a predominance of the SNS. HRV measures the specific changes in time (or variability) between successive heart beats and it shown the continuing interplay between SNS and PNS. The time between beats is measured in milliseconds (ms) and is called an "RR interval" which provides information about autonomic tone. Different physiological factors may influence HRV such as gender, age, circadian rhythm, respiration and body position. The most common HRV indexes are calculated from the mean and standard deviation of the R-R interval (i.e. rMSSD, SDNN) (Sztajzel, 2004).

The hypothalamus-pituitary-adrenal axis (HPA axis)

The HPA axis, together with the ANS system, represents system through which the brain influences all body organs during exposure to threatening stimuli. The principal hypothalamic stimulus to the HPA axis is the corticotropin-releasing hormone (CRH), a 41 amino acid peptide first isolated in 1981 by W. Vale (Vale, Spiess, Rivier, & Rivier, 1981). Through the CRH, the hypothalamus secrets the adrenocorticotropin hormone (ACTH) from the anterior pituitary. In turn, ACTH stimulates the release by the adrenal cortex of glucocorticoid hormones (GCs). GCs are the final product of the HPA axis and contribute to the control of whole body homeostasis and the organism's response to stress (Tsigos & Chrousos, 2002). The principal GC in humans is the cortisol which can be measured through blood or saliva.

Unlike HR and HRV which responses are fast and peak almost immediately, cortisol concentrations reach the peak around 15-30 minutes after the stress stimuli (Engert et al., 2011). In the same way, whereas HR and HRV return to baseline some minute after stress is finished, cortisol needs almost 60 minute to return to the original concentrations (Ulrich- Lai & Herman, 2009; Dickerson & Kemeny, 2004). Chronic stress and elevated levels of cortisol might have negative effect on human health (McEwen, 1998). As a matter of fact, it has been observed that high levels of cortisol interfere with learning and memory (de Quervain, Schwabe, & Roozendaal, 2017), it leads to lower immune function and bone density , high cholesterol and heart rate, among others (Lundberg, 2005). Moreover, elevated cortisol

levels also increase risk of depression, mental illness, and lower life expectancy (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013).

1.2.2. Psychological components

Several theories have explained how people stress' perception may affect their physiological and behavioral responses. As Cannon (1932) states, negative emotions are key factors in triggering physiological responses to cope with environmental challenges. Similarly, the way people appraise the situations generates a series of physiological and behavioral changes that are at the bases of their individual way to cope with stress (Carver & Connor-Smith, 2010). Thus, the first stress response is the appraisal of the stress (Carver & Connor-Smith, 2010; Dickerson & Kemeny, 2004; Schlotz, Hammerfald, Ehlert, & Gaab, 2011). Major stressful situations (i.e. unemployment or chronic illnesses) may influence the way people appraise the situation and cope with that. Consequently, the appraisal of stress has an impact on physiological and behavioral changes as a response to stress (Miller, Chen, & Zhou, 2007; Ockenfels, Porter, Smyth, Kirschbaum, Hellhammer, & Stone, 1995). In the same line, personality traits have been observed to play an important role in the psychophysiological responses to stress (Puig-Pérez, Villada, Pulopulos, Almela, Hidalgo, Salvador, 2015).

Cognitive appraisal of stress

In the Transactional model of Stress and Coping, Richard Lazarus (1966) defined stress as a relationship between the person and the environment that is appraised as personally significant. Individuals feel stress when external situations overload their ability to cope with that. The theory

emphasizes the importance of two processes, the appraisal of the stressful stimuli (primary appraisal) and the perception of the ability to cope with it (secondary appraisal), as mediators of the ongoing relationship between the person and the environment.

During primary appraisal process people ask “everything is ok with my well-being?”, whereas during secondary appraisal process individuals ask “Can I cope with that?” Thus, according to primary appraisal people may evaluate the stimulus as threatening or challenges for their own well-being. Threat appraisal refers to the evaluation of the situation as potentially harmful or as a source of failure. By contrast, challenge appraisal refers to the evaluation of the situation as an opportunity for self-growth and beneficial to our well-being (Folkman, 1993). Coping refers to the thoughts and actions people use to manage distress. Thus, appraisal of stress is influenced by the individuals coping resources such as psychological, social, environmental, and material resources, as well as the nature of the situation (i.e. whether its outcome is controllable or not) (Lundberg, 2005). Situations that are appraised as high in personal relevance and low in controllability are usually appraised as threats, and situations that are high in personal relevance and high in controllability are appraised as challenges (Folkman, 1993). When a person is faced with a stressor, the cognitive stress appraisal is considered the difference between the primary and secondary appraisal (Drach-Zahavy & Erez, 2002).

The biopsychosocial model (BPS) of challenge and threat (Blascovich & Tomaka, 1996) is one of the most complete theories that highlights how appraisals shape stress responses. More specifically, depending on the way

stress is evaluated as a challenge or threat, cognitive stress appraisal triggers different physiological responses to stress (e.g., Blascovich, Mendes, Hunter, & Salomon, 1999). Moreover, these chains of events have an impact on performance (i.e. cognition and behavior) (Blascovich & Tomaka, 1996). Although both evaluations have been associated with physiological responses, the perception of the situation as a challenge has shown a more efficient autonomic reactivity and better performance. On the contrary, the perception of the situation as threat has shown an inefficient autonomic reactivity and poor performance (Blascovich & Tomaka, 1996).

Thus, the appraisal of stress is the primary step in stress responses. It generates a series of physiological responses that in turn influence individuals' performances (Blascovich & Tomaka, 1996). Moreover, some major chronic situations or factors such as personality traits may influence the way people appraise the situation (David, & Suls, 1999; Kirschbaum, Bartussek, & Strasburger, 1992; Miller, et al., 2007; Ockenfels, et al., 1995).

1.2.3. Behavioral component

Behavior is considered one of the last sequential outcomes of the stress response. However, only recently more attention has been paid on this significant human stress related performance. During several years, the human stress response has been considered, both physiologically and behaviorally, characterized by "fight-or-flight" reaction (Cannon, 1932). The fight-or-flight response, first described by Cannon in 1932, is characterized physiologically by SNS activation that innervates the adrenal medulla, producing a hormonal cascade that results in the secretion of catecholamines,

mainly norepinephrine and epinephrine, into the bloodstream. Additionally to its physiological concomitants, fight-or-flight has been used to describe human behavior in responses to stress. Specifically, whether a human (or animal) fights or flees in response to sympathetic arousal is considered to depend on the nature of the stressor and their appraisal to that. If individuals consider that they have a realistic chance of overcoming the challenge, then fight is likely. In circumstances in which the threat is perceived too much to handle for their coping abilities, then flight is more probable. Nevertheless, although fight-or-flight has been considered the primary physiological responses to stress for both males and females, recently it has been proposed that females differ from males' responses. Thus, females' responses to stress have been related to a pattern of "tend and befriend."

The "tend-to-befriend" response, described by Taylor and colleagues (2000) affirms that to face a stressful situations women are more likely to express affiliative social behavior. They tend to quiet and care for offspring and blending into the environment. It may be effective for addressing a broad range of threats. Moreover, they are inclining to befriend the enemy, whether there is an enemy causing the stress, or to look for social support from their family members or friends. Physiologically, instead of releasing large amounts of norepinephrine and cortisol into the bloodstream as men do, women respond to stress by secreting more endorphins (a peptide hormone that inhibits the transmission of pain signals and produces a feeling of euphoria during social interactions) and oxytocin (a neurohormone that regulates social interaction – particularly maternal-infantor bonding - and sexual reproduction).

During the last years, several behaviors have been studied in relation to stress, such as affiliative, submissive, assertive, displacement or escape behaviors, among others. Each behavior has been related to different emotional states and social attitudes. Moreover, several systems have been developed in order to detect and assess this kind of behaviors (Mohiyeddini, Bauer, & Semple, 2013a-b; Troisi, 2000). Particularly, a strong relationship with physiological stress responses (HR and cortisol) and emotion (i.e. anxiety state and trait) has been found with displacement behaviors, defined as a set of movements focused on one's body such as hand-face touching or scratching (Mohiyeddini et al., 2013a; Pico-Alfonso et al., 2007; Sgoifo et al., 2003, Villada, Hidalgo, Almela, Mastorci, Sgoifo, & Salvador, 2014). However, little has been explored about the role of different personality trait and stress appraisal on it.

1.3. Aim of the thesis

The main aim of this dissertation was to assess the role of some potential antecedents and consequents of psychological and physiological responses to stress in healthy young adult people. In order to do that, we carry out a semi-experimental study subjecting individuals to a standardized laboratory stressor, the Trier Social Stress Test (TSST). Moreover, we used a multi-method approach performing physiological laboratory analysis, using self-repost questionnaires, observing and computing ethological behavior, and subjecting participants to a cognitive test. In the following paragraphs the main aim of each study is described:

Study 1.

Nowadays, the high unemployment rate present in different European countries makes it more difficult for individuals to find a job in a short period of time. For this reason, people can spend a very long time (months, years) looking for work. It implies being subjected to different acute evaluative stress situations such as job interviews. Unemployment along with job search is one of the biggest causes of stress especially for young adult individuals (Knabe & Rätzel, 2011). Being unemployed looking for a job has been shown to have negative psychological and physical health consequences (Ali, Ryan, Lyons, Ehrhart & Wessel, 2016; Urbanos-Garrido & Lopez-Valcarcel, 2014). Although several studies have been carried out to understand the role of unemployment on health consequences (i.e. Roelfs, Shor, Davidson, & Schwarts, 2011; Wamberg, 2012), little attention has been paid on the psychophysiological mechanisms related to it. Thus, the aim of this study was to investigate the effect of being unemployed job seeker on cognitive stress appraisal and physiological responses to job interview. Particularly, we wanted to verify the impact of being unemployed looking for a job on cognitive threat appraisal and cardiac responses. Moreover, we wanted to assess the mediating role of cognitive threat appraisal between being unemployed looking for a job and the cardiac responses to stress.

Study 2.

Nowadays the feeling of stress is increasing in our societies, particularly in young adult people subjected to social evaluative situations in highly competitive academic and work contexts. Threat appraisal is a primary

and fundamental reaction when people face a stressful situation (Folkman, 2013). The way people assess stress might be influenced from some personality trait (Penley & Tomaka, 2002), as well as it might influence consequent individuals' behaviors (Troisi, 2002). The main aim of this study was to investigate the role of a positive personality trait (dispositional optimism) on threat appraisal of stress, and the mediated role of threat appraisal between dispositional optimism and a stress related behavior (displacement activities). Also, given fundamental role of physiological activity in stress responses, it was also studied the moderated role of HR and cortisol on the relationship between threat appraisal and displacement behaviors.

Study 3.

Stress response has been observed to have an important role on cognitive performance (Wolf, Atsak, Quervain, Roozendaal, & Wingenfeld, 2016). Moreover, sex has been considered an important moderated factor in the relationship between stress and cognitive performance, particularly working memory performance (Almela, Hidalgo, Villada, Espín, Gómez-Amor, & Salvador, 2011). Nevertheless, the results about sex differences on WM are strongly contradictory, and the impact of cognitive stress appraisal on working memory performance has not received much attention. The aim of this study was to investigate the role of physiological responses (heart rate and cortisol) and cognitive stress appraisal on working memory (WM) performance in males and females.

CHAPTER II

METODOLOGY

2. Participants and sampling process

The sample used in the three articles was collected during the academic year 2012/2013. Individuals were recruited from a wide range of classes at the University of Valencia, from the campus office of the Occupational and Labour Agency (SERVEF), and from the Labour Insertion Observatory of the University (OPAL). The recruitment process took two phases:

First screening. Potential participants were initially screened (i.e. age, level of education) by an expert psychologist through a telephone interview.

Second screening. Subjects considered suitable to pass to the second phase of recruitment were asked to fulfill a self-repost with more specific inclusion criteria and send it back by e-mail (for more specific information about the inclusion criteria see the "Participant" section presented in the three studies). Totally, 162 individuals were assessed. Subjects who fulfilled all the criteria were asked to attend sessions that took place in a laboratory at the Faculty of Psychology.

The final sample for the three studies was composed of 82 healthy young adults (37 males and 45 females) from 20 to 39 years old (mean ± SEM: Age = 24.98 ± 0.55). Participants were students finalizing bachelor's studies (32,1%) or graduate (58%) and post graduate (9,9%) in a wide range of majors at the University of Valencia. The 6,9% of them considered their economic situation medium/high. All participants were volunteers;

nevertheless they were offered to receive feedback from an expert interviewer about how to improve their individual performance on a job interview. It was provided at the end of the experimental session. More information about the specific characteristics of the sample has been provided in each "participant" section presented in the three articles.

2.1. Experimental design

Experimental sessions were performed during the academic year 2012/2013. Experimental procedure lasted approximately 90 min. and took place between about 16.00 and 19.00h from Monday to Friday avoiding civil or religious festivities.

The experimental sessions were composed by different phases:

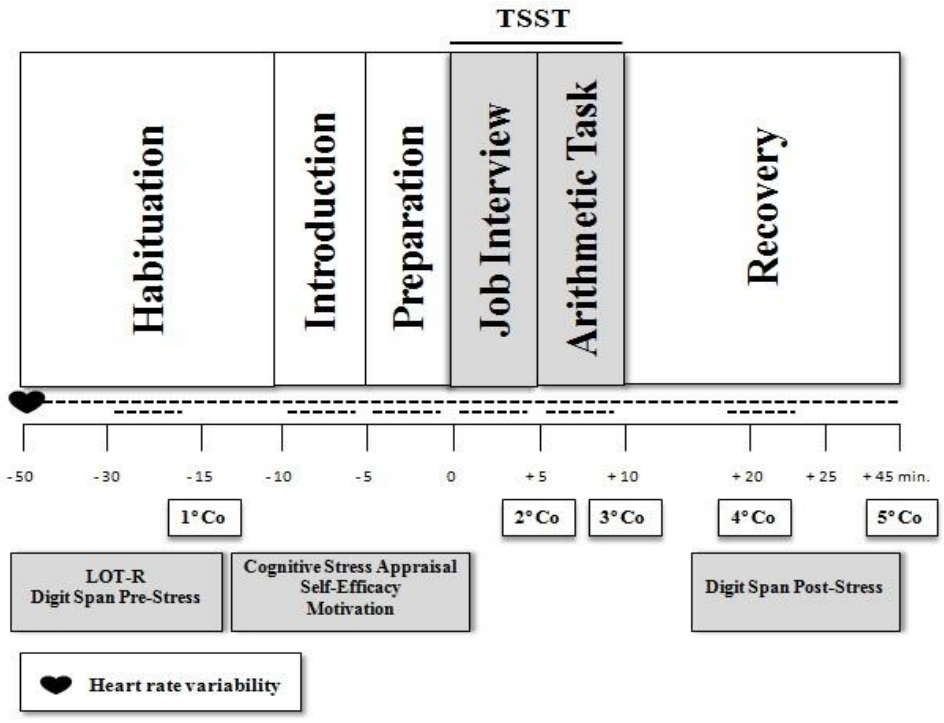

Figure 1. General protocol.

Habituation

The session started with a 40 minutes habituation phase. During this time, participants had to fill out a general questionnaire that included a battery of questionnaires and tests to measure psychological states and traits and cognitive performance. Particularly, the psychological questionnaires participants had to fulfill during habituation phase were: Life Orientation Test Revised (LOT-R; Scheier, Carver, & Bridges, 1994) and State-Trait Anxiety Inventory (STAI-T) (Spielberg, Gorsuch, & Lushene, 1970). Moreover, cognitive performance pre-stress was tested though the Digit Span numbers pre-stress. Physiological variables were started to be measured: HRV was recorded continuously during the whole experimental procedure, while five salivary cortisol intakes were collected along the session. The first cortisol sample was taken during habituation phase (-15 min.).

Introduction

During this phase the interviewer introduced to the participants the stressful tasks of the TSST: job interview and an arithmetic task. Immediately after that, they had to fill out the Primary Appraisal Secondary Appraisal scale (PASA, Gaab, Rohleder, Nater, & Ehlert, 2005) and a general Self-Efficacy and Motivation scale based on Bandura's (1997) recommendations (Costa, Serrano, & Salvador, 2016; van der Meij, Buunk, Almela, Salvador, 2010).

Preparation and TSST

To produce stress, participants were subjected to a modified version of the Trier Social Stress Test (TSST, Kirschbaum et al., 1993). The modifications from the original were: (i) all the phases took place in the same room, whereas in the original test two separate rooms are used (one room for introduction phase and TSST and another room for the rest of the phases); (ii) the committee was composed of only one person (female) who had been introduced as an expert in human resources, while in the original protocol the committee was composed by two people (male and female).

The TSST protocol consisted of 5 minutes of free speech (job interview), followed by a 5 minutes arithmetic task. During the TSST participants stood at a distance of 1.5 meters from the interviewer and a video camera, a microphone, and a monitor where subjects could see their performance were clearly visible. Both the speech and arithmetic tasks were videotaped.

Before starting the stress tasks, a preparation phase is performed. During this phase, it was asked to the participants to write down their main ideas about what they would like to say during the job interview. However, they could not use these notes during the speech task. Preparation phase lasted 5 minutes.

Job interview

During job interview, the main purpose of the participants was to convince the expert interviewer that he/she was the best candidate for their

"dreamed job". The expert introduced the job interview telling to the subjects that his/her professional curriculum had been positively evaluated. However, for a final evaluation they needed to explain what characteristics and personal competencies they had to fill that position. The task lasted 5 minutes. The interviewer was allowed to ask questions only whether the subjects were unable to speak for the entire duration of the phase. After completing the job interview, the second cortisol sample was taken (+5 min.).

Arithmetic task

The subjects had to perform an arithmetic calculation for 5 minutes in front of the evaluator. At the end of the arithmetic task, the third cortisol sample was taken (+10 min.).

Recovery

The recovery phase lasted 35 minutes. The main purpose of this phase was to measure post stress physiological and cognitive variables. At the middle of this phase, the fourth cortisol was taken (+20 min.). Moreover, cognitive performance post stress was tested though the Digit Span numbers post-stress. At the end of this phase, a sample of the fifth cortisol sample was taken (+45 min.), and weight and height were measured in order to calculate participants' body mass index (BMI).

2.2. Measures

In the following, the battery of questionnaires, test and behavioral and individual variables used along the three articles presented in the thesis are

described. At the end of the section the Table 1 is included, summarizing the variables used in each empirical study of the present thesis.

2.2.1. Physiological variables

Heart Rate and Heart Rate Variability

Data for Heart Rate (HR) and Heart Rate variability (HRV) were continuously recorded during the entire session using a Polar©RS800cx watch (Polar CIC, USA), which consists of a chest belt for detection and transmission of heartbeats and a Polar watch for collection and storage of the data. The transmitter is located on the chest belt, which is placed on the solar plexus and transmits HR and HRV information to the receiver (Polar watch). The data collected by the Polar watch were downloaded and stored in the Polar ProTrainer5TM program in the computer and analyzed using HRV software Kubios Analysis (Biomedical Signal Analysis Group, University of Kuopio, Finland).

HR and HRV parameters were used in the three studies: Particularly, in the first investigation the following parameters from time domain analysis were used: the average HR in beats per minute (bpm) as an indicator of sympathetic activation and a selecting root mean square of successive differences (rMSSD 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. In the second and third study the average HR in beats per minute (bpm) parameter was used.

Neuroendocrine response

We measured the activity of the HPA axis analyzing the cortisol from the five salivary samples taken through the session (see Figure 1). Participants provided saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany). They were instructed to keep the cotton swab in their mouths for exactly 2 min, without chewing it, and move the swab around in a circular pattern to collect saliva from all salivary glands. The samples were centrifuged at 3000 rpm for 5 min, resulting in a clear supernatant of low viscosity that was stored at 80°C until the analyses were performed in the Central Research Unit (Unidad Central de Investigación) of the Faculty of Medicine, University of Valencia (Spain). The salivary cortisol samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Spectria Cortisol RIA (cat. Nu 06119) from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/L, and within- and inter-assay variation coefficients were all below 8%.

In order to verify the hypotheses of the second and third study, salivary cortisol measures were used. On the contrary, it was not assessed in first investigation.

2.2.2. Psychological variables

Anxiety Trait

Anxiety Trait was evaluated by the Spanish version (Seisdedos, 1988) of the STAI-T (Spielberger et al., 1970). It consists of 20 items (e.g. ‘I feel at

ease', 'I feel upset') with a 4-point scale ranging from 0 (not at all) to 3 (extremely).

Self-efficacy

Self-efficacy was measured based on Bandura's (1997) recommendations. Particularly, two items were used: (i) How capable are you of successfully perform this task? (ii) How much confidence do you have that you will successfully perform this task? The scale was from 1 (none) to 100 (very much). The average of these two items made it possible to obtain a situational self-efficacy score (Costa et al., 2016; van der Meij et al., 2010).

Motivation

Motivation was evaluated by asking (again on a scale from 1 to 100): How important is it for you to successfully perform this task?

Anxiety Trait, Self-efficacy and Motivation were used in the preliminary analysis of the first study to verify the possible confounding effect of it on the results.

Cognitive stress appraisal

Cognitive stress appraisal was evaluated with the PASA scale (Gaab et al., 2005). This scale was employed to assess cognitive appraisal processes before performing the TSST. The PASA scale is composed of the "Primary appraisal" subscale, which includes two situation-specific subscales assessing 'Threat' (e.g., I do not feel threatened by the situation) and 'Challenge' (e.g., The situation is not a challenge for me), and the "Secondary appraisal" subscale, which includes two situation-specific scales assessing 'Self-Concept of Own Competence' (e.g., In this situation I know what I can do)

and ‘Control Expectancy’ (e.g., It mainly depends on me whether the experts judge me positively). Each scale has four items rated on a 6-point Likert scale ranging from “strongly disagree” to “strongly agree”.

Threat appraisal sub-scale was used in order to evaluate the hypotheses of both first and second studies, whereas in the third study the four situation-specific subscales composing primary appraisal and secondary appraisal were assessed. Moreover, based on the transactional stress paradigm (Lazarus & Folkman, 1984), the “Tertiary appraisal” variable (also called the “global index of cognitive stress appraisal”) was calculated using the formula proposed by Gaab and colleagues (2005).

Life Orientation Test Revised (LOT-R; Scheier et al., 1994)

LOT-R is composed by 10 items and two subscales: optimism and pessimism. Three items measure dispositional optimism (e.g. “In uncertain times, I usually expect the best”), and three other items measure dispositional pessimism (e.g. “If something can go wrong for me, it will”); the remaining items are distractors. Items are answered on a 5-point Likert scale from 0 (strongly disagree) to 4 (strongly agree).

Dispositional optimism was used to verify the hypothesis of the second article. Pessimism items were reversed to obtain a one-dimensional measure of dispositional optimism. We employed the Spanish version of LOT-R (Otero, Luengo, Romero, Gómez, & Castro, 1998).

2.2.3. Cognitive test

Working Memory test: Digit Span subtest

The Digit Span subtest of the Wechsler Memory Scale III (Wechsler, 1997) was performed before (habituation phase) and after (recovery phase) TSST. Specific information about the test is provided on pages 130-131.

Digit Span test pre stress and post stress was used to assess the effect of stress on cognitive performance in the third article.

2.2.4. Behavioral variable

Displacement behavior

Displacement behaviors were analyzed using modified version of the Ethological Coding System for Interviews (ECSI) (Troisi, 2002). Two main behavioral categories were analyzed: 1. Hands movements' displacement behaviors; 2. Body movements' displacement behaviors. Each displacement behavior has been assessed as present = 1 or no present = 0 with a frequency of 20". Each video lasted 5 minutes (job interview). The total score indicates the frequency of displacement behaviors in each category. Detailed information about displacement behaviors analysis is presented on pages 100-101.

Displacement behaviors were used to evaluate the hypotheses of the second article.

2.2.5. Individual variables

Unemployed job seeker

This variable was used to compare two groups: unemployed job seeker and inactive people (i.e. students). For more information about how this variable was evaluated see “Participants” on page 68.

Unemployed job seeker variable was used to verify the hypotheses of the first article.

Sex

This variable was used to compare males and females in cognitive performance. Moreover, it was taken into consideration a possible influence of menstrual cycle on the results (for more information about how it was measured see page 68).

Sex variable was used to assess males and females differences in cognitive performance in the third article.

Summary of the variables used in the three empirical studies presented in the thesis

	Study 1	Study 2	Study 3
Physiological variables			
HR	X	X	X
r-MSSD	X		
Salivary cortisol		X	X
Psychological variables			
Anxiety Trait	X		
Self-Efficacy	X		
Motivation	X		
LOT_R		X	
Cognitive threat appraisal	X	X	X
Challenge appraisal			X
Self-concept of own competence			X
Control expectancy			X
Tertiary appraisal			X

Cognitive test			
WM Test: Digit Span subset			X
Behavioral variable			
Displacement behavior		X	
Individual variables			
Unemployed job seeker	X		
Sex			X

Table 1. Summary of the variables used in the three empirical studies

2.2.6. Statistical Analysis

Preliminary analyses

In the three empirical studies were tested data normal distribution and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests before the statistical procedures were applied. These analyses revealed significant deviations in the HR, rMSSD and Cortisol values. Therefore, data were square-root-transformed in the first article, whereas they were logarithm transformed in the second and third articles. Both data's transformations are valid procedures of replacement that changes the shape of the data's distribution ensuring its normality and homogeneity (Bartlett, 1936; Bartlett & Kendall, 1946).

Outliers were evaluated in the three empirical studies. Outliers are generally considered to be data points that are far outside the norm for a variable or population (e.g., Jarrell, 1994). Evaluate outliers avoid to include inflated error rates and consequently substantial distortions of parameter and statistic estimates (Osborne, & Overbay, 2004). Two different outlier evaluation procedures were used in the present thesis: in the first study outliers were evaluated on the basis of Mahalanobis distance D^2 (De Maesschalck, Jouan-Rimbaud, & Massart, 2000), in the second and third

articles were considered outliers subjects which indexes differed more than 3 S.D. from the total sample mean (Seo, 2006).

In order to perform correlation and regression analysis with physiological variables, in the first article we calculated the areas under the total curve with respect to the ground (AUCg) for HR and rMSSD using the trapezoid formula specified in Pruessner, Kirschbaum, Meinlschmidt, and Hellhammer (2003). The use of AUCg simplifies the statistical analyses by transforming the multivariate into univariate data, especially when the numbers of repeated measurements are high and there is a need to summarize the information (Fekedulegn et al., 2007). In the second and third articles a Delta changes (Δ) for HR and Cortisol were calculated by subtracting baseline levels (HR: habituation; cortisol: -15 min) from the highest indexes (HR: job interview; cortisol: +20 min). Also in this case, Δ variable permits to transform multivariate into univariate data. Particularly, it indicates the effect of stress on physiological responses estimating the change from baseline to the highest indexes.

Moreover, the possible effects of demographic, anthropometrics and/or psychological variables on the principal variables studied were also investigated. In order to do that, a series of analyses of variance (ANOVAs) and Pearson' correlations were performed (for more details on the analyses see the sections "Statistical analysis" of each one of the three studies).

In the three studies, the effect of TSST on HR, rMSSD and/or cortisol during the experimental session was evaluated performing ANOVAs or Analyses of Covariance (ANCOVAs) for repeated measures.

In all cases, when ANOVAS or ANCOVAs were performed, Greenhouse–Geisser procedure was used when the requirement of sphericity 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 $p < .05$.

All the analyses were performed with SPSS 20.0 to perform the statistical analyses.

Specific statistical analysis for the three studies

The specific statistical analyses carried out in order to verify the hypothesis for each one of the three studies are indicated in the following.

Study 1.

In order to create the variable "unemployed job seekers" a dummy variable (unemployed job seekers: 1 and unemployed non-job seekers: 0) was computed. It was considered as independent variable. Moreover, a mediation procedure based on nonparametric resampling, known as bias-corrected bootstrapping, was conducted to assess the mediating role of cognitive threat appraisal in the relationship between being an unemployed job seeker and AUCg HR and rMSSD (PROCESS model number 4) (Preacher and Hayes, 2004). Bootstrapping allows gathering many alternative versions of a single statistic that is usually only calculated from one sample. The analysis was based on 10,000 bootstrap iterations, and the CI was set at 95%, as recommended by Mallinckrodt et al. (2006).

Study 2.

To assess the mediated effect of threat appraisal in the relationship between dispositional optimism and displacement behavior a bias-corrected bootstrapping mediation procedure was conducted (PROCESS model number 4; Preacher and Hayes, 2004). Successively, we performed moderated mediation analyses to estimate the moderator effect of ΔHR and ΔCortisol on the relationship between threat appraisal and displacement behavior, being threat appraisal a mediated variable between dispositional optimism and displacement behavior (PROCESS model number 14; Preacher and Hayes, 2004). As in the case of study 1, the analysis was based on 10,000 bootstrap iterations and the CI was set to 95%, as recommended by Mallinckrodt et al. (2006).

Study 3.

To verify sex differences in the several factors composing cognitive stress appraisal (Threat appraisal, Challenge appraisal, Self-concept of Own Competence, Control expectancy, Tertiary PASA) Multivariate Analysis of Variance (MANOVA) was performed. ANCOVA for repeated measures were used to assess sex differences in cortisol, HR, and WM performance (DS-Forward and DS-Backward). Sex (males vs. females) was stated as between-subject factor and Time (cortisol: - 15, + 5, + 10, + 20, + 45; HR: habituation, preparation, job interview, arithmetic task and recuperation; WM: pre and post) as a within-subject factor. Moreover, hierarchical regression analyses were performed to investigate the role of Δcortisol/ HR and cognitive stress appraisal factors were predictor variables of WM

performance. Finally, following the self-regulation perspective (Thompson, Morgan, & Jurado, 2015) and based on Miller and colleagues (2013), the sample was split into Increases, Decreases and No-changers (for more information about how it was split see page 140). Thus, in order to verify possible differences among this three groups on Cortisol, HR, and cognitive stress appraisal, and on WM performance, ANCOVA for repeated measures were conducted. Group (increasers vs. decreasers vs. no changers) and Sex (males vs. females) were stated as between-subject factors, and Time (cortisol: - 15, + 5, + 10, + 20, + 45; HR: habituation, preparation, job interview, arithmetic task and recuperation; WM: pre and post) as a within-subject factor. In all this analyses Age, BMI were used as covariate variable. In addition to that, in the last ANCOVA for repeated measures described before the basal levels of cortisol and HR were also used as covariate variables

CHAPTER III

STUDY 1

Searching for a job: Cardiac responses to acute stress and the mediating role of threat appraisal in young people

The main results of this study have been published in:

Zandara, M., Garcia-Lluch, M., Villada, C., Hidalgo, V., & Salvador, A. (2017). Searching for a job: Cardiac responses to acute stress and the mediating role of threat appraisal in young people. *Stress and Health*. (in press). doi: 10.1002/smi.2757

3. Introduction

Within the current economic panorama, youth unemployment has become an increasing problem for many European countries (Thern, de Munter, Hemmingsson, & Rasmussen, 2017). Indeed, leaving university to find employment is a difficult transition for young people (Bell & Blanchflower, 2011; Bergmark & Palme, 2003). Furthermore, it has been observed that the fear of future unemployment is detrimental to the well-being of young adults and might be a cause of stress in itself, particularly in countries with high unemployment rates (Knabe & Rätzel, 2011). Several studies found that youth unemployment can decrease physical and mental health (Ali, Ryan, Lyons, Ehrhart, & Wessel, 2016; Brown et al., 2003; Koziel, Lopuszańska, Szklarska, & Lipowicz, 2010; Gallo et al., 2004; Sullivan & von Wachter, 2009; Roelfs, Shor, Davidson, & Schwartz, 2011; Urbanos-Garrido & Lopez-Valcarcel, 2014; Wanberg, 2012). Other studies have shown that the negative effects of youth unemployment on mental health can remain during adulthood, even without experiencing unemployment again (Fergusson, McLeod, Horwood, 2014; Hammarström & Janlert, 2002). In addition to the unemployment condition, individuals have to take active steps every day to find a job, with the accompanying uncertainty about their positive outcomes (Boswell, Ryan, Zimmerman, Brian, & Swider, 2012). Searching for a job is a difficult, stressful task for many young people, and it includes several activities carried out to look for employment (Ali et al., 2016; Boswell et al., 2012). Particularly, it may involve being subjected to successive job interviews and facing repeated

social evaluative threat (SET) situations. A SET situation occurs when an aspect of the self can be negatively judged by others (Dickerson & Kemeny, 2004); consequently, it triggers many important psychophysiological responses. However, surprisingly, and despite its relevance in modern society, no attention has been paid to the physiological consequences of the stress of being an unemployed young person looking for a job (Duffy, Bott, Allan, & Torrey, 2013).

Regarding physiological responses to SET situations, one of the most important biomarkers of the stress response is the cardiac response measured through heart rate (HR) and HR variability (HRV) parameters. They reflect the activity of the autonomic nervous system (ANS) and are specifically the result of the interplay between the excitatory sympathetic nervous system (SNS) and the inhibitory parasympathetic nervous system (PNS). In normal situations, the continuous interaction between these two subsystems helps people to adequately respond to specific challenges and situations (Ganzel, Morris, & Wethington, 2010; McEwen & Gianaros, 2011). However, recent investigations have pointed out that in conditions of ongoing stress, HRV can become less flexible and respond less robustly (Chatkoff, Maier, & Klein, 2010; McEwen & Gianaros, 2011; Porges, 1995; Seeman, Epel, Gruenewald, Karlamangla, & McEwen, 2010). In addition, several studies have observed that subjecting people to constant stressful situations might produce an attenuated sympathetic response to acute stressful situations, mostly in moderate sympathetic activity (al'Absi, Bongard, Buchanan, Pincomb, Licinio, & Lovallo, 1997; Hughes, Howard, James, & Higgins, 2011; Kelsey, Blascovich, Leitten, Schneider, Tomaka, & Wiens, 2000; Kelsey, Soderlund

& Arthur, 2004; Kelsey, Blascovich, Tomaka, Leitten, Schneider, & Wiens, 1999). In addition, it was recently pointed out that this lower sympathetic reactivity is a maladaptive response to stress, rather than benign and protective, and might have a harmful impact on health and behavior (Phillips, Ginty, & Hughes, 2013). All these investigations are based on Selye's work on adaptation, which indicates that individuals experiencing ongoing stress become depleted and fail to react adequately to environmental demands (Selye, 1993). Thus, being an unemployed person looking for a job is a stressful situation (Bell & Blanchflower, 2011; Bergmark & Palme, 2003) that might have a negative impact on the physiological capability to respond to acute social stress.

SET responses seem to be influenced, among other psychological dimensions (i.e. anxiety trait, Gonzalez-Bono et al., 2002; motivation, Phillips et al., 2013; self-efficacy, Gerin, Litt, Deich, & Pickering, 1995), by mental events such as cognitive threat appraisal (Gaab, Rohleder, Nater, & Ehlert, 2005; Kudielka, Hellhammer & Wüst, 2009). From an evolutionary perspective, cognitive threat appraisal may be necessary to survive and respond adaptively to stress stimuli (LeDoux, 1996). Cognitive threat appraisal is a pervasive, common, core stressor that occurs when a person anticipates personal harm due to challenges or novel situations, or expects that loss is imminent (Feldman, Cohen, Hamrick, & Lepore, 2004). Specifically, in a job interview people see themselves as being judged by others, knowing that the judgement might be negative. However, it has also been observed that some threatening stimuli such as SET situations might be considered less threatening over time (Quigley, Barrett, & Weinstein, 2002;

Tomaka, Blaschovich, Kelsey, & Leitten, 1993; Thayer, Ahs, Fredrikson, Sollers III, & Wager, 2012). As a process of emotion regulation, people tend to reappraise the affective meaning of the stressful stimuli using different coping strategies (i.e. external locus of control), coming to perceive the stimuli as less threatening (Mantler, Matejicek, Matheson, & Anisman, 2005). In the same way, unemployed job seekers may adjust to the threatening situation of being an unemployed job seeker and appraise it as less threatening over time.

Cognitive threat appraisal has also been found to influence cardiovascular responses during SET situations (Thayer et al, 2012). For example, cognitive threat appraisal during public speaking tasks or a mock job interview has been found to lead to higher cardiovascular reactivity (Feldman et al., 2004), and the anticipation of a threatening situation might be a source of stress in itself, showing increases in cardiac activity during job interviews (Everson, Kaplan, Goldenberg, & Salonen, 1996). Ongoing cognitive threat appraisal might lead to maladaptive physiological functioning (Thayer et al., 2012), although the appraisal could change over time as a result of prolonged stress or long-lasting exposure to the same stressor. Therefore, its threatening value would diminish over time (Quigley et al., 2002; Tomaka et al., 1993), leading to decreases in HR responses (Cacioppo, Rourke, Marshall-Goodell, & Tassinary, 1990; Kelsey et al., 1999; 2000; 2004; Olff, Langeland, & Gersons, 2005). Moreover, several authors have claimed that cardiac responses are associated with neural structures involved in threat appraisal (Thayer & Lane, 2009; Thayer et al., 2012). When a subject is facing a threat, some brain areas such as the medial

prefrontal cortex become hypoactive. This hypoactive state creates a lack of inhibition of the sympathoexcitatory circuits, which are indispensable for energy mobilization (Thayer et al., 2012). However, over time, this process produces wear and tear on these circuits, causing a decrease in physiological responses (i.e. lower sympathetic activity) (McEwen, 1998). Thus, considering that unemployed job seekers might lower their cognitive threat appraisal of SET situations due to stress and/or habituation, their cardiac responses might change accordingly (the lower the cognitive thrteat appraisal, the lower the cardiac responses).

With all of this in mind, the aim of this study was to analyze the cardiac response and cognitive threat appraisal of unemployed young-adult people looking for a job. For this purpose, we compared a group of unemployed job seekers to a group of unemployed non-job seekers, and we subjected them to a standardized social evaluative stressor. Based on the idea that being unemployed and looking for a job is a difficult and stressful situation for many young adults (Bell & Blanchflower, 2011; Bergmark & Palme, 2003; Thern et al., 2017; Knabe & Rätzel, 2011), and that being subjected to repeated stressful situations might lead to lower physiological responses (al'Absi et al., 1997; Hughes et al., 2011; Kelsey et al., 1999; 2000; 2004), we expect that (H1) unemployed young adults looking for a job will show lower sympathetic (HR) and higher parasympathetic (rMSSD) reactivity to a SET situation, compared to young-adult non-job seekers. Moreover, based on the emotion regulation process, we expect that (H2) unemployed job seekers will show lower cognitive threat appraisal to a SET situation than non job-seekers (Mantler et al., 2005; Quigley et al., 2002;

Tomaka et al., 1993; Thayer et al., 2012). Finally, we expect that (H3) cognitive threat appraisal will mediate the relationship between being an unemployed job seeker and overall stress-induced cardiac output (AUCgHR and rMSSD) (Cacioppo et al., 1990; Feldman et al., 2004; Kelsey et al., 1999; 2000; 2004; Olff et al., 2005; Thayer et al, 2012).

3.1. Method

Participants

The final sample was composed of 82 healthy young adults divided into two groups: unemployed job seekers (N= 42; 21 men and 21 women) and unemployed non-job seekers (N= 40; 17 men and 23 women). The general inclusion criteria were: (i) Spanish nationality; (ii) age between 20 and 40; (iii) an educational level between secondary school and a Ph.D.; (iv) not smoking more than five cigarettes per day; (v) no alcohol or any other drug abuse; (vi) absence of visual, speech, or hearing problems; (vii) absence of sympathetic, endocrine, neurological, or psychiatric diseases; (viii) not having been under general anaesthesia once or more than once in the past year; (ix) not having experienced a major stressful life event during the past year; (x) not using any medication directly related to cardiac, emotional, or cognitive function, such as glucocorticoids or b-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies or psychotropic substances. None of the women had gynecological problems, they all had regular menstrual cycles of between 24 and 36 days, and they reported the last date of their menses. This information was used to identify women who

were in the follicular (N=12), luteal (N=12), or menstrual (N=9) phase, or oral contraceptive users (OC) (N=12).

Furthermore, some additional inclusion criteria were used to differentiate between the two groups. The inclusion criteria for the job seeker sample were: (i) being unemployed; (ii) actively seeking a job for at least two weeks prior to the selection process for the study; (iii) having been in previous contact with a public employment office with the purpose of finding work; (iv) having been in contact with a private office (temporary employment office, specialized contracting company, etc.) with the purpose of finding job; (v) having sent a job application directly to employers; (vi) having inquired via personal contacts, unions, etc.; (vii) having posted an ad or answered ads; (viii) having studied employment offers; (ix) having participated in a test, competition, or interview in the context of a hiring process; and (x) being able to work [Economically Active Population Survey (EAPS)]. Of the job seekers, 71% stated that they had been actively looking for a job for ten months to more than one year prior to the study.

Likewise, the inclusion criteria for the non-job-seekers were that, at least two weeks before the study, they could NOT: (i) be employed or (ii) actively look for a job. The criteria to identify whether they were non-job seekers were the same as for the job seekers, but they did not need to be actively looking for a job [Economically Active Population Survey (EAPS)]. 80 volunteers were excluded from participation for not meeting the general or job-related inclusion criteria. Subjects who met the criteria were asked to attend sessions that took place in a laboratory at the Faculty of Psychology.

Job seekers were recruited at the University campus office of the Occupational and Labour Agency (SERVEF) and at the Labour Insertion Observatory of the University (OPAL). Non-job seekers were recruited through an oral presentation in different classes at the University. Potential participants were first screened by a trained psychologist through a telephone interview and selected according to the general inclusion criteria. Then, the participants were considered job seekers or non-job seekers based on the specific criteria for each group.

Before the individual session, participants were asked to maintain their general habits, sleeping as much as usual, refraining from heavy physical activity the day before the session, and not consuming alcohol since the night before the session. Additionally, they were instructed to drink just water, and not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session. Upon arrival at the laboratory, all the participants received verbal and written information about the study and signed an informed consent form. All the participants were volunteers, and at the end of the experimental session they received feedback from an expert interviewer about how to improve their individual performance on a job interview. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the Ethics Research Committee of the University.

Procedure

The study involved an experimental session that lasted approximately 90 min and took place between 16.00 and 19.00h. The experimental sessions

were composed of different phases (see Figure 1). Upon arrival at the laboratory, the experimenter checked whether they had followed the instructions given previously. Moreover, at the end of the session, the weight and height of the participants were measured.

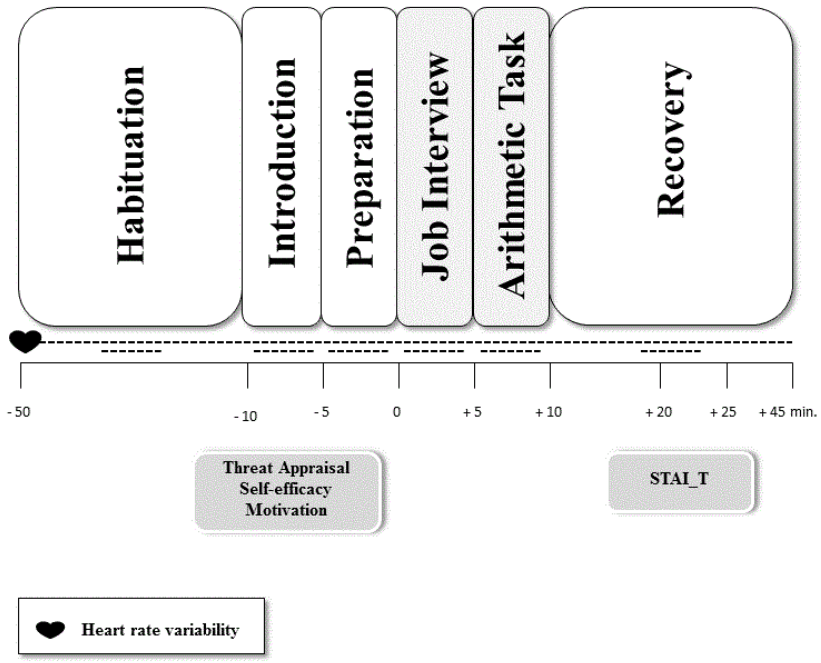

Figure 1. Timeline of the TSST. Dotted line depicts the time of HR and rMSSD collection. STAI_T = Trait Anxiety Inventory

To induce stress, we employed a modified version of the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993), which consists of 5 minutes of free speech (job interview) followed by a 5-minute arithmetic task, performed in front of an expert interviewer (different from the experimenter). The modification of the original TSST protocol consisted of having only one evaluator. In order to create a more real job interview situation, participants were told that it was carried out by a personnel

selection expert. In the job interview (5 min.), the participant's main goal was to convince the interviewer that he/she was the best candidate for his/her "dream job", describing his/her personal skills to perform the job. The participants remained standing at a distance of 1.5 meters from the interviewer. In addition, a video camera, a microphone, and a monitor were placed where subjects could see their performance clearly. Both the speech and arithmetic tasks were filmed. In all cases, the experimenter and the interviewer were women. At the end of the session, the expert had to communicate his/her evaluation and make some recommendations about interview competencies. .

3.2. Measures

Heart Rate and Heart Rate Variability

Data for Heart Rate (HR) and Heart Rate variability (HRV) were continuously recorded during the entire session using a Polar©RS800cx watch (Polar CIC, USA), which consists of a chest belt for detection and transmission of heartbeats and a Polar watch for collection and storage of the data. The transmitter is located on the chest belt, which is placed on the solar plexus and transmits HR and HRV information to the receiver (Polar watch). The data collected by the Polar watch were downloaded and stored in the Polar ProTrainer5TM program in the computer and analyzed using HRV software Kubios Analysis (Biomedical Signal Analysis Group, University of Kuopio, Finland). The following parameters from time domain analysis were quantified: the average HR in beats per minute (bpm) as an indicator of sympathetic activation, selecting 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. A low value indicates high stress. Following the recommendations of the Task Force (1996), we analyzed HR and rMSSD in periods of five minutes. Whereas the job interview and arithmetic tasks lasted five minutes each, the habituation, preparation, and recovery phases lasted longer than five minutes, and we chose the central five minutes of each phase.

Psychological questionnaires

Cognitive threat appraisal

Cognitive threat appraisal was evaluated with the Primary Appraisal Secondary Appraisal (PASA) scale (Gaab et al., 2005). This scale was constructed to assess threat appraisal while performing the TSST, based on transactional stress theory (Lazarus & Folkman, 1984). We employed the situation-specific subscale assessing 'Threat' (e.g., I do not feel threatened by the situation), which contains four items rated on a 6-point Likert scale ranging from "strongly disagree" to "strongly agree" (e.g., I do not feel threatened by the situation). Cronbach's alpha in our sample for the 'Threat' subscale was 0.76. It was administered just at the end of the introduction phase.

Anxiety Trait

Anxiety Trait was evaluated by the Spanish version (Seisdedos, 1988) of the Trait Anxiety Inventory (STAI-T, Spielberger, Gorsuch, & Lushene,

1970). It consists of 20 items (e.g. ‘I feel at ease’, ‘I feel upset’) with a 4-point scale ranging from 0 (not at all) to 3 (extremely). Cronbach’s alpha was 0.82.

Self-efficacy and motivation

Self-efficacy was evaluated by the following two items, both on a scale from 1 (none) to 100 (very much): (i) How capable are you of successfully perform this task? (ii) How much confidence do you have that you will successfully perform this task? These items are based on Bandura’s (1997) recommendations. The scores on both items were averaged and had a Cronbach's alpha of 0.86. The average of these items made it possible to obtain a situational self-efficacy score (Costa et al., 2016; van der Meij et al., 2010).

Motivation

Motivation was evaluated by asking (again on a scale from 1 to 100): How important is it for you to successfully perform this task?

3.3. Statistical Analysis

Data were tested for normal distribution and homogeneity of variance using Kolmogorov–Smirnov and Levene’s tests before the statistical procedures were applied. These analyses revealed significant deviations in the HR and rMSSD values; therefore, they were square-root-transformed. One-way ANOVAs were used to investigate group demographic differences between job seekers and non-job seekers. Moreover, a t-test was used to assess Group differences in threat appraisal.

In order to evaluate whether the application of the TSST had an effect on all the physiological variables measured (HR, rMSSD), repeated-measures ANCOVAs were performed, with Time (habituation, preparation, job interview, arithmetic task, and recovery phases for HR and rMSSD) as a within-subject factor and Group (unemployed job seekers vs. unemployed non job seekers) as a between-subject factor. The habituation phase was used as covariate in order to avoid the effect of the baseline on the results. In a first moment, we used age as a covariate variable due to significant differences between groups. Nevertheless, this variable did not have a significant effect on HR or rMSSD, and, therefore, it was excluded from the final analysis. We calculated the areas under the total curve with respect to the ground (AUCg), using the trapezoid formula specified in Pruessner, Kirschbaum, Meinlschmidt, and Hellhammer (2003) for HR and rMSSD, in order to use them as dependent variables. To control the effect of the influence of the menstrual cycle or oral contraceptive intake on Cognitive threat appraisal, AUCgHR and AUCgrMSSD, Pearson's Correlation analysis was performed. None of the correlations were statistically significant (all p's>.09). Thus, all analyses were performed on the basis of the interest groups (unemployed job seekers vs. non-job seekers).

Five participants (four job seekers and one non-job seeker) were removed from the statistical analyses of HR and rMSSD due to problems in data recording. In addition, two outliers were removed from the statistical analyses of HR and rMSSD (one job seeker and one non-job seeker). Outliers were evaluated on the basis of Mahalanobis distance D^2.

In addition, in order to assess whether cognitive threat appraisal mediates the relationship between being an unemployed job seeker and the overall physiological response, we employed AUCg for HR and rMSSD as dependent variables. Then the unemployed job seekers variable was created by computing a dummy variable (unemployed job seekers: 1 and unemployed non-job seekers: 0), in order to use it as an independent variable. Furthermore, a mediation procedure based on nonparametric resampling, known as bias-corrected bootstrapping, was conducted to assess the mediating role of Cognitive threat appraisal in the relationship between being an unemployed job seeker and AUCg HR and rMSSD (PROCESS model number 4) (Preacher and Hayes, 2004). Bootstrapping makes it possible to gather many alternative versions of a single statistic that is ordinarily only calculated from one sample. Bootstrap data resampling procedures establish confidence intervals (CIs) for testing the statistical significance of an indirect effect (Shrout & Bolger, 2002). The analysis was based on 10,000 bootstrap iterations, and the CI was set at 95%, as recommended by Mallinckrodt et al. (2006).

We used the Greenhouse–Geisser procedure when appropiate. 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). For ease of interpretation of the figures, the values are represented in raw values, and not in square-root-transformed values. SPSS 20.0 was used to perform the statistical analyses.

3.4. Results

Characteristics of groups

There were differences between groups in age (job seekers: *M* = 26.83, *SEM*± 0.6; non-job seekers: M = 24.70, SEM ± 0.6; $p<0.001$), but not in body mass index (BMI) (job seekers: *M* = 24.18, *SEM*± 0.5; non-job seekers: M = 22.91, SEM ± 0.5; $p=0.119$), subjective socio-economic status (SES scale: Adler et al., 2000) (job seekers: *M*= 6.07, *SEM*± 0.1; non-job seekers: *M* = 6.15, *SEM*± 0.1; $p=0.756$), marital status (86.6% were single; $p=0.815$), or educational level (98.8% of participants had a university education or were in the last year of college in a wide range of studies; $p=0.978$)[1].

Moreover, there were no significant differences in Anxiety trait (job seekers: *M* = 17.67, *SEM*± 1.2; non-job seekers: *M* = 18.83; *SEM*± 1.2), Self-efficacy (job seekers: *M* = 70.23, *SEM*± 2.2; non-job seekers: *M* = 66.87, *SEM*± 2.2), or Motivation (job seekers: *M* = 78.33, *SEM*± 2.8; non job seekers: *M* = 74.50; *SEM*± 2.9) (all $p>0.290$).

Heart rate (HR)

Repeated-measures ANOVA on HR showed main effects of Group ($F(1,74)=4.640$, $p=0.034$), with higher values in the non-job seekers, and Time ($F(2.982,220.982)=1287.352$, $p<0.001$). HR increased immediately after the preparation phase (habituation vs. preparation phase, $p<0.001$) and continued to increase until reaching its peak rate in the job interview phase

(habituation vs. job interview, $p<0.001$); afterwards, HR decreased, without reaching baseline levels in the recovery phase (habituation vs. recovery phase, $p=0.004$). Moreover, a significant effect of the Time x Group interaction was found ($F(2.982,220.982)=2.844$, $p=0.039$); post hoc analyses indicated that HR was significantly lower in job seekers than in non-job seekers during the job interview phase ($p=0.021$) (see Figure 2).

Heart rate variability (rMSSD)

Repeated-measures ANOVA showed a main effect of Time on rMSSD ($F(3.301,244.306)=21.359$, $p<0.001$); it decreased immediately after the preparation phase (habituation vs. preparation phase, $p<0.001$) and continued to decrease until reaching its lowest rate in the arithmetic task phase (habituation vs. arithmetic phase, $p<0.001$). Afterwards, rMSSD increased, but without reaching baseline levels, in the recovery phase (habituation vs. recovery phase, $p<0.001$). Furthermore, a significant effect of the Time x Group interaction was found ($F(3.301,244.306)=2.818$, $p=0.035$). Post hoc analyses indicated that rMSSD was higher in job seekers than in non-job seekers during the job interview phase ($p=0.040$) (see Figure 2). Nevertheless, no effect of Group was observed ($p>0.553$).

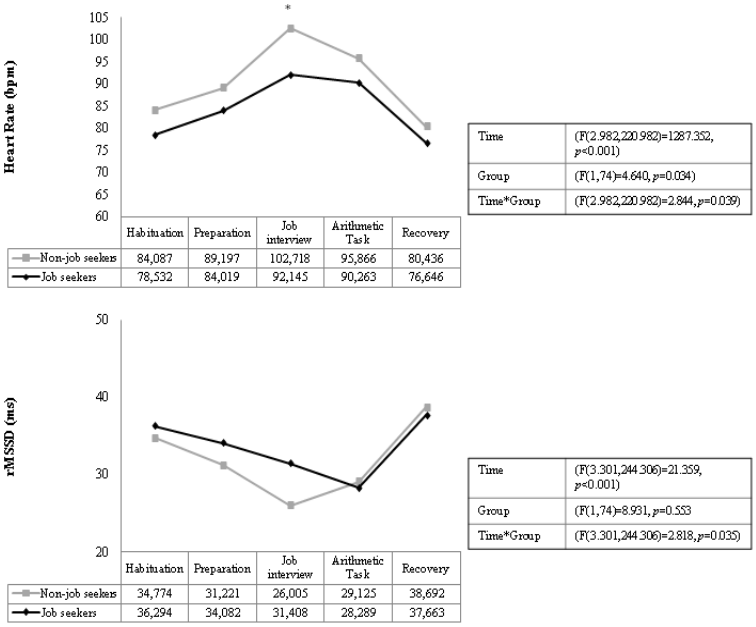

Figure 2. Mean (±SEM) for HR (bpm) and rMSSD (ms) during a modified version of the TSST for unemployed job seekers (42) and non-job seekers (40). Anovas for repeated measures have shown a statistically significant Time and Group interaction both for HR (p=0.039) and rMSSD (p=0.035). Post hoc analysis indicated that HR was lower in job seekers than in non-job seekers during the job interview phase (p=0.021) and rMSSD was higher in job seekers than in non-job seekers during the job interview phase (p=0.040).

Threat appraisal

T-tests showed a main effect of Group on cognitive threat appraisal (t(79)=2.419, p=0.018), as unemployed job-seekers perceived the job interview as less threatening (mean = 2.76, ±SEM = 0.7) than non-job seekers (mean = 3.19, ±SEM = 0.8).

Mediation models

The direct effect of being a job seeker on AUCgHR was not statistically significant (β = -15.36, SE =.78, t = -1.74, p = 0.08). However, the results showed that Cognitive threat appraisal had a positive relationship

with the AUCgHR (β = 11.13, SE = 5.54, t = 2.00, *p* = 0.04), showing that a higher Cognitive threat appraisal produces higher sympathetic (HR) responses to SET situation. Moreover,, a negative relationship was found between being an unemployed job seeker and Cognitive threat appraisal (β = -0.36, SE = 0.17, t = -2,052, *p* = 0.04).

A Bootstrapping procedure (Shrout & Bolger, 2002) was performed to verify the significance of the indirect effect of Cognitive threat appraisal on the relationship between being a job seeker and AUCgHR. The conditional indirect effect of being a young unemployed job seeker and AUCgHR through Cognitive threat appraisal is the product of β = -15.36, SE = 8.78, 95% CI = 0.41 to 11.50. Thus, Cognitive threat appraisal fully mediated the relationship between being an unemployed job seeker and AUCgHR (see Figure 3).

The direct effect of being a job seeker on AUCgrMSSD was not statistically significant (β = 3.24, SE =3.14, t = 1.03, *p* = 0.30). However, the results showed that Cognitive threat appraisal had a negative effect on AUCgrMSSD (β = -4.11, SE = 1.97, t = -2.08, *p* = 0.04), showing that the higher the Cognitive threat appraisal, the lower the parasympathetic (rMSSD) reactivity to a SET situation. Furthermore, a negative relationship was found between being an unemployed job seeker and Cognitive threat appraisal (β = -0.37, SE = 0.17, t = -2.08, *p* = 0.04).

A bootstrapping procedure (Shrout & Bolger, 2002) was performed to verify the significance of indirect effect of Cognitive threat appraisal on the relationship between being an unemployed job seeker and AUCgrMSSD. The

conditional indirect effect of being an unemployed job seeker and AUCgrMSSD through Cognitive threat appraisal is the product of β = 3.24, SE = 3.14, 95% CI = 0.59 to 4.63. Thus, Cognitive threat appraisal fully mediated the relationship between being a job seeker and AUCgrMSSD (see Figure 3).

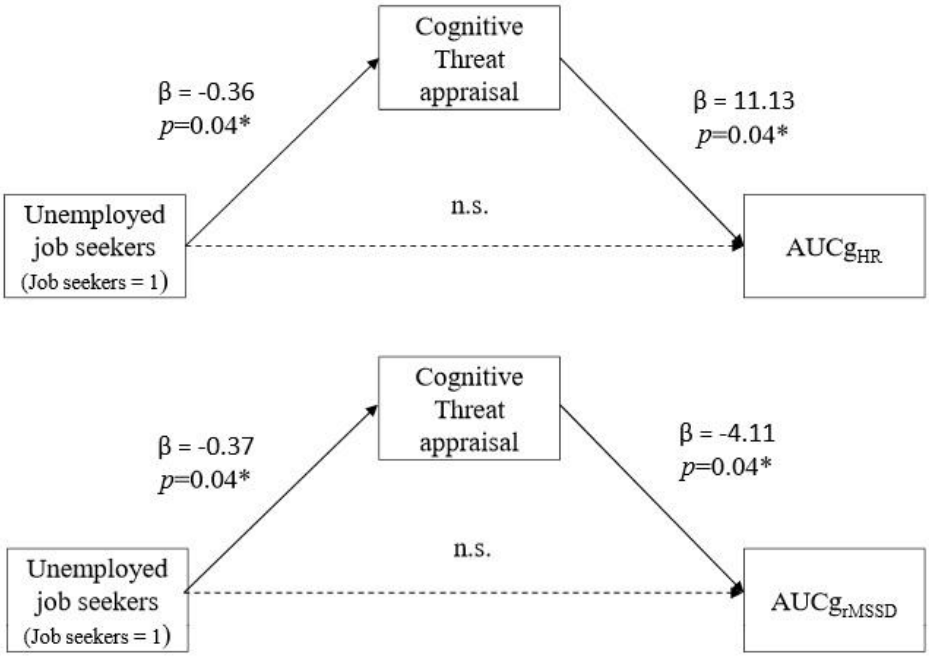

Figure 3. Mediation analysis of the association between Unemployed job seekers, Cognitive Threat appraisal and (i) AUCgHR (ii) AUCg$_{rMSSD}$. The conditional indirect effect of being young unemployed job seekers and AUCgHR through Cognitive Threat Appraisal is the product of β = -15.36, SE = 8.78, 95% CI = 0.41 to 11.50. The conditional indirect effect of being young unemployed job seekers and AUCgHR through Cognitive Threat Appraisal is the product of β = 3.24, SE = 3.14, 95% CI = 0.59 to 4.63.

3.5. Discussion

The aim of this study was to investigate the impact of being unemployed and looking for a job on the cardiac stress response to an acute stressor (a simulated job interview), and on the cognitive threat appraisal. In addition, we aimed to verify the mediating role of cognitive threat appraisal in the relationship between being an unemployed job seeker and the cardiac

response to a SET situation. In order to do so, a group of young-adult unemployed job seekers and non-job seekers were subjected to a modified version of the TSST**.** This modified version of the TSST focused on performing an evaluated speaking task emulating a job interview, where the participants expected an expert's evaluation. Therefore, in this "unreal" situation, where they have to demonstrate effective skills during a "job interview", they might feel a higher emotional impact because this type of task could determine their professional successful in the future. Our results show that unemployed job seekers manifest lower cardiac responses and lower cognitive threat appraisal than non-job seekers when faced with this psychosocial stressor. Moreover, we found that being unemployed and looking for a job is not directly related to cardiac responses. In fact, cognitive threat appraisal fully mediates the relationship between being an unemployed job seeker and cardiac responses.

Previous investigations have shown that repeated stressful situations might lead to a reduction in the cognitive threat appraisal (Quigley et al., 2002; Tomaka et al., 1993) and, consequently, to maladaptive physiological functioning over time (Thayer et al., 2012). Specifically, studies have found that a decrease in cognitive threat appraisal might lead to a reduction in HR responses to acute stress (Cacioppo et al., 1990; Kelsey et al., 1999; 2000; 2004; Olff, Langeland, & Gersons, 2005). Along these lines, as mentioned above, when a subject appraises a situation or task as threatening, the hypoactivity of some brain areas creates a lack of inhibition of the sympathoexcitatory circuits (Thayer et al., 2012), ultimately producing reduced physiological responses. Being unemployed and looking for a job is

a very stressful situation for many young people (Thern et al., 2017), and the fear caused by conditions of uncertainty after leaving the university might be a source of stress in itself (Knabe & Rätzel, 2011). Therefore, the stress experienced by unemployed young people looking for a job could affect the way they experience the job interview and, in turn, the stress response systems.

Another possible explanation for our results is that unemployed job seekers might have acquired more experiences in SET situations, with our results showing a process of habituation. These results might also suggest that they have learned how to self-regulate their emotions during these tasks; consequently, they evaluate the job interview as less threatening than non-job seekers. In this habituation process, people tend to reappraise repeated stressful stimuli using different coping strategies, thus perceiving the stimuli as less threatening (Mantler et al., 2005). These results agree with Lazarus' stress theory (1984), which explains that habituation might reduce cognitive threat appraisal, and as a result, this reappraisal would lead to a decrease in sympathetic activity (Cacioppo, et al., 1990; Kelsey et al., 1999; 2000; 2004). In this direction, Schommer et al. (2003) found that after repeated psychosocial exposure (TSST), people increased their perception of controllability of the situation and decreased their subjective perception of novelty and predictability, as well as their physiological response (HR). Thus, diminished cardiac responses in unemployed job seekers might be the result of a lower cognitive threat appraisal due to a habituation process produced by repeated exposure to the same stimuli (job interviews).

Our results might also be due to third variables not taken into consideration in this cross-sectional study (i.e. regional unemployment rate or motivation in the job search behavior). According to the Expectancy-value theory (EVT) (Feather, 1990), the individual's level of motivation to strive for a certain goal is the product of the expectations of attaining the desired goal and the incentive value or valence of that particular goal (as in finding a job). For example, an unemployed person could have a strong expectation that he or she would perform well on a job interview, thereby meeting the main requirement for successful performance (i.e. addressing the questions asked in the interview). Thus, unemployed people expect that succeeding at the interview would yield positive consequences, such as being hired for the job. However, this is not easy in some countries where the rate of unemployment is high and the likelihood that a person looking for a job will become employed appears to be very low. Thus, being unemployed during a period of high unemployment might buffer the negative consequences of unemployment because failures can be attributed to external causes (McKee-Ryan, Song, Wanberg, & Kinicki, 2005). In addition, negative feedback from the environment might negatively influence job search behavior and motivation (Latack, Kinicki, & Prussia, 1995). In our study, the lower cognitive threat appraisal by the unemployed job seekers might imply a loss of motivation and interest in the interview and in learning how to perform better on job interviews (at the end of the experimental session, an expert in human resources offered them various tips on how to improve their individual performance on the job interview). Therefore, a loss of motivation or interest in job search activities might lead to lower cognitive threat

appraisal of SET situations and, thus, decreased cardiac responses. However, we did not find differences in motivation between the two groups of subjects, and further studies are needed to investigate this possible explanation.

Overall, our study provides some new insights into the effect of being a young unemployed job seeker on psychophysiological responses to SET situations. Some practical implications can also be identified. Our study provides evidence for the need to support unemployed people who are looking for a job during this difficult period of time. On the one hand, it might be useful to monitor the stress experienced by unemployed job seekers and its effect on cognitive threat appraisal and cardiac responses. This might be one of the reasons for the high incidence of physiological and psychological diseases in this population (Ali et al., 2016; Brown et al., 2003; Koziel et al., 2010; Gallo et al., 2004; Siegrist & Klein, 1990; Sullivan & von Wachter, 2009; Roelfs et al., 2011; Urbanos-Garrido & Lopez-Valcarcel, 2014; Wanberg, 2012). There is a need to prevent health consequences in unemployed people and monitor specific health risks that arise due to this condition, through psychological support and periodic health check-ups. On the other hand, it would be useful to provide job search interventions that include teaching job search skills, improving self presentation, boosting self-efficacy, encouraging proactivity, promoting goal setting, and enlisting social support. These skills would help to increase the motivation and job search self-efficacy of unemployed job seekers, aspects closely related to an adaptive stress response.

The limitations of this study reveal possible future lines of research. Although our sample was composed of unemployed young adults who had

mostly been looking for a job for ten months to one year (71%), we were not able to detect physiological effects reflecting chronic stress. Furthermore, due to this quasi-experimental design and the characteristics of the sample, which is relatively small and composed of young people, these results cannot be considered conclusive or extrapolated to other age ranges that are more at risk (over 50 years old). However, despite these limitations, we are able to show some relevant psychophysiological differences between groups, highlighting the relevance of appraisal in the physiological response to this important kind of social stress. Therefore, future longitudinal and cross-cultural investigations would be useful to determine the psychophysiological impact of being unemployed and looking for a job over time and in different regions.

In conclusion, our study shows that healthy university-educated young people who are relatively recently unemployed and looking for jobs respond to and appraise stress produced by a job interview differently from their non job-seeking counterparts. This result was found in an environment that gives them negative feedback due to the high regional unemployment rate. These physiological and psychological differences produced by being unemployed and looking for a job highlight the importance of supporting and monitoring the health and motivation of this vulnerable group of young individuals. Further studies are needed with people in other high risk situations, such as long-term unemployed people and those with fewer resources, and with a broader age range, in order to develop prevention programs.

CHAPTER IV

STUDY 2

Assessing the antecedents and consequences of threat appraisal of an acute psychosocial stressor: the role of optimism, displacement behavior, and physiological responses

The main results of this study are under review in *Stress* Journal:

Zandara, M., Villada, C., Hidalgo, V., Salvador, A. Assessing the antecedents and consequences of threat appraisal of an acute psychosocial stressor: the role of optimism, displacement behavior, and physiological responses.

4. Introduction

The feeling of stress is increasing in young adults who are constantly subjected to social evaluative situations (i.e. being exposed to several job interviews/exams/performance evaluations) due to increased competitiveness in the current job market. Generally, individuals feel stress when they perceive that external situations exceed their ability to cope. Anything that might be a challenge or a threat to well-being is a stressor, although some stressors are motivating and good for people lives. In fact, the short-term stress response enables the organism to mobilize energy to fight or flee from a threat, enhancing its chances of survival (Folkman, 2013). However, some stressors are maladaptive, especially if they are prolonged over time, producing negative health consequences (McEwen & Gianaros, 2011). Traditionally, human studies have focused on the assessment of physiological responses to the threat appraisal of stress. On the one hand, due to their relevance in explaining individual differences in the stress response, personality traits emerge as one of the most important antecedents of threat appraisal. On the other hand, in understanding the stress response, behavior as a consequence of stress and the influence of the physiological stress response in the relationship between the appraisal and behavior seem to be essential.

Threat appraisal is a stress-related mental state where a person anticipates personal harm due to challenges or novel situations or expects that loss is imminent (Feldman, Cohen, Hamrick, & Lepore, 2004). As Lazarus

and Folkman's (1984) Transactional model of stress and coping proposes, people perceive the situation as threatening when they feel that events become too much to handle. They are overloaded and wonder whether they can cope with the pressures placed upon them. The more they appraise the situation as threatening and feel they do not have sufficient resources to cope, the greater the threat appraisal will be (Folkman, Lazarus, Dunkel-Schetter, DeLongis, & Gruen, 1986).

Nevertheless, not all individuals perceive challenging situations in the same way. In fact, some studies have shown that personality might influence the threat appraisal of stress (Penley, & Tomaka, 2002). Specifically, dispositional optimism has been found to positively influence the way people assess stressful events (Endrighi, Hamer, & Steptoe, 2011; Penley & Tomaka, 2002). Scheier and Carver (1985) considered dispositional optimism to be a trait of a balanced personality, across time and in various situations, that influences the way people come to terms with present, past, and future life events. Optimistic individuals are positive about events in daily life, frequently have more protective attitudes, are more resilient to stress, and believe they have the coping strategies to face the challenges of everyday situations (Carver, Scheier, & Segerstrom, 2010). Optimism can even have a protective role in modulating stress-related autonomic and neuroendocrine responses (Puig-Pérez et al., 2017). Dispositional optimism was found to be positively correlated with coping strategies related to the elimination, reduction, or control of stressors, and negatively correlated with strategies employed to ignore, avoid, or distance oneself from stressors and emotions (Nes & Segerstrom, 2006). This distinction is particularly interesting

considering that threat appraisal is influenced by the individual's assessment of the relationship between the situational demands (i.e. amount of effort required, danger, and uncertainty involved in the particular performance situation) and his/her own coping resources. The less the individual believes s/he has the coping strategies to overcome a stressful situation, the higher the threat appraisal experienced (Blascovich & Tomaka, 1996; Tomaka, Blascovich, Kelsey, & Leitten, 1993). Considering all these points, dispositional optimism might have an important role in influencing the threat appraisal of stress.

In addition, the Biopsychosocial (BPS) model of arousal regulation (Blascovich & Tomaka, 1996) takes threat appraisal into consideration as a fundamental part of the stress response. According to this perspective, threat appraisal first influences physiological responses to stress, which in turn moderate individuals' behavioral responses to stress. Specifically, physiological responses differ depending on whether or not the situation is perceived as threatening (Blascovich, Mendes, Tomaka, Salomon, & Seery, 2003; Gaab, Rohleder, Nater, & Ehlert, 2005; Seery, 2011; Tomaka, Blascovich, Kelsey, & Leitten, 1993; Tomaka, Blascovich, Kibler, & Ernst, 1997; Wirtz, von Känel, Emini, Suter, Fontana, & Ehlert, 2007). In fact, using heart rate (HR) and cortisol release as indexes of physiological responses to stress, several studies have observed that these parameters increase during stressful tasks such as public speaking (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004a-b). Specifically, their increase begins when threat is appraised or, in other words, before the stressful task begins (during the anticipation phase) (Feldman et al., 2004;

Gaab et al., 2005; Schlotz, Hammerfald, Ehlert, & Gaab, 2011). Thus, threat appraisal has been found to positively influence HR and cortisol release during acute stress (Feldman et al., 2004; Gaab et al, 2005; Schlotz et al., 2011; Tomaka et al, 1993; Tomaka et al., 1997; Wirtz et al., 2007). As mentioned above, according to the BPS model, threat appraisal not only directly affects the physiological response to stress, but the interaction between threat appraisal and the physiological response to stress might also play an important role in the resulting human behavior. Displacement activities are important stress-related behaviors. Displacement behaviors have been defined as stress-related actions or movements focused on one's body, such as grooming behavior (i.e. head scratching, licking and biting lips, etc.), iterative movements, and manipulation of objects (repeated body movement forward and backward, twisting and fiddling with fingers, touching and twisting rings, etc). Several studies have shown a high correlation between psychophysiological stress responses and displacement behaviors (Mohiyeddini, Bauer, & Sample, 2013a-b, Mohiyeddini & Sample, 2013; Pico-Alfonso et al., 2007; Troisi, 2002; Kortmulder, 1998). Other studies have found a link between psychological responses to stress and displacement activities. Indeed, people with higher scores on negative mood and/or anxiety prior to or during a public speaking task have shown higher percentages of displacement behaviors (Shreve, Harrigan, Kues, Kagas, 1988; Troisi, Delle Chiaie, Russo, Mosco, & Pasini, 1996; Villada, Hidalgo, Almela, Mastorci, Sfoifo & Salvador, 2014). Analyzing 25 patient-doctor interactions, Shreve and colleagues (1988) showed that hand to body self-touching was significantly higher in patients during doctors' presentations of anxiety-

producing topics. Several studies carried out by Troisi and colleagues found that anxiety enhances displacement behavior in patients with alexithymia (Troisi et al., 1996), schizophrenia (Troisi, Spaleta, & Pasini, 1998), and pathological anxiety and depression disorders (Troisi, Belsanti, Bucci, Mosco, Santi, & Verducci, 2000). Recently, Villada and colleagues (2017) showed that the anxiety experienced prior to a public speech led to an increase in displacement behaviors in women, mostly during the early follicular phase. On the other hand, a relationship has been observed between some parameters of the physiological responses to stress and displacement activities. In fact, Mohiyeddini and colleagues (2013a) found that displacement behaviors were associated with lower cardiac responses in men, but not in women. The relationship between behavior and the physiological stress response in women seems to be modulated mostly by the subjective perception of stress (Mohiyeddini et al., 2013b). Villada and colleagues (2014) reported a negative relationship between displacement behaviors and cardiac responses in a sample consisting only of women. Moreover, Pico-Alfonso and colleagues (2007) observed that cardiac (RR and rMSSD) responses to stress correlated positively with displacement activities. In other words, individuals exhibiting more displacement during the interview were also characterized by a lower heart rate during post-stress recovery. Some studies have also assessed the relationship between cortisol and displacement behaviors, although no significant relationships were found (Pico-Alfonso, 2007; Villada et al., 2014). Hence, these findings reveal that displacement behaviors may be acting as indicators of negative mood states and cardiac

activation, as well as confirming their de-arousing properties during social stress situations.

Based on the aforementioned arguments, the main aim of this study was to investigate the antecedent (dispositional optimism) and consequences (displacement behavior) of threat appraisal of an acute psychosocial stressor. Moreover, we aimed to verify the moderating role of physiological responses to stress (HR and cortisol) in the relationship between threat appraisal and displacement behavior. Thus, we expect that: (H1) Optimism will negatively predict threat appraisal; (H2) Threat appraisal will positively predict displacement behavior; (H3) Threat appraisal will fully mediate the relationship between Optimism and Displacement behavior; and finally, given the importance of the interaction between the psychological and physiological responses to stress in behavior, we want to explore whether (H4a) HR and (H4b) Cortisol moderate the relationship between threat appraisal and displacement behavior.

4.1. Methods

Participants

The final sample was composed of 82 healthy young adults (37 men and 45 women) from 20 to 39 years old (mean ± SEM: Age = 24.98 ± 0.55). They were graduate and postgraduate students in a wide range of majors at the University of Valencia. All participants were volunteers, and at the end of the experimental session they received feedback from an expert interviewer about how to improve their individual performance on a job interview.

The following inclusion criteria were examined through self-reports: (i) Spanish nationality; (ii) age between 20 and 40 years; (iii) educational level between Secondary school and postgraduate studies; (iv) not smoking more than five cigarettes per day; (v) no alcohol or any other drugs; (vi) no visual or hearing problems; (vii) no cardiovascular, endocrine, neurological, or psychiatric diseases; (viii) not having been under general anesthesia once or more than once in the past year; (ix) not having experienced a major stressful life event during the past year; (x) not using any medication directly related to cardiac, emotional, or cognitive function, one that was able to influence hormonal levels, such as glucocorticoids or β-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, or psychotropic substances. Volunteers were assessed to verify that they met the experiment's inclusion criteria. Subjects who fulfilled the criteria were asked to attend sessions that took place in a laboratory at the Faculty of Psychology.

Before each individual session, participants were asked to maintain their general habits, sleep as much as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to drink only water, and not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the Ethics Research Committee of the University of Valencia. Upon arrival at the laboratory, all the participants received verbal and written information about the study and signed an informed consent form.

Procedure

The study involved an individual session that lasted approximately 90 min and took place between about 16.00 and 19.00h. The experimental sessions were composed of different phases (see Figure 1). Upon arrival at the laboratory, the experimenter verified that participants had followed the instructions given previously.

To produce stress, we subjected the participants to a modified version of the Trier Social Stress Test (TSST, Kirschbaum et al., 1993). This test includes a highly competitive component where the subject has to persuade the committee that s/he is ‘‘the best applicant’’ for the position (Salvador & Costa, 2009). The modifications were: (i) all the phases of the TSST took place in the same room, and (ii) the committee was composed of only one person who had been introduced as an expert in human resources. The session started with a 40-minute habituation phase. During that time, participants had to fill out a general questionnaire related to demographics and anthropometric data and the Life Orientation Test Revised (LOT-R, Scheier et al., 1994); for the last 10 minutes, they were left alone to rest mentally and physically. Next, during the introduction phase, participants were told about the job interview task. Immediately after that, they had to fill out the Primary Appraisal Secondary Appraisal scale (PASA) (Gaab et al., 2005). Before the beginning of the TSST, participants had 5 minutes to prepare their presentations. During this preparation phase, individuals had to write down their main ideas about what to say during the job interview. However, they could not use these notes during the speech task. The TSST protocol consisted of 5 minutes of free speech (job interview), followed by a

5- minute arithmetic task. In the job interview, the participant's main goal was to convince the interviewer that s/he was the best candidate for his/her "dream job". The participants stood at distance of 1.5 meters from the evaluator. In addition, a video camera, a microphone, and a monitor where subjects could see their performance were clearly visible. Both the speech and arithmetic tasks were videotaped, and subsequently the displacement behavior was assessed.

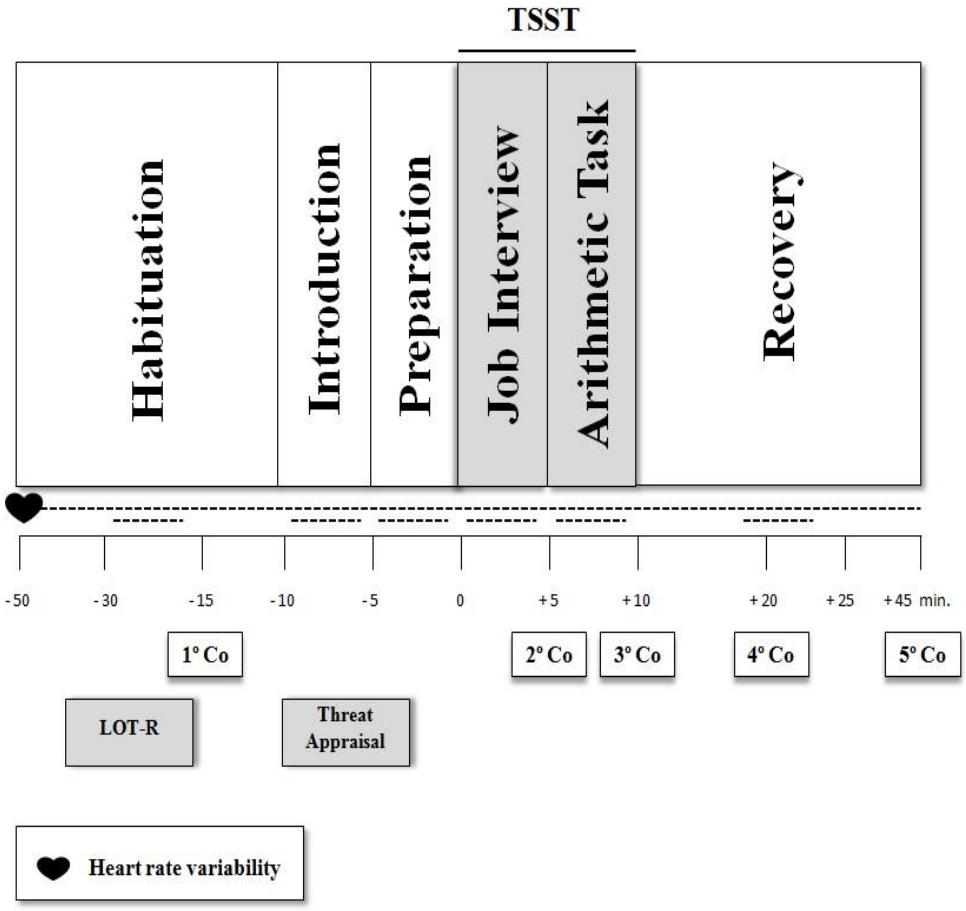

Figure 1. Timeline of the TSST. Dotted lines depict the time of HR collection. Salivary cortisol samples = 1°Co, 2°Co, 3°Co, 4°Co. LOT-R, Life Orientation Test Revised.

4.2. Measures

Demographic and anthropometric measures

We collected demographic and anthropometric data such as age, height, weight, and subjective socioeconomic status (SES) (Adler, Epel, Castellazzo, & Ickovics, 2000).

Displacement behavior

Three observers (two men and one woman) were trained to interpret and reliably rate the participants' behavior from the video recordings on the basis of a modified version of the Ethological Coding System for Interviews (ECSI) (Troisi, 2002). We analyzed two main behavioral categories: 1. Hand movement displacement behaviors (head scratching, twisting and fiddling with fingers, touching and twisting rings, constant arrangement of clothing, covering their mouth with their hands and touching their lips, clicking their fingers); 2. Body movement displacement behaviors (repeated rocking forward and backward, licking and biting lips, repeated arm movements. Each displacement behavior was assessed as present = 1 or not present = 0, with a frequency of 20". Each video lasted 5 minutes (job interview). The total score indicates the frequency of displacement behaviors in each category. The observers rated the full set of videos on two separate occasions, with the second rating taking place three weeks after the first rating. To assess the repeatability of the ratings from a given observer, we first calculated an intra-observer correlation coefficient (ICC) for each item, based on the two separate ratings nested within each subject. Then, we averaged the observers' first and second ratings for each item, and we used these average

scores to assess the degree of inter-observer ICC among the three observers. The repeatability of the ratings of intra and inter-observers' ICC ranged from 0.78 to 0.95 (0.84±0.15) for the three observers.

Life Orientation Test Revised (LOT-R; Scheier et al., 1994)

LOT-R is composed of 10 items answered on a 5-point Likert scale ranging from 0 (strongly disagree) to 4 (strongly agree). Three items measure optimism (e.g. "In uncertain times, I usually expect the best"), and three items measure pessimism (e.g. "If something can go wrong for me, it will"); the remaining items are distractors. Pessimism items were reversed to obtain a one-dimensional measure of dispositional optimism. We employed the Spanish version (Otero, Luengo, Romero, Gómez, & Castro, 1998), which has shown adequate reliability ($\alpha = .78$) (Ferrando, Chico, & Tous, 2002).

Threat appraisal

Threat appraisal was evaluated using a situation-specific subscale of the PASA (Gaab et al., 2005). This subscale was employed to assess threat appraisal processes before performing the TSST, based on transactional stress theory (Lazarus & Folkman, 1984). The subscale has four items, rated on a 6-point Likert scale ranging from "strongly disagree" to "strongly agree" (e.g., I do not feel threatened by the situation). The scale was translated into Spanish and back-translated. In our sample, Cronbach's alpha for the scale was 0.76. The PASA scale was administered at the end of the introductory phase of the TSST.

Heart Rate

HR data were continuously recorded during the entire session using a Polar©RS800cx watch (Polar CIC, USA), which consists of a chest belt for detection and transmission of heartbeats and a Polar watch for data collection and storage. The transmitter is located on the chest belt, which is placed on the solar plexus and transmits HR information to the receiver (Polar watch). The data collected by the Polar watch were downloaded, stored in the Polar ProTrainer5TM program in the computer, and analyzed using HRV Kubios Analysis software (Biomedical Signal Analysis Group, University of Kuopio, Finland). Following the recommendations of the Task Force (1996), we analyzed HR in periods of five minutes. Whereas the job interview and arithmetic task phases lasted five minutes each, the habituation, preparation, and recovery phases lasted longer than five minutes; for this reason, we chose the central five minutes of each phase. HR analysis failed to detect the HR measurements in two women and three men; therefore, these subjects were excluded from the HR statistical analyses.

Cortisol

We measured the activity of the hypothalamic-pituitary-adrenal (HPA) axis by analyzing the cortisol from the five salivary samples taken throughout the session (see Figure 1). Participants provided saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany). They were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all salivary glands. The samples were centrifuged at 3000 rpm for 5 min,

resulting in a clear supernatant of low viscosity that was stored at 80 C until the analyses were performed in the Central Research Unit (Unidad Central de Investigación) of the Faculty of Medicine, University of Valencia (Spain). The salivary cortisol samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Spectria Cortisol RIA (cat. Nu 06119) from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/L, and within- and inter-assay variation coefficients were all below 8%.

4.3. Statistical Analysis

All calculations were carried out using SPSS v.20 (SPSS, Inc., Chicago, IL, USA). Data are presented as mean ± SD. HR values were logarithmic transformed because they did not have a normal distribution after the Kolmogorov–Smirnov and Levene tests were applied. Delta changes (Δ) for HR and Cortisol were calculated by subtracting baseline levels (HR: habituation; cortisol: -15 min) from the highest indexes (HR: job interview; cortisol: +20 min). Three outliers in the cortisol data (one female and two males) and one outlier in the HR data (one female) were removed from the analyses because their indexes differed by more than 3 S.D. from the total sample mean.

Possible Sex differences in HR and cortisol were assessed using Analysis of Variance (ANOVA) for repeated measures, with Sex (men vs. women) as between-subject factor and Time (cortisol: - 15, + 5, + 10, + 20, + 45 min; HR: habituation, preparation, job interview, arithmetic task, and recovery) as a within-subject factor.

Following Preacher and Hayes (2004), first a mediation procedure based on nonparametric resampling, known as bias-corrected bootstrapping, was conducted to assess the mediating effect of threat appraisal in the relationship between dispositional optimism and displacement behavior (PROCESS model number 4). Then, we performed moderated mediation analyses to estimate the moderator effect of ΔHR and ΔCortisol in the relationship between threat appraisal and displacement behavior, with threat appraisal as a mediator variable between dispositional optimism and displacement behavior (PROCESS model number 14). Bootstrapping makes it possible to gather many alternative versions of a single statistic that is usually only calculated from one sample. Bootstrap data resampling procedures establish confidence intervals (CIs) for testing the statistical significance of an indirect effect (Shrout & Bolger, 2002). The analysis was based on 10,000 bootstrap iterations, and the CI was set to 95%, as recommended by Mallinckrodt and colleagues (2006).

4.4. Results

In order to discover possible Sex influences in our analyses, MANOVA analysis was performed. No sex differences were observed for age (men: *M* = 25.81, *SEM* ± 0.9; women: *M* = 24.31, *SEM* ± 0.6; F(1,80)=1.831, *p*= .18) or SES (men: *M* = 5.89, *SEM* ± 0.1; women: *M* = 6.28, *SEM* ± 0.1; F(1,80)=2.540, *p*= .11), but men had a higher BMI than women (men: *M* = 25.13, *SEM* ± 0.6; women: *M* = 22.27, *SEM* ± 0.4, F(1,80)=14.265, *p*= .001). In addition, no sex differences were found for threat appraisal (men: *M* = 4.52, *SEM* ± 0.1; women: *M* = 4.33, *SEM* ± 0.1; F(1,80)=0.238, *p*= .62), Optimism (men: *M* = 22.56, *SEM* ± 4.3; women: *M* =

22.13, *SEM* ± 3.2; F(1,80)=3.829, *p*= .60), or Displacement behaviors (men: *M* = 6.98, *SEM* ± 2.1; women: *M* = 6.77, *SEM* ± 1.4; F(1,80)=0.921, *p*= .73).

Moreover, repeated-measures ANOVAs were performed. For HR, the analysis showed a main effect of Sex (F(1,74) = 6.341, *p*= .01). Overall, women had a higher HR than men. The main effect of Time (F(3.084,228.212) = 130.390, *p*< .001) was also significant. HR increased immediately after the habituation phase (habituation vs. preparation phase, *p*< .001), and it continued to increase until reaching its peak rate during the job interview phase (habituation vs. job interview phase, *p*< .001). Then, HR decreased (habituation vs. recovery phase, *p*= .09). The interaction between Time and Sex was not statistically significant (*p*= .20).

For cortisol, analysis showed a main effect of Sex (F(1,75) = 21.416, *p*= .01). Overall, men had higher cortisol than women. Moreover, the main effect of Time (F(1.852,138.931) = 5.572, *p*= .006) was also significant. Cortisol concentrations increased immediately after the job interview (-15 min sample vs. +5 min sample, *p*=.01), and they continued to increase until reaching peak levels 20 min after the onset of the stress task (- 15 min sample vs. + 20 min sample, *p*= .007). Afterwards, cortisol levels decreased, reaching baseline levels in the last saliva sample (-15 min sample vs. +45 min sample, *p*= .84). The interaction between Time and Sex was not statistically significant (*p*= .13)[2].

[2] The following means ± SEM will be reposted for each phase of TSST: HR: habituation = 80.806 ± 1.3, preparation = 86.269 ± 1.4, job interview = 97.062, arithmetic task = 92.729 ± 1.4, recuperation = 78.214 ±1.1. Cortisol: -

Due to Sex and BMI differences, they were included in the mediation and moderated mediation analyses with ΔHR and ΔCortisol. However, no main effects or interactions were found (all p> .12). Thus, they were excluded from the final analyses.

Mediation analysis

Although the direct effect of dispositional optimism on displacement behavior was not statistically significant (β = - 0.13, SE = 0.08, t = -1.67, *p* = 0.09), H1 and H2 were supported. Indeed, the results show that threat appraisal has a positive relationship with displacement behavior (β = 0.84, SE = 0.39, t = 2.13, *p* = 0.03). Moreover, dispositional optimism was found to have a negative relationship with threat appraisal (β = -0.02, SE = 0.02, t = -2.13, *p* = 0.03).

The bootstrapping procedure (Shrout & Bolger, 2002) was performed to verify the significance of the full effect of threat appraisal on the relationship between dispositional optimism and displacement behavior. The conditional indirect effect of dispositional optimism on displacement behavior through threat appraisal was significant (β = -0.04, SE = 0.02, 95% CI = -0.20 to -0.05), as the 95% CI does not include zero. Thus, H3 was also confirmed.

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15 = 11.560 ± 0.6, + 5 = 11.636 ± 0.5, + 10 = 12.680 ± 0.6, + 20 = 13.494 ± 0.7, + 45 = 11.592 ± 0.6.

Moderated mediation analysis with ΔHR

Figure 2 depicts the results of the Moderated mediation analysis for ΔHR. Although the direct effect of dispositional optimism on displacement behavior was not statistically significant (β = - 1.15, SE = 0.08, t = -1.82, p = 0.07), H4a was supported. Indeed, results show that threat appraisal has a positive relationship with displacement behavior (β = 2.34, SE = 0.79, t = 2.94, p = 0.004), and that dispositional optimism has a negative relationship with threat appraisal (β = -0.04, SE = 0.02, t = -2.02, p = 0.04).

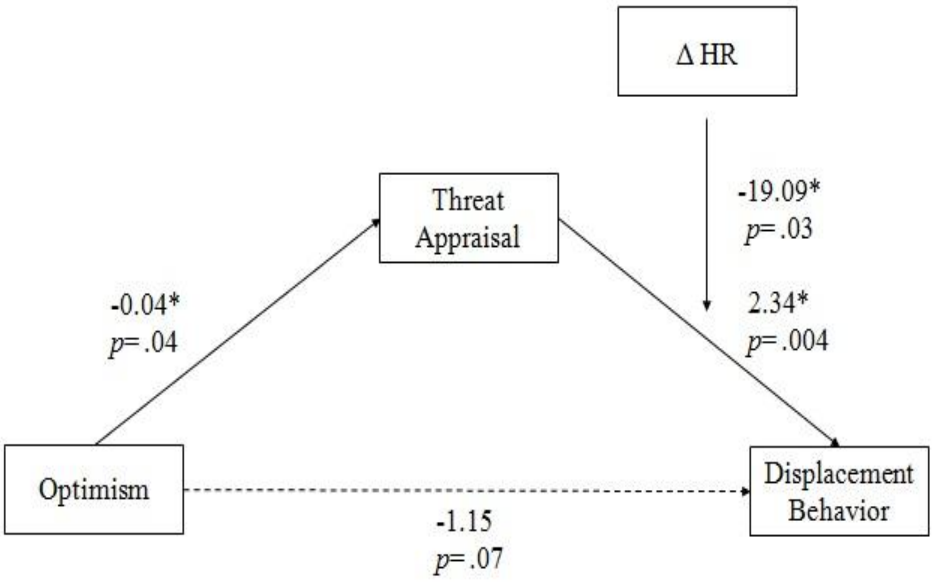

Figure 2. Moderation mediation analysis with ΔHR using bias-corrected bootstrapping in conjunction with multiple regression analysis. Solid lines represent significant direct effect; dashed lines indicate non-significant effects. Numbers on the lines show β and p values.

Moreover, moderated mediation analysis showed that threat appraisal significantly interacted with ΔHR to predict displacement behavior (β = -19.09, SE = 8.91, t = -2.14, p = 0.03). Threat appraisal moderated the effect of dispositional optimism on displacement behavior when ΔHR was low (0.03, 95% CI = -0.18 to -0.016), moderate (0.07, 95% CI = -0.10 to -0.004),

and high (0.12, 95% CI = 0.07 to -0.03). Thus, as Figure 3 shows, the relationship between threat appraisal and displacement behavior was slightly negative when ΔHR was high, whereas the relationship was strongly positive when ΔHR was low.

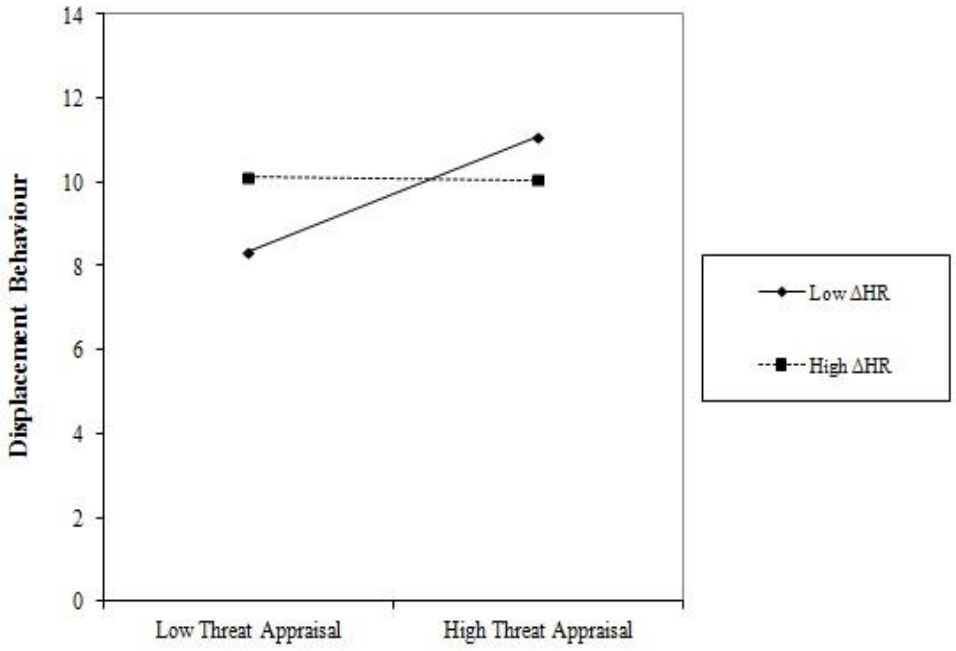

Figure 4. Moderation role of ΔHR in the relationship between threat appraisal and displacement behavior.

Moderated mediation analysis with ΔCortisol

Figure 4 depicts the results of the Moderated mediation analysis for ΔCortisol. The direct effect of dispositional optimism on displacement behavior was not statistically significant (β = - 0.11, SE = 0.08, t = -1.43, *p* = 0.15). However, results show that threat appraisal has a positive relationship with displacement behavior (β = 0.97, SE = 0.41, t = 2.32, *p* = 0.02), and that dispositional optimism has a negative relationship with threat appraisal (β = -0.04, SE = 0.02, t = -2.13, *p* = 0.03).

Moderated mediation analysis showed that threat appraisal did not significantly interact with ΔCortisol to predict Displacement behavior (β = -2.68, SE = 2.98, t = -0.89, p = 0.37. Thus, H4b was not supported.

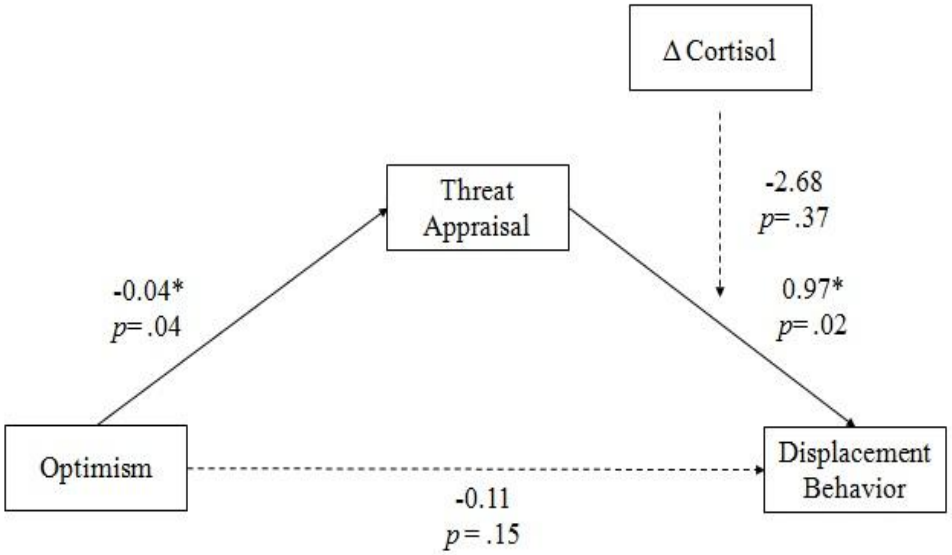

Figure 4. Moderation mediation analysis with Δ Cortisol using bias-corrected bootstrapping in conjunction with multiple regression analysis. Solid lines represent significant direct effect; dashed lines indicate non-significant effects. Numbers on the lines show β and p values.

4.5. Discussion

The main aim of this study was to investigate the mediation role of a threat appraisal of stress between dispositional optimism and displacement behaviors. Moreover, we aimed to analyze the moderating role of the physiological responses to stress in the relationship between threat appraisal and displacement behavior. To do so, we combined the Trier Social Stress Test (TSST) with ethological assessment, self-report questionnaires, and physiological measures (cortisol and heart rate). Specifically, we first assessed the effect of dispositional optimism on threat appraisal. Second, we verified the link between threat appraisal and displacement behaviors. Third, we verified the mediation role of threat appraisal in the relationship between

dispositional optimism and displacement behavior. Fourth, we investigated the moderating role of HR and cortisol between threat appraisal and displacement behavior.

As expected, the social evaluative test employed, the TSST, was able to produce a significant stress response in the study participants. In fact, according to previous studies (Espin, Almela, Hidalgo, Villada, Salvador, & Gomez-Amor, 2013; Hidalgo, Villada, C., Almela, Espín, Gómez-Amor, & Salvador, 2012; Villada et al., 2014), preliminary analyses showed that the TSST was effective in triggering an acute stress response because of the activation of the autonomic nervous system (HR) in the anticipation phase (immediately before the speech) and the HPA axis (cortisol) during the speaking task. Moreover, in agreement with prior investigations, the TSST was considered threatening by participants (i.e. Gaab et al., 2005; Frisch, Häusser, & Mojzisch, 2015).

As hypothesized, our results show that people with higher scores on optimism assessed stressful tasks as less threatening. This result agrees with several studies that pointed out that dispositional optimism has a positive effect on people's stress appraisal (Endrighi et al., 2011; Penly & Tomaka, 2002). This result also coincides with Scheiner and Carver's (1985) theory indicating that optimistic people trust their coping strategies in facing challenging situations in everyday life. In fact, optimism has also been found to have an impact on coping strategies. Optimistic people seem to have coping strategies related to the elimination, reduction, or control of the stressors (Nes & Segerstrom, 2006). Along the same lines, people who believe they have the coping strategies to overcome a challenge perceive

stress as less threatening (Lazaruz & Folkman, 1984). Overall, this result supports the idea of a health protection role of optimism in stressful situations (Carver & Scheier, 1990; Carver et al., 2010; Scheier & Carver, 1993). In fact, some studies have observed that people with high dispositional optimism manifest greater physical and mental health, even in pathological situations such as type 2 diabetes (Puig-Perez, Hackett, Salvador, & Steptoe, 2016; Steptoe et al., 2014). Previously, Puig-Perez et al. (2015) studied optimism and pessimism separately as two different factors in older people, showing that only pessimism was associated with the situational stress experienced. Thus, assuming that a one-dimensional perspective considers pessimistic people to be those with lower scores on dispositional optimism, their result reinforces our finding in healthy young people.

In our study, threat appraisal showed a positive relationship with displacement behavior. When the task was appraised as more threatening, more displacement activities were displayed. In addition, mediation analysis indicated that threat appraisal fully mediated the relationship between dispositional optimism and displacement behavior. No direct relationship between optimism and displacement behaviors was found. To the best of our knowledge, our study is the first one to take displacement behaviors into consideration as a consequence of threat appraisal. However, previous literature supports this finding, suggesting that some other negative mental states (i.e. depression) might influence the level of displacement behaviors during stressful tasks (Shreve et al., 1988; Troisi et al., 1996; Villada et al., 2014). Hence, we considered that threat appraisal, as a temporary negative mental state, might influence the level of displacement behavior during a

social evaluative stress situation. Particularly, according to these authors, displacement behaviors are manifested to re-establish an internal equilibrium when high psychological activation (i.e. anxiety) exists. Along these lines, several investigations carried out in recent years have pointed out that the role of displacement activities is to regulate the internal homeostasis when it is compromised by an external stressor (Troisi, 2002; Villada et al., 2014). Thus, the perception of stress, as psychological activation in response to stress, plays a fundamental role in displacement behavior, and so displacement behaviors might require medium/high psychological responses to stress in order to be manifested. However, because dispositional optimism is not a stressful state of activation, but rather a positive personality trait, displacement behavior is not directly influenced by it. Hence, as discussed above, dispositional optimism influences threat appraisal, which, in turn, influences the use of displacement behaviors to reestablish internal equilibrium in response to stress.

Interestingly, our results also show that HR reactivity moderates the relationship between threat appraisal and displacement behavior when threat appraisal is a mediating variable between dispositional optimism and displacement behavior. The same result was not found for cortisol reactivity. Regarding HR, the relationship between threat appraisal and displacement behavior is positive when low HR reactivity exists. However, the relationship between threat appraisal and displacement behavior is slightly negative when HR reactivity is high. In other words, when HR reactivity is low, people who perceive low threat manifest low displacement behavior, and people who perceive high threat manifest high displacement. By contrast, when HR

reactivity is high, people who perceive either high or low threat manifest a similar (high) level of displacement behavior. This result suggests that people engaged in high threat appraisal and high sympathetic activity might use displacement behavior to re-establish an internal homeostasis. In agreement with Pico-Alfonso and colleagues' (2007) study, engagement in displacement behavior during a social evaluative task reduces sympathetic activity and increases parasympathetic activity. Thus, people with higher threat appraisal and HR reactivity might show more displacement behavior because they need to regulate their psychophysiological stress-related activation. Moreover, this result also indicates that even with low HR reactivity, displacement behaviors are displayed when high threat appraisal is perceived. As discussed above for the mediation result, displacement behavior might regulate the individual level of arousal created by a high level of threat appraisal, even without greater sympathetic activation. Thus, high/medium levels of threat appraisal lead to displacement behaviors, even when cardiovascular activation is low. This might be the case of the three types of psychophysiological responses to cognitive tasks described by Boudarene, Legros, and Timsit-Berthier (2002). They indicate "biological silence" as a psychophysiological reaction to a stressful task where subjects manifest high emotional responses without increases in physiological responses. Thus, a "biological silence" reaction might lead people to use displacement behavior to regulate their psychological response to stress. According to Troisi (2002), displacement activities are likely to be behavioral elements of the adaptive psychophysiological stress response, possibly having an anxiolytic effect.

Particularly, they are manifested in response to autonomic and threat appraisal activation.

Regarding cortisol responses, we did not find any moderating role in the relationship between threat appraisal and displacement behavior. Therefore, cortisol does not strengthen or weaken the link between threat appraisal and displacement behavior. Taking into account that cortisol increases in this study could be considered moderate, these behavioral patterns may not be necessary to regulate HPA axis activity. This result is in line with Campbell and Ehlert (2012), who reviewed a total of 49 studies in which the paradigm of social stress used was always the TSST. They concluded that stress appraisal and physiological reactions are not always correlated. That is, cortisol does not seem to reflect a greater experience of stress or anxiety. Moreover, as Villada et al. (2016) stated, a moderate cortisol increase might have a positive function, reflecting the response of preparing to deal with stress. Therefore, behavioral strategies (i.e. displacement) would not be necessary to reestablish homeostasis.

This study provides new insights into the role of optimism and displacement behavior as antecedent and consequence of the threat appraisal of stress. Moreover, it is the first study to assess the moderating role of HR and cortisol in the relationship between threat appraisal and displacement behaviors. Additionally, our study provides insight into the role of displacement behaviors in communicating information about the individual's emotional state and personality traits. Indeed, displacement behaviors are believed to reflect the state of tension brought about by the social context (Maestripieri, Schino, Aureli, & Troisi, 1992), and they provide more

accurate information about the subject's emotional state than verbal statements and facial expressions (Troisi, 2002). In agreement with Mohiyeddini and Sample (2013), displacement behaviors might be considered “cues” rather than “signals”, and they might be quite useful during various evaluative situations such as clinical or job interviews. In fact, they might help clinicians or psychologists to detect people’s personality traits and coping strategies, or help recruiters to detect candidates’ stress management abilities, which would be beneficial for the company’s hiring process.

Whether and how threat appraisal mediates the relationship between different personality traits and other stress-related behaviors remains poorly understood. Further studies in this area are required to provide new insight into the relationships among threat appraisal, personality traits, and behavioral stress responses in interpersonal communication. Moreover, due to the nature of the study (experimental design and protocol), its internal validity is high, but its external validity is limited. Thus, our study greatly contributes to understanding the relationships among threat appraisal, dispositional optimism, displacement behavior, and physiological responses. Nevertheless, further studies are needed taking natural settings into consideration.

CHAPTER V

STUDY 3

Acute stress and working memory: the role of sex and cognitive stress appraisal

The main results of this study have been published in:

Zandara, M., Garcia-Lluch, M., Pulopulos, M. M., Hidalgo, V., Villada, C., & Salvador, A. (2016). Acute stress and working memory: The role of sex and cognitive stress appraisal. *Physiology & Behavior*, *164*, 336-344.

5. Introduction

Acute stress triggers physiological and psychological responses that affect cognitive performance, especially memory performance (Lupien, Maheu, Fiocco, & Schramek, 2007). From a physiological perspective, acute stress influences memory performance by eliciting the activation of the Hypothalamus–Pituitary–Adrenal axis (HPA-axis) and the resulting secretion of glucocorticoids (GCs) (primarily cortisol in human) (Sapolsky, Romero, & Munck, 2000). Studies in humans indicate that cortisol affects memory through the GC receptors located especially in the prefrontal cortex (PFC), hippocampus, and amygdala (see Wolf, 2009). Therefore, these brain regions have been considered the focal point of the stress effect and, consequently, related to memory performance (Joëls, Karst, DeRijk, & de Kloet, 2008; Patel, Katz, Karssen, & Lyons, 2008).

Specifically, working memory (WM) has been defined as a prefrontal cortex (PFC) dependent ability that allows both (i) the temporary retention of a limited amount of information online in the external environment for a short period of time (attention and maintenance component of the WM) and (ii) the executive function of manipulating and/or processing this information (executive component of the WM) (D'Esposito, 2007). To date, most studies on the effects of stress on WM have found a negative effect (Duncko, Johnson, Merikangas, & Grillon, 2009; Elzinga and Roelofs, 2005; Gärtner, Rohde-Liebenau, Grimm, & Bajbouj, 2014; Luethi, Maier, & Sandi, 2009; Oei, Everaerd, Elzinga, van Well, & Bermond, 2006; Schoofs, Preuss, & Wolf, 2008; Schoofs, Wolf, & Smeets, 2009; Terfhr et al., 2011; Wolf et al.,

2001; Young, Sahakian, Robbins, & Cowen, 1999), although some studies have shown a positive effect (Cornelisse, van Stegeren, & Joéls, 2011; Duncko et al., 2009; Stauble, Thompson & Morgan, 2013; Weerda, Muehlhan, Wolf, & Thiel, 2010), or even no effect (Hoffman & al'Absi, 2004; Smeets, Jelicic, & Merckelbach, 2006; Kuhlmann, Piel, & Wolf, 2005).

Recently, sex has been considered an important moderating factor in the relationship between stress and WM (e.g. Almela et al. 2011; Wolf, 2008; Schoofs et al., 2013). Nevertheless, most of the previous investigations with young adults have focused on the stress-related WM performance of males, whereas females have hardly been considered. Likewise, most studies have investigated the executive component of WM, rather than the attention and maintenance component. On the one hand, the executive component of WM has been shown to be negatively affected by acute stress, in both males (Oei et al., 2006; Schoofs et al., 2008; Schoofs et al., 2009; Young et al., 1999; Wolf et al., 2001) and females (Schoofs et al., 2013). However, other studies have found that acute stress positively affects the executive component of WM only in males (Cornelisse et al., 2011; Schoofs et al., 2013; Weerda et al., 2010), and some have shown no effect or no sex-related differences in the executive component of WM (Hoffman & al'Absi, 2004; Smeets et al., 2006; Kuhlmann et al., 2005). On the other hand, the attention and maintenance component has been found to be positively affected by acute stress in males (Smeets et al., 2006; Stauble et al., 2013), but no effects and sex-related differences have also been observed (Elzinga & Roelofs, 2005; Hoffman & al'Absi, 2004; Kuhlmann et al., 2005). Thus, the results are currently inconclusive, and the mechanisms underlying sex differences are still unclear.

In order to better understand these sex-related differences in WM in response to acute stress, a perspective formulated by Taylor and colleagues (2000) might be useful. According to these authors, males and females might present different biobehavioral patterns of stress responses. Females have evolved toward a "tend and befriend" stress response pattern, in contrast to the classic "fight or flight" stress response characteristic of males. The "tend and befriend" response to stress is related to females' need to protect and nurture their offspring and their need for affiliation with social groups to maximize the survival of the species in times of adversity. This female behavior pattern has been described as oriented toward cooperation rather than competition. Thus, some studies have proposed that the "tend and befriend" stress response is concomitant, due to the mediating role of oxytocin, with a "down-regulation" of HPA activation. Specifically, oxytocin release in response to stress has been found to be mediated by estrogen (McCarthy, 1995), and its release has been shown to be higher in females than males (Jezova, Jurankova, Mosnakova, Kriska, & Skultetyova, 1996). Thus, whereas in females the neuroendocrine secretion in response to stress would be buffered by the "tend and befriend" response, in males the "fight or flight" response pattern is characterized by elevated neuroendocrine activation (Taylor et al., 2000). Therefore, these sex dissimilarities might be associated with sex-related differences in the relationship between the stress response and WM performance.

In addition to the differences in physiological responses, cognitive stress appraisal might be another important moderating factor in the relationship between sex and WM. According to the transactional model,

cognitive stress appraisal is the result of a complex mental process consisting of three main aspects: the threatening and/or challenging appraisal of a stressful stimulus (primary appraisal), and the perception of our own ability to cope with it (secondary appraisal). Threat appraisal refers to the evaluation of the situation as potentially harmful or as a source of failure. By contrast, challenge appraisal refers to the evaluation of the situation as an opportunity for self-growth and beneficial to our well-being. When a person is faced with a stressor, the cognitive stress appraisal is considered the difference between the primary and secondary appraisal (Drach-Zahavya & Erezb, 2002). The more he/she appraises the situation as threatening and feels lacking in sufficient resources to cope with it, the greater the cognitive stress appraisal will be (Folkman, Lazarus, Dunkel-Schetter, DeLongis, & Gruen, 1986). Some findings have associated the cognitive threat appraisal with impaired cognitive performance (e.g., Blascovich, Mendes, Hunter, & Salomon, 1999; McEwen & Sapolsky, 1995). Moreover, another study showed a tendency where an increase in the cognitive threat appraisal led to WM performance impairment (Ell, Cosley, & McCoy, 2011). Furthermore, even though coping strategies have been positively related to WM (i.e. Putman & Roelofs, 2011; Snyder, Barry, & Valentino, 2014), sex differences in coping strategies have been widely observed. Indeed, several studies have observed that women suffer more stress than men, and their coping style is more emotion-focused than that of men (Pilar, 2014). Moreover, women perceive having inadequate resources for coping with a threatening situation more often than men do, and they also see a stressful situation as unchangeable and tend to turn to others for support (Banyard & Graham-Bermann, 1993).

With all this in mind, the aim of this study was to investigate sex-related differences in WM performance after a stress task. In addition, we examined the role of cognitive stress appraisal and physiological responses to acute stress in WM performance in males and females. To reach our aims, we subjected healthy young adult males and females to a modified version of the TSST, and we assessed WM performance before and after the stressful task

5.1. Methods

Participants

162 individuals were assessed by an expert interviewer to verify that they met the experiment's inclusion criteria. The inclusion criteria were examined through self-report, and they were: (i) Spanish nationality; (ii) age between 20 and 40 years; (iii) educational level between Secondary school and postgraduate studies; (iv) not smoking more than five cigarettes per day; (v) no alcohol or any other drugs of abuse; (vi) no visual or hearing problems; (vii) no cardiovascular, endocrine, neurological, or psychiatric diseases; (viii) not having been under general anesthesia once or more than once in the past year; (ix) not having experienced a major stressful life event during the past year; (x) not using any medication directly related to cardiac, emotional, or cognitive function, one that was able to influence hormonal levels, such as glucocorticoids or β-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, or psychotropic substances. Subjects who fulfilled the criteria were asked to attend sessions that took place in a laboratory at the Faculty of Psychology. Females reported the last date of their menstruation. This information was used to identify participants who

were in the follicular (N=12), the luteal (N=12), or menstrual (N=9) phase, and oral contraceptive users (OC) (N=12).

Before each individual session, participants were asked to maintain their general habits, sleep as much as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to drink only water, and not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the Ethics Research Committee of the University of Valencia. Upon arrival at the laboratory, all the participants received verbal and written information about the study and signed an informed consent form.

The final sample was composed of 82 healthy young adults (37 males and 45 females) from 20 to 39 years old. They were graduate and post graduate students in a wide range of majors at the University of Valencia. All participants were volunteers, and at the end of the experimental session they received feedback from an expert interviewer about how to improve their individual performance on a job interview.

Procedure

The study involved an individual session that lasted approximately 90 min and took place between about 16.00 and 19.00h. The experimental sessions were composed of different phases (see Figure 1). Upon arrival at the laboratory, the experimenter verified that participants had followed the

instructions given previously. In order to calculate each participant's body mass index (BMI), his/her weight and height were measured at the end of the session.

To produce stress, we subjected the participants to a modified version of the Trier Social Stress Test (TSST, Kirschbaum et al., 1993). The modifications were: (i) all the phases of the TSST took place in the same room, and (ii) the committee was composed of only one person (female), who had been introduced as an expert in human resources. The session started with a 40-minute habituation phase. During that time, participants had to fill out a general questionnaire related to demographics and anthropometric data, and for the last 10 minutes they were left alone to rest mentally and physically. Next, during the introduction phase, participants were told about the job interview task. Immediately after that, they had to fill out the Primary Appraisal Secondary Appraisal scale (PASA) scale. Before the beginning of the TSST, participants had 5 minutes to prepare their presentations. During this preparation phase, individuals had to write down their main ideas about what to say during the job interview. However, they could not use these notes during the speech task. The TSST protocol consisted of 5 minutes of free speech (job interview), followed by a 5- minute arithmetic task. In the job interview, the participant's main goal was to convince the interviewer that he/she was the best candidate for his/her "dream job". The participants stood at a distance of 1.5 meters from the evaluator. In addition, a video camera, a microphone, and a monitor where subjects could see their performance were clearly visible. Both the speech and arithmetic tasks were filmed. The Digit Span Test was administered before (- 30 min. pre stress) and after (+ 15 min.

post stress) the TSST. Moreover, we measured salivary cortisol, Heart Rate (HR) responses, and cognitive stress appraisal, in order to evaluate their relationship with WM performance.

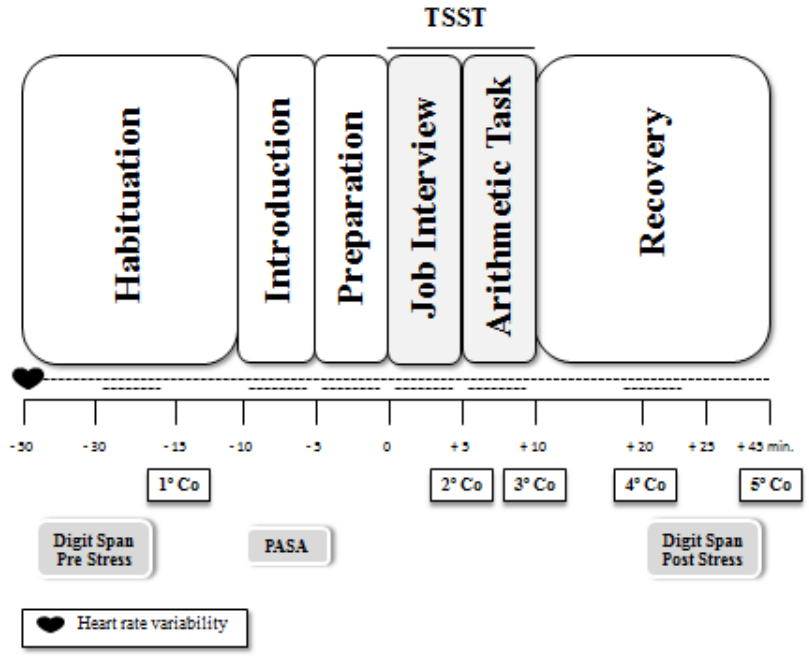

Figure 1. Timeline of the TSST. Dotted lines depict the time of HR collection. Salivary cortisol samples = 1°Co, 2°Co, 3°Co, 4°Co. PASA, Primary Appraisal and Secondary Appraisal.

5.2. Measures

Demographic and anthropometric measures

To assess possible differences between groups, we collected data such as age, BMI, and subjective socioeconomic status (SES) (Adler, et al., 2000).

Neuroendocrine response

We measured HPA-axis activity by analyzing the salivary cortisol levels. Participants provided saliva samples by using salivettes (Sarstedt, Nümbrecht, Germany). Salivary samples provided during the session were

stored and analyzed as described in detail in Pulopulos, Almela, Hidalgo, Villada, Puig-Perez, and Salvador (2013).

Heart Rate

HR data were continuously recorded during the entire session using a Polar©RS800cx watch (Polar CIC, USA), which consists of a chest belt for detection and transmission of heartbeats and a Polar watch for data collection and storage. The transmitter is located on the chest belt, which is placed on the solar plexus and transmits HR information to the receiver (Polar watch). The data collected by the Polar watch were downloaded, stored in the Polar ProTrainer5TM program in the computer, and analyzed using HRV Kubios Analysis software (Biomedical Signal Analysis Group, University of Kuopio, Finland). Following the recommendations of the Task Force (1996), we analyzed HR in periods of five minutes. Whereas the job interview and arithmetic task phases lasted five minutes each, the habituation, preparation, and recovery phases lasted longer than five minutes; for this reason, we chose the central five minutes of each phase. HR analysis failed to detect the HR index in the samples of two females and three males; therefore, these subjects were excluded from the HR statistical analyses.

Cognitive stress appraisal

Cognitive stress appraisal was evaluated with the PASA (Gaab et al., 2005). This scale was employed to assess cognitive appraisal processes before performing the TSST, based on transactional stress theory (Lazarus & Folkman, 1984). The PASA scale is composed of the "Primary appraisal" subscale, which includes two situation-specific subscales assessing 'Threat'

(e.g., I do not feel threatened by the situation) and 'Challenge' (e.g., The situation is not a challenge for me), and the "Secondary appraisal" subscale, which includes two situation-specific scales assessing 'Self- Concept of Own Competence' (e.g., In this situation I know what I can do) and 'Control Expectancy' (e.g., It mainly depends on me whether the experts judge me positively). Moreover, based on the transactional stress paradigm (Lazarus & Folkman, 1984), the "Tertiary appraisal" scale (also called the "global index of cognitive stress appraisal") was calculated using the formula proposed by Gaab and colleagues (2005). Each scale has four items, rated on a 6-point Likert scale ranging from "strongly disagree" to "strongly agree". The scale was translated into Spanish and back-translated. In our sample, Cronbach's alphas for the four scales were 0.76, 0.82, 0.75 and 0.78. The PASA scale was administered at the end of the introductory phase of the TSST.

WM test: Digit Span subtest

The Digit Span subtest of the Wechsler Memory Scale III (Wechsler, 1997) was given before and after the stress task. Participants listened to a series of numbers of increasing length (from 4 to 8 numbers on the Digit Span Forward (DS-Forward), and from 3 to 7 numbers on the Digit Span Backward (DS-Backward)), at the rate of one digit per second. Each series of numbers had to be repeated in the same order (DS-Forward) or in reverse order (DS-Backward). When the participant failed to repeat one list of digits, a second attempt was made with another list of numbers of the same length. After two successful attempts, the number of digits was added up. When the participant failed to reproduce two series of digits of the same length (e.g., failing to reproduce a 6-item list on two successive trials), the task ended.

The maximum score possible in each test condition was 16. Two parallel versions of the test were administered, and the order of presentation was counterbalanced. The data from the DS-Forward and DS-Backward express the maximum number of digits recalled. DS-Forward was specifically used as a measure of the attention and maintenance component of WM, whereas DS-Backward was used as a measure of attention and maintenance and the executive component of WM (Lezak, Howieson, Loring, Hannay, & Fischer, 2004).

5.3. Statistical Analysis

Cortisol and HR values were logarithmic transformed because they did not have a normal distribution after Kolmogorov–Smirnov and Levene's tests were applied. We used Multivariate Analysis (MANOVA) to investigate sex differences in demographic and anthropometric measures. We used BMI and age as covariate variables for each physiological and memory assessment. Preliminary bivariate Pearson's correlation analyses showed that both BMI and age had a relationship with one or more of the dependent variables we used for the analyses in males and/or females[3]. In females, age had a positive relationship with the DS-Backward (r=0.409, *p*= .005) and a

[3] We also analyzed the influence of the variable "number of cigarettes smoked". However, only 13 participants were smokers (8 females and 5 males; mean cigarettes smoked per day = 3.5), and no differences were found between males and females (Mann Whitney U test, U= 17,550, *p*= .72), and there were no significant correlations between the "number of cigarettes smoked" and cortisol release (r=0.079, *p*= .79).

tendency toward a positive relationship with the DS-Forward (r= 0.271, *p*= .06) (see Table 1). In males, BMI had a positive relationship with the post-stress DS-Forward (r=0.327, *p*= .04). Therefore, age and BMI were used as covariate variables, based on previous studies that indicated their potential effect on the HPA-axis and memory performance (e.g., Abraham, Rubino, Sinaii, Ramsey, & Nieman, 2013; Cournout et al., 2006; Fisk & Warr, 1996; Wang et al., 2011). Three outliers in the cortisol data (one female and two males) and one outlier in the HR data (one female) were removed from the analyses because their indexes differed by more than 3 S.D. from the total sample mean.

MANOVA were used to assess sex differences in the "Tertiary PASA", "Threat appraisal", "Challenges appraisal", "Self-concept of Own Competence" and "Control expectancy" measures.

Sex differences in cortisol, HR, and WM performance (DS-Forward and DS-Backward) were assessed using Analysis of Covariance (ANCOVA) for repeated measures, with Sex (males vs. females) as between-subject factor and Time (cortisol: - 15, + 5, + 10, + 20, + 45; HR: habituation, preparation, job interview, arithmetic task and recuperation; WM: pre and post) as a within-subject factor.

We used the Greenhouse–Geisser procedure when the requirement of sphericity in the ANCOVAs 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 $p < .05$.

When not otherwise specified, results shown are means ± 1 standard error of means (SEM).

Furthermore, delta changes (Δ) in cortisol and HR were calculated by subtracting baseline levels of cortisol and HR levels (cortisol: - 15; HR: Habituation) from the highest levels (cortisol: + 20 min; HR: job interview). Hierarchical regression analyses were performed to investigate whether Δcortisol and ΔHR, Tertiary PASA (global index of cognitive stress appraisal), Cognitive Threat and Challenges Appraisal, Self-concept of Own Competence, and Control expectancy were predictor variables of DS-Forward or DS-Backward performance. Preliminary one-way ANOVA revealed Sex differences in baseline cortisol $F(1.75)=18.968$, $p> .001$. Males had higher baseline cortisol concentrations than females. However, when we performed a correlation between the baseline cortisol and the Δcortisol, the correlation was not statistically significant (p=.66), indicating that the change in cortisol was not affected by the baseline level. However, to avoid any basal level effect, we added baseline Cortisol and HR as covariate variables in the hierarchical regression analysis. Thus, for each regression analysis, we entered (i) the control variables age and BMI, and baseline cortisol and HR in the first step and (ii) Δcortisol and ΔHR, Tertiary PASA, Cognitive Threat and Challenge Appraisal, Self-concept of Own Competence, and Control expectancy performance in the second step. In order to avoid Type II error, the regression analyses were performed separately for males and females.

2.4. Results[4]

Preliminary analysis

There were no sex differences in age (males: *M* = 25.81, *SEM* ± 0.9; females: *M* = 24.31, *SEM* ± 0.6; F(1,80)=1.831, *p*= .18) or SES (males: *M* = 5.89, *SEM* ± 0.1; females: *M* = 6.28, *SEM* ± 0.1; F(1,80)=2.540, *p*= .11), but males had a higher BMI than females (males: *M* = 25.13, *SEM* ± 0.6;

[4] The influence of the menstrual cycle or oral contraceptive intake was investigated with repeated-measures ANOVAs, as cortisol, HR responses to stress, and WM performance may differ in females with different sex hormones levels. However, when we repeated the analyses with this factor as an independent factor, no main effects or interactions were detected (all *p's*> .15).

females: *M* = 22.27, *SEM* ± 0.4, F(1,80)=14.265, *p*= .001) (see Table 1).

Males

Variable	M	SD	1	2	3	4	5	6	7	8	9	10	11
1. Age	25.81	5.56	-										
2. BMI	25.13	3.68	.07	-									
3. ΔCortisol	0.37	0.71	.20	-.13	-								
4. ΔHR	-1.37	1.54	-.08	.16	.11	-							
5. DS-Forward post	6.27	1.09	.20	.32*	-.18	-.30	-						
6. DS-Backward post	4.91	1.13	.05	.17	-.04	-.38	.53**	-					
7. Threat stress appraisal	3.02	0.79	-.10	-.15	-.09	.10	.10	.03	-				
8. Challenge stress appraisal	3.57	0.77	-.08	-.24	-.36	-.23	.27	.30	.25	-			
9. Self- Concept of Own Competence	3.94	0.93	-.03	.01	.07	-.09	.13	.03	-.55**	.10	-		
10. Control Expectancy	4.45	0.77	-.22	.07	-.04	-.02	.16	.01	.01	.57**	.40**	-	
11. Tertiary appraisal	-0.90	0.95	-.02	-.20	-.19	-.03	.01	.11	.79**	.22	-.85**	-.37**	-

Females

Variable	M	SD	1	2	3	4	5	6	7	8	9	10	11
1. Age	24.31	4.47	-										
2. BMI	22.27	3.17	.15	-									
3. ΔCortisol	0.15	0.51	.29*	-.01	-								
4. ΔHR	-0.77	1.44	.19	.08	-.03	-							
5. DS-Forward post	6.28	1.17	.27	-.38	-.32*	-.12	-						
6. DS-Backward post	4.91	1.18	.40**	.09	-.01	-.08	.57**	-					
7. Threat stress appraisal	2.93	0.80	.05	-.13	.22	.14	-.40**	.02	-				
8. Challenge stress appraisal	3.40	0.62	.19	-.01	.21	.01	-.01	.03	.46**	-			
9. Self- Concept of Own Competence	3.97	0.76	.15	-.09	-.08	.27	.40	.06	-.35*	.01	-		
10. Control Expectancy	4.45	0.70	-.21	-.09	-.06	.07	.13	.20	-.11	.16	.17	-	
11. Tertiary appraisal	-1.01	0.91	.08	.04	.20	-.13	-.39**	-.10	.76**	.49**	-.67**	-.41**	-

Table 1. Descriptive statistics and correlations among the study variables.

Cognitive stress appraisal

The MANOVA did not find significant Sex differences in Cognitive Threat Appraisal (males: *M* = 3.02, *SEM* ± 0.1; females: *M* = 2.93, *SEM* ± 0.1; F(1,80)=0.238, *p*= .62), Challenge Appraisal (males: *M* = 3.57, *SEM* ± 0.1; females: *M* = 3.40, *SEM* ± 0.1; F(1,80)=1.201, *p*= .27), Self-concept of Own Competence (males: *M* = 3.94, *SEM* ± 0.1; females: *M* = 3.97, *SEM* ± 0.1; F(1,80)=0.029, *p*= .86), Control expectancy (males: *M* = 4.45, *SEM* ± 0.1; females: *M* = 4.45, *SEM* ± 0.1; F(1,80)=0.003, *p*= .95), or the Tertiary

PASA index (males: *M* = -0.90, *SEM* ± 0.1; females: *M* = -1.01, *SEM* ± 0.1; F(1,80)=0.276, *p*= .60) (see Table 1).

Salivary cortisol

The repeated-measures ANCOVA with salivary cortisol concentrations as the dependent variable showed a main effect of Sex (F(1,73) = 17.324, *p*< .001). Overall, males had higher cortisol concentrations than females. Moreover, the main effect of Time (F(1.786,130.362) = 4.563, *p*= .01) was also significant. Thus, cortisol concentrations increased immediately after the job interview (-15 min sample vs. +5 min sample, *p*=.01) and continued to increase until reaching peak levels 20 min after the onset of the stress task (- 15 min sample vs. + 20 min sample, *p*= .005). Afterwards, cortisol levels decreased, reaching baseline levels in the last saliva sample (-15 min sample vs. +45 min sample, *p*> .99) (see figure 2). Moreover, the Time and Age interaction was also significant (F(1.786,130.362) = 5.876, *p*= .005). The interaction between Time and Sex and the main effect of Age and BMI were not significant (all *p's*> .38).

Heart rate

The repeated measures ANCOVA with HR as the dependent variable showed a main effect of Sex (F(1,72) = 6.284, *p*= .01). Overall, females had higher HR than males. Moreover, the main effect of Time (F(2.998,215.888) = 6.972, *p*< .001) was also significant. HR increased immediately after the habituation phase (habituation vs. preparation phase, *p*< .001), and it continued to increase until reaching its peak rate during the job interview phase (habituation vs. job interview phase, *p*< .001). Then, HR decreased,

reaching lower levels than in the habituation phase (habituation vs. recovery phase, *p*= .01) (see figure 2). Moreover, the Time and Age interaction was also significant (F(2.998,215.888) = 3.475, *p*= .01). The interaction between Time and Sex and the main effect of Age and BMI were not significant (all *p's*> .08).

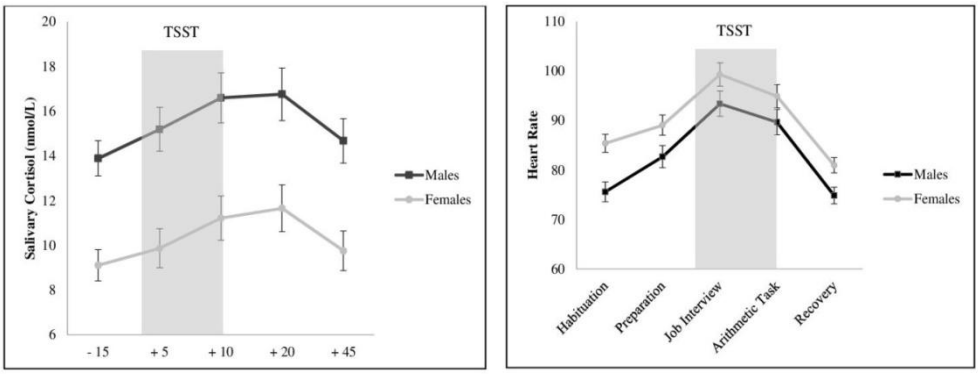

Figure 2. Salivary Cortisol concentrations (i) and Heart Rate (ii) for Males and Females during TSST.

WM test: Digit Span subset

DS-Forward (attention and maintenance). The repeated-measures ANCOVA with the number of digits on the DS-Forward as the dependent variable showed that the Sex, Time and BMI factors were not significant (both *p's*> .55), whereas Age was (F(1,78) = 5.152, *p*= .02). However, a significant interaction was found between Time and Sex (F(1,78) = 4.144, *p*= .04). Post hoc analysis revealed that females had better performance after the task than before it (*p*= .04), but this did not occur in males (*p*= .31). Moreover, there were no sex differences before the stress task (pre-stress task, *p*= .27) or after the stress task (post-stress task, *p*= .46) (see figure 3).

However, the Time and Age interaction and the Time and BMI interaction were not statistically significant (all *p's*>.41).

DS-Backward (maintenance and executive component). The repeated-measures ANCOVA with the number of digits on the DS-Backward as the dependent variable did not show any main effects of Sex, Time, Age or BMI, and there were no interactions among these factors (all *p's*> .09) (See figure 3).

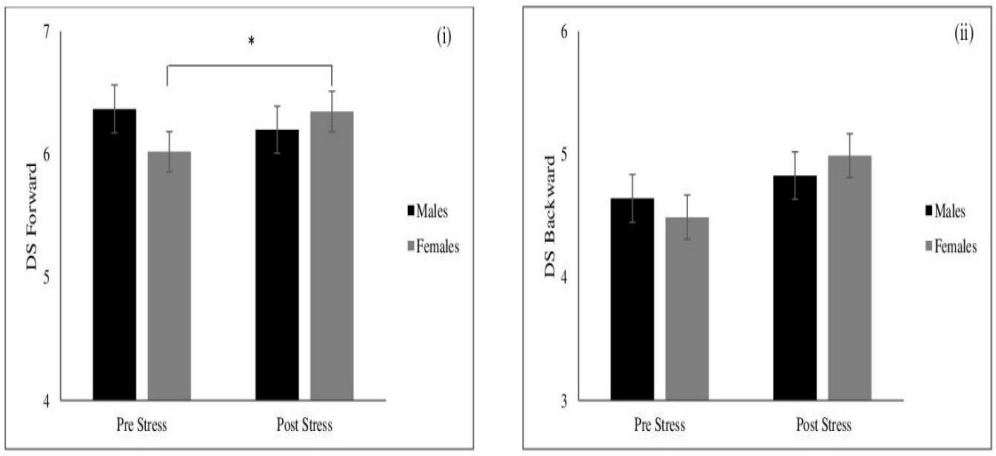

Figure 3. Performance on Digit Span Forward (DS-Forward) (i) and Digit Span Backward (DS-Backward) (ii) for Males and Females before and after TSST.

Relationship between cognitive stress appraisal and WM performance

None of the associations studied for males were significant (all *p's*> .073), except the relationship with BMI (β=-0.397, *p*= .03). In females, regression analysis showed a significant negative relationship between the Tertiary PASA and the DS-Forward (β=-0.380, *p*= .009), whereas there was no significant relationship with the DS-Backward (β=-0.094, *p*= .51).

Moreover, a significant negative relationship was also found between Cognitive Threat appraisal and the DS-Forward (β=-0.390, *p*= .007), whereas a positive linear relationship was observed between Self-concept of Own Competence and the DS-Forward (β=0.391, *p*= .008). Furthermore, in females, there were no significant associations between Cognitive Threat and Challenge Appraisal, Self-concept of Own Competence, Control Expectancy, Age or BMI and the DS-Forward and DS-Backward (all *p's*> .18), except between Age and the DS-Backward (β=0.433, *p*= .009).

Relationships among cortisol, HR and WM performance

In males, there were no significant associations between Δcortisol, ΔHR, Age, or the basal levels of cortisol and HR and the DS-Forward or Backward (all *p's*> .25). However, we found a significant relationship between BMI and the DS-Forward (β=0.334, *p*= .05). In females, regression analysis showed a significant negative relationship between Δcortisol and the DS-Forward (β=-0.335, *p*= .03), and between Age and the DS-Forward (β=-0.383, *p*= .01). However, no associations were found between Δcortisol, BMI, the basal level of cortisol and HR, and the DS-Backward. Moreover, none of the associations between ΔHR and the DS-Forward and DS-Backward were significant (all *p's*> .21). Furthermore, we did not observe any significant relationships between BMI, the basal level of cortisol and HR, and the DS-Forward (all *p's*> .50).

Differences between increasers, decreasers, and no-changers on Cortisol, HR, and cognitive stress appraisal, and on the DS-Forward.

Thompson and colleagues (2015) recently posited that there are three main adaptive responses to stress: up regulation, down regulation, and no

change responses (floating near a homeostatic set point), and cognitive performance (in infants) has been shown to be positively related to an HPA pattern of "down-regulation" of responses in decreasers, but not in increasers or no-changers. Thus, to better understand the relationship between cortisol and WM observed in our study, the sample was split according to the self-regulation perspective (Thompson, Morgan, & Jurado, 2015) proposal. We divided males and females into increasers (males=20, females=15), decreasers (males=8, females=6), and no-changers (males=8, females=24). Based on Miller and colleagues (2013), responders had an increase of at least + 1.5 nmol/l in their salivary cortisol concentrations from the baseline levels (-15 min) to the fourth cortisol sample (+20 min); decreasers showed a decrease of more than - 1.5 nmol/l; and no-changers remained in a range between +1.5 and -1.5 nmol/l. Importantly, we considered the perspective formulated by Taylor and colleagues (2000), where females' neuroendocrine secretion in response to a stress task is associated with a "down-regulation" rather than the "up-regulation" found in males. This statistical approach might allow us to obtain more detailed and valuable results about the relationship between HPA self-regulation and cognitive performance in males and females than by merely splitting the sample into responders and non-responders.

ANCOVA for repeated measures were conducted, with Group (increasers vs. decreasers vs. no changers) and Sex (males vs. females) as between-subject factors, and Time (cortisol: - 15, + 5, + 10, + 20, + 45; HR: habituation, preparation, job interview, arithmetic task and recuperation; WM: pre and post) as a within-subject factor. Age, BMI and the basal levels

of cortisol and HR were used as covariate variables. Cortisol analysis showed a significant effect of Time (F(2.218, 153.030) = 4.953, *p*= .006) and a main effect of Sex (F(1,69) = 10.039, *p*= .002) and Group (F(1.69) = 10.220, *p*= .000). Moreover, we observed a marginally significant Time and Sex and Group interaction (F(2.218,153.030) = 2.770, *p*= .08) (see Figure 4.). No significant effects were found for Age, BMI, Sex or Group, or for Time and BMI, Time and Age, and Time and Sex (*p's*> .61). HR analysis showed no significant differences in Age, BMI, Sex, Group, Time and BMI, Time and Sex, Time and Group, or Time and Sex and Group (*p's*> .65). However, we observed a significant interaction between Time and Age (F(2.893,193.805) = 4.502, p= .000) and Sex and Group (F(1.67) =3.581, p= .03), where female increasers have higher HR than male increasers (p< .001)[5].

[5] One-way ANOVA did not show a significant difference in number between female increasers, decreasers, and no changers in relation to the menstrual cycle or oral contraceptive use (*p*=.33): increasers (follicular phase (N=4), the luteal phase (N=5), the menstrual phase (N=4), and oral contraceptive users (OC) (N=2)); decreasers (follicular phase (N=2), the luteal phase (N=2), the menstrual phase (N=1), and oral contraceptive users (OC) (N=1)); no changers (follicular phase (N=6), the luteal phase (N=5), the menstrual phase (N=5), and oral contraceptive users (OC) (N=8)).

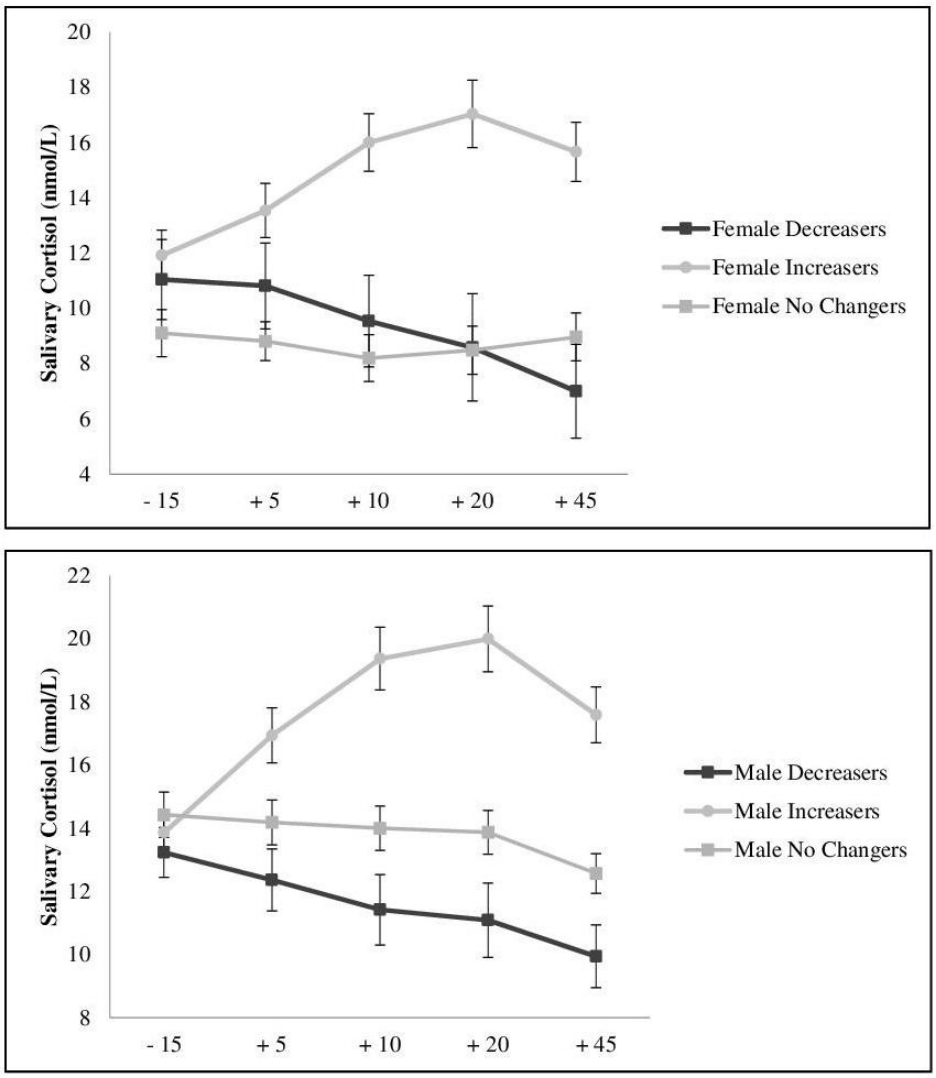

Figure 4. Salivary Cortisol concentrations during TSST for Female and Male Increasers, Decreasers and No Changers.

Moreover, MANCOVA were performed, with Group (increasers vs. decreasers vs. no changers) and Sex (males and Females) as between-subject factors and the cognitive stress appraisal factors as a within-subject factor. Age, BMI and the basal levels of cortisol and HR were used as covariate variables. MANCOVA did not show any significant differences in Age, BMI, Sex, Group or the Sex and Group interaction (p's> .94).

Moreover, ANCOVA for repeated measures were performed, with Group (increasers vs. decreasers vs. no-changers) and Sex (males vs. females) as between-subject factors and DS-Forward (DS-Forward Pre task vs. DS-Forward Post task) as a within-subject factor. We used age and BMI as covariate variables. A significant interaction was found between Time and Sex (p= .003), Time and Group (p= .01), and Time and Sex and Group ($F(1,73) = 4.191$, p= .01). Post hoc analysis of the Time and Sex interaction revealed that females (p< .001) performed better after the TSST, but males did not (all *p's*> .45). Post hoc analysis of the Time and Group interaction revealed that decreasers (p= .003) performed better than increasers and no changers after the TSST. Moreover, post hoc analysis of the Time and Sex and Group interaction reveals that only the females in the decreasers group performed better after the TSST (p< .001), whereas female and male increasers and no-changers and male decreasers did not change their performance after the stressful task (all *p's*> .25) (see figure 5).

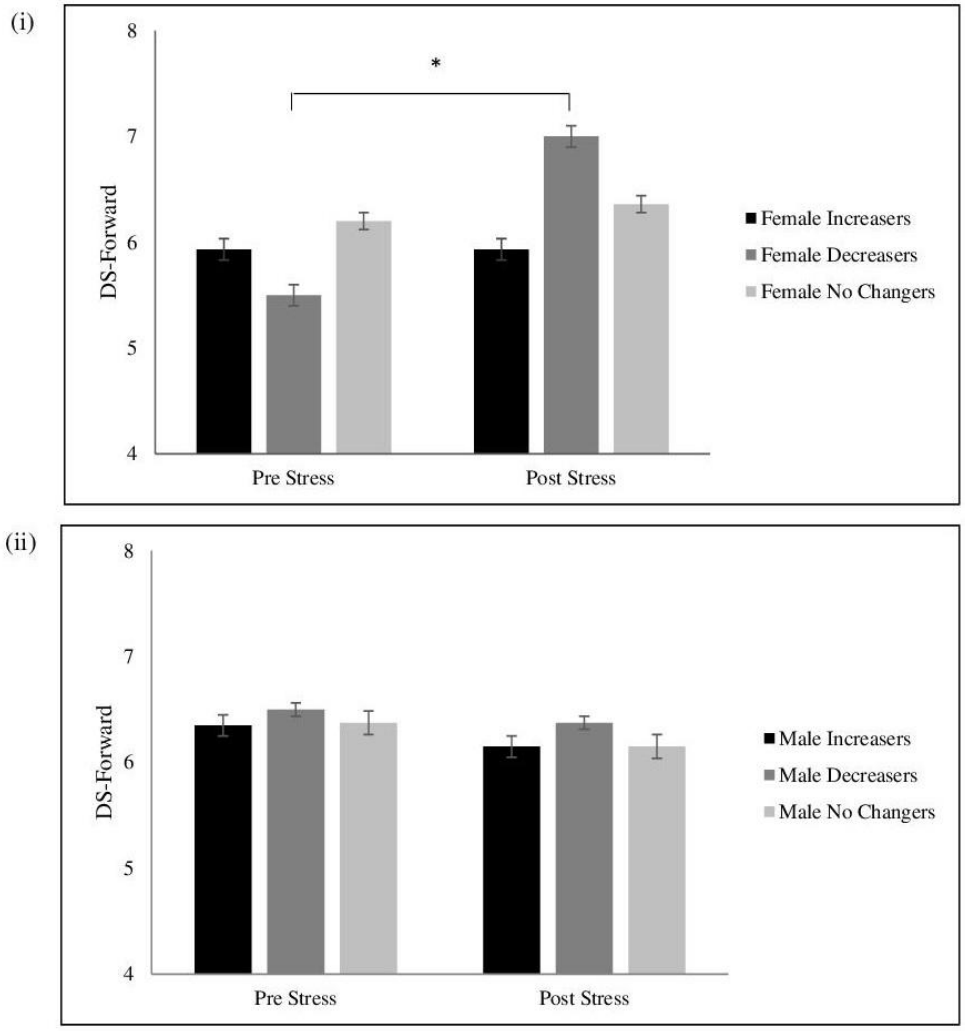

Figure 5. Performance on Digit Span Forward for Female (i) and Male (ii) Increasers, Decreasers and No Changers.

5.5. Discussion

The aim of the present study was to investigate sex-related differences in WM performance after the TSST. The effect of HPA-axis and SNS responses was examined, as well as the effect of cognitive stress appraisal on WM. We observed that females performed better on the DS-Forward after the stress task, whereas males' performance remained stable over time. Moreover, we divided males and females into cortisol increasers, decreasers,

and no changers, and we observed that females in the decreaser group enhanced their performance after the stress task. However, female and male increasers and no changers and male decreasers did not show any change in their performance over time.

The results of this study agree with the findings of Thompson and Trevathan (2008, 2009) and Thompson and colleagues (2015) showing that infants with a pattern of decreasing cortisol reactivity showed better cognitive performance after stress (separation from their mothers). The same association between a pattern of decreasing cortisol reactivity and better cognitive performance was found at different ages and with different experimental paradigms. Furthermore, when they assessed infants at 12 months, they found that female decreasers showed better cognitive performance than males (discriminating familiar auditory sequences better than non-familiar). Our results extend these previous findings and show that similar results can be observed in young adults.

Importantly, our results agree with the self-regulation perspective (Thompson et al., 2015), which proposes that there are three main adaptive responses to stress: cortisol can move upward from, fall down to, or float near a homeostatic set point. Based on this perspective, cognitive performance has been positively related to a pattern of "up- regulation", followed by "down-regulation" (Thompson et al., 2015). This response pattern was found in a study by Blair and colleagues (2005), who observed better cognitive performance when children showed moderate activation of the HPA system followed by a reduction while engaged in the assessment. Thus, in our study the cortisol may have been experiencing a downward trend during the

cognitive task, leading females in the decreaser group to perform better on attention and maintenance performance.

Moreover, as Thompson and colleagues (2015) state, decreasers, and in this case female decreasers, may operate with an optimal ratio of mineralocorticoid receptors/glucocorticoid receptors (MR/GR) (de Kloet, Oitzl, & Joëlin, 1999) in the hippocampus, leading to a positive effect on cognitive performance. Thus, this optimal operation of the MR/GR ratio might explain why our female decreasers showed better DS-Forward performance. Overall, considering that males had higher cortisol concentrations than females, male decreasers may not have shown an enhancement in attention and maintenance performance due to a lack of optimal MR/GR occupational levels in the hippocampus during the assessment. Moreover, it is also possible that the optimal MR/GR ratio and the low cortisol level would allow female decreasers to enhance DS-Forward performance due to a practice effect (Elzinga & Roelof, 2005), whereas an enhancement in WM is not observed in increasers and no changers due to a possible effect of cortisol and stress on WM in these two groups.

In females we also observed a negative relationship between Δcortisol and DS-Forward, but not in males, which might be due to a sex-related difference associated with their different patterns of response to stress. Indeed, it was recently proposed that the classic "fight or flight" response described by Cannon may not be the characteristic stress response in female, as it is in males. Taylor and colleagues (2000) suggested that females have evolved to a "tend and befriend" response to stress that is related to HPA down-regulation during stress. Thus, this characteristic female stress response

pattern might be what leads our female decreasers to perform better on attention and maintenance after the stress task. Therefore, a down-regulation (low levels of cortisol) might be beneficial for females' performance, and it might positively affect attention and maintenance in females, whereas an up-regulation would not. However, we did not observe any effect of HR on attention and maintenance, or any HR difference between increasers, decreasers, and no changers of either sex. Thus, future investigations are needed to verify the impact of other sympathetic indexes (i.e. *Alpha-amylase*) on both sexes.

Our results do not coincide with studies that have shown no effect of a stress task on WM in females (Hofmann et al., 2004; Smeets et al., 2006; Schoofs et al 2012), or with a study that found a decrease, although not statistically significant, in WM performance after a stress task in females (Schoofs et al., 2013). These different results might be due to the fact that these studies included fewer females than our study did (e.g., Hofmann et al., 2004: females = 15; Smeets et al., 2006: females = 30; Schoofs et al., 2013: females = 29). Thus, the lower number of females might not have provided a sufficient number of increasers, decreasers, and no changers to observe a difference between them. Moreover, Schoofs et al. (2013) was carried out in the morning, when the cortisol level was high. Thus, in comparison to our study, which was performed in the afternoon, the level of cortisol in the females might have been too high to have an optimal MR/GR ratio in the hippocampus and, consequently, observe better WM performance.

We observed that female cortisol decreasers improve their DS-Forward performance after the stress task, which could be due to a practice

effect in this group, but not in cortisol decreasers and no-changers. However, we did not observe any effects of stress on the DS-Backward in males or females. This result coincides with several studies that found no effects of acute stress on this component in either sex (Elzinga & Roelofs, 2005; Hoffman et al., 2004; Kuhlmann et al., 2005). Moreover, one possible explanation for the different DS-Backward and DS-Forward results in female decreasers may be related to some differences in the two tasks. The DS-Forward assesses the attention and maintenance component of WM, whereas on the DS-Backward, an executive component is added to the assessment of attention and maintenance. Thus, the differences might be due to the greater complexity of the DS-Backward compared to the DS-Forward task (Lezak et al., 2004) and, consequently, less possibility of showing a practice effect. Only one study found an impairing effect of stress on WM in females (Schoofs et al., 2013). However, this study was conducted in the morning, a time period related to high cortisol concentration and high inter-individual variance (Dickerson & Kemeny, 2004), when the cortisol concentration could affect WM performance more than in the afternoon (Maheu, Collicutt, Kornik, Moszkowski, & Lupien, 2005).

Finally, cognitive stress appraisal might play a more important role in the effect of stress on attention and maintenance performance than the cortisol release itself. In fact, in females, but not in males, we found that the cognitive threat appraisal was negatively related to attention and maintenance, whereas self-concept of one's own competence was positively related to it. Our results coincide with Ell and colleagues (2011), who found a negative association between cognitive threat appraisal and WM performance

in a sample composed mainly of young females (n=33, 31 females, Age mean= 22.70 years old). Moreover, several functional magnetic resonance imaging (fMRI) studies have shown a negative relationship between cognitive threat appraisal and WM performance, and a stronger activation of emotion-related brain areas (i.e. amygdala and the orbitofrontal cortex (OFC)) in females compared to males (in males brain regions were activated related to cognition and control, i.e., prefrontal and superior parietal regions, Koch et al., 2007). This study and others (Matud, 2004; Thayer, Rossy, RuizPadial, & Johnsen, 2003), suggest that cognitive threat appraisal might activate more emotion-related brain areas and have a negative relationship with WM performance in females, but not in males. By contrast, we did not find any association between cognitive stress appraisal and DS-Backward performance in males or females. Cognitive stress appraisal may not have a direct role in the executive component of WM, but it interacts with some physiological variables that were not taken into consideration in this study (i.e. alpha-amylase) in causing an effect. Moreover, in this study the cognitive stress appraisal may not have been strong enough to produce an effect on the DS-Backward. However, further investigations are needed to assess this relationship.

Furthermore, our results coincide with previous studies showing that coping strategies are positively related to WM (i.e. Putman & Roelofs, 2011; Snyder, Barry, & Valentino, 2014). Oxytocin has been related to a higher self-concept of one's own competences in both sexes (Cardoso, Linnen, Joober, & Ellenbogen, 2011). However, in females, their overall higher level of oxytocin might make them more sensitive to the association between their

self-concept of their own competences and WM performance. Further studies are needed to more fully understand the role of cognitive stress appraisal in attention and maintenance.

It is worth noting that we found that Δcortisol and cognitive stress appraisal have a significant impact on the DS-Forward after the stress task in females. However, other factors not controlled in this design may affect the enhancement of DS-Forward performance in females. In fact, although our experimental design includes Digit Span measures before and after the stress task, we did not include a non-stress control condition. Another limitation is that we used a committee with only one member, a female, during the TSST, which may have led to the low number of participants who showed cortisol release (35 out of 82) after the stress task. However, this low rate of cortisol responses allowed us to comparably split the male and female samples into increasers, decreasers and no changers, leading to interesting and valuable results. Moreover, although we made a great effort to control the number of females in each phase of the menstrual cycle, their number was still small when we split the sample into increasers, decreasers and no changers and performed the analyses. Future research should include a larger number of females per group and verify the existence of possible differences between the menstrual cycle phases and different self-regulatory HPA patterns.

In conclusion, the present study provides interesting results about sex differences in WM performance. In young females, a down-regulation of the HPA responses was related to better attention and maintenance performance. Moreover, both cortisol reactivity and cognitive threat appraisal were negatively related to DS-Forward performance in females, but not in males.

Moreover, also in females, Self-Concept of Own Competences was positively related to attention and maintenance performance. Together, our findings provide empirical support for the idea that sex plays an important role in WM performance after acute stress. Moreover, the different patterns of stress and emotion adaptation might explain the differences in WM performance between males and females after the stress task. Furthermore, cognitive stress appraisal was revealed to be an important factor to take into consideration when investigating the effects of stress on WM.

CHAPTER VI

GENERAL DISCUSSION

6. General discussion

Stress is a very common feeling that individuals experiment different times in their own life. Generally, stress triggers a series of psychophysiological responses to it. First, people appraise the stressor as challenge or threat for their own well-being (Blascovich & Mendes, 2000). Consequently, according to their appraisal process, organism reacts to stress mobilizing a wide range of physiological functions (i.e. ANS and HPA axis). These physiological responses enhance people chances to overcome the stressful situation and help the body to re-establish the internal homeostasis unbalanced from stress (Folkman, 2013).

Psychological and physiological responses to stress have been extensively studied (Chida, & Hamer, 2008), however, investigated further investigations are needed to clarify contradictory results. Throughout this thesis, new evidences have been provided about some important factors related to psychological and physiological responses to stress. Specifically, the antecedent role of being unemployed looking for a job and dispositional optimism on psychological and physiological responses to stress (i.e. cognitive appraisal of stress, heart rate variability and cortisol) have been tested. Moreover, the consequences of psychological and physiological responses to acute stress on working memory and behavioral displacement behavior have been also studied. In order to do that, we subjected people to an experimental session which included the TSST. Moreover, we used a multi-method study approach administering several self-report

questionnaires, measuring physiological variables along the experimental protocol, subjecting people to cognitive tests and performing ethological analysis of people behavior. Relevant results have been found.

Unemployment and Dispositional Optimism as individuals' antecedents of psychological and physiological responses to stress

In order to better understand the individual difference that might affect the psychological and physiological responses to stress, the first and second studies have investigated two antecedents of stress. The first individual factor influencing psychophysiological responses to stress studied along this thesis has been one of the most common situations among young adults around Europe: to be unemployed looking for a job (study 1). The study of unemployment is particularly important considering past empirical evidences that have pointed out as the stress to be unemployed is related to negative physical and mental health (Ali et al., 2016; Brown et al., 2003; Koziel, Lopuszańska, Szklarska, & Lipowicz, 2010; Gallo et al., 2004; Sullivan & von Wachter, 2009; Roelfs et al., 2011; Urbanos-Garrido & Lopez-Valcarcel, 2014; Wanberg, 2012), and the fear of future unemployment might be a source of stress itself having a detrimental to the well-being of young adults, particularly in countries with high unemployment rates (Knabe & Rätzel, 2011). Although several studies have focused their attention on the health effect of unemployment, no previous studies have taken into consideration the role of to be unemployed job seeker on psychophysiological responses to stress. Understanding about how it affects people stress responses might help to prevent negative health consequences and provide appropriate psychological support to those unemployed.

The first study has shown that unemployed job seekers have lower cardiac responses, along with a lower cognitive threat appraisal, compared to non-job seekers. Moreover, we observed a fully mediating role of cognitive threat appraisal on the relationship between being an unemployed job seeker and cardiac responses to stress. These results might have several explanations. First, according to some studies people subjected to prolonged stressful situation might see reduced the way they appraise the situation as threatening (Quigley et al., 2002; Tomaka et al., 1993), and consequently might lead to reduced physiological responses to that (Thayer et al., 2012). Particularly, it might be due to an Allostatic load response where initial psychophysiological hypoactivity responses might lead over time to a reduction in their responses. This reduced response might result in excessive activity of other allostatic systems such as the inflammatory cytokines (McEwen 1998). In this study the 71% of the active unemployed were looking for a job from ten months to one year. Although, it is not possible to affirm that they are into chronic stress, it might be possible that the unemployment condition affects people's cognitive appraisal of stress that, in turn, affects cardiac response. Another reason might be that unemployed job seekers might show a process of habituation. Indeed, according to previous studies, people exposed to a prolonged stressful situation might learn over time new coping strategies, thus perceive the stimuli as less threatening (Cacioppo et al., 1990; Mantler et al., 2005; Kesley et al., 1999; 2000; 2004; Schommer et al., 2003). In this line, unemployed looking for a job might simply have learned how to self-regulate their emotions during job interview and for this reason they evaluate it as less threatening than non-job seekers.

Consequently, this self-regulation influences physiological responses during job interview. Also, our results might also be affected by other variables not taken into consideration in this study (i.e. job search motivation). According to some studies, given that the chance to find a job in a region with high unemployment rate is quite low, people lose their motivation to make an effort to achieve it (McKee-Ryan et al., 2005; Latack et al., 1995) Thus, less motivation might imply less threat appraisal and consequently lower cardiac responses. Overall, this study highlights an important role of unemployment and looking for a job on psychophysiological responses to stress. However, further investigations using additional variables are needed to better understand this relationship.

A second individual factor influencing the psychological responses to stress studied along this thesis has been dispositional optimism (study 2). Particularly, the role of optimism on threat appraisal has been studied what has added new support to the few previous investigations on the topic. Indeed, to the best of our knowledge, just two studies have previously analyzed the relationship between dispositional optimism and threat appraisal. Moreover, these studies studied this relationship in a sample of older people (Endrighi et al., 2011; Puig-Pérez et al., 2017). The study presented in this thesis has shown that people who scored high in dispositional optimism perceived the stress task as less threatening. This result confirms the positive relationship between optimism and stress appraisal previously found (Endrighi et al., 2011; Puig-Pérez et al., 2017). Optimistic people feel to have the coping strategy to cope with stressors (Nes

& Segerstrom, 2006), and that might be the reason why optimistic people perceive less threatening the social evaluative test.

Displacement behaviors and working memory as consequences of psychological and physiological responses to stress, and the moderated role of HR

Throughout the thesis two different consequences of psychological and physiological responses to stress has been investigated: displacement behavior and working memory performance.

Displacement behaviors have been defined as stress related activities such as grooming behaviors and interactive movements (Troisi, 2002; McFarland, 1966). Although some study have investigated its relationship with psychological and physiological responses to stress (i.e. Mohiyeddini et al, 2013 a-b; Mohuyeddini & Sample, 2013; Pico-Alfonso et al., 2007; Troisi, 2002; Villada et al., 2014), no previous studies have studied the mediated role of threat appraisal of stress between dispositional optimism and displacement behaviors. Moreover, in the last years the interest on the personality trait and its relationship with psychophysiological effects of stress and displacement behavior is steadily growing. Indeed, it might furnish important information to practitioners such as psychologists, psychiatrists, recruiters, among others, about individuals' emotional state or stress management abilities. Particularly, the study has shown that threat appraisal fully mediates the relationship between dispositional optimism and displacement behavior. As previously discussed, the relationship between dispositional optimism and threat appraisal is in agreement with several studies which have indicated that

optimistic people perceive stressful situations as less threatening (Endrighi et al., 2011; Puig-Pérez et al., 2015). This result is in agreement with previous studies that have pointed out as other negative mental states such as anxiety or depression are related with higher level of displacement behavior during stress tasks (Sharve et al., 1988; Troisi et al., 1996; Villada et al., 2014). Furthermore, some studies observed that people with lower cardiovascular activation after stress were also the ones that exhibited more displacement behavior during stress. They concluded that displacement behaviors might have a re-balancing role when the internal homeostasis of the body has been compromised by stress (Troisi, 2002; Villada et al., 2014). Accordingly, this result shows the influence of dispositional optimism on threat perception of stress, that in turn affect the level of displacement behavior exhibited with the purpose of reestablishing internal balance impaired by stress.

Not only threat appraisal has an impact on displacement behavior. Indeed, our result has shown that HR has a moderating role between threat appraisal and displacement behavior, when threat appraisal is a mediated factor between dispositional optimism and displacement behavior. It was observed that when HR is low only people with high threat appraisal manifest displacement behaviors, while when HR is high both people with low or high threat appraisal display displacement behavior. This result confirms that, displacement behaviors are stress related, and indicated that it is needed medium/high level of psychological and/or physiological responses to stress to be exhibited. Indeed, only when threat appraisal is high (even with low HR) or HR is high (even with low threat appraisal) displacement behaviors are used. As discussed before, the reason of it might be that in both cases

when the psychological or physiological stress is high, displacement behaviors are needed to re-equilibrate the body's internal functioning.

Another consequent factor to physiological and physiological responses to stress studied in this thesis has been WM performance considering its two functions: attention and maintenance component of the WM and executive function (D'Esposito, 2007). In addition to it, we analyzed this relationship comparing males and females. During the years, working memory has been widely studied in young adult people (i.e. Cornelisse et al., 2011; Duncko et al., 2009; Gärtner et al., 2014; Luethi et al., 2009; Oei et al., 2006; Schoofs et al., 2009; Stauble et al., 2013; Terfhr et al., 2011), however results are very contradictory and no consensus on the bases of this relationship exists particularly regarding possible sex differences (Elzinga & Roelofs, 2005; Hoffman & al'Absi, 2004; Kuhlmann et al., 2005; Smeets et al., 2006; Stauble et al., 2013). Results have point out that females showed higher attention and maintenance performance after the TSST, whereas males did not. However, following the self-regulation perspective (Thompson et al., 2015) the sample was divided into increasers, decreasers and no changers and assessed WM performance in these three kinds of stress responses. According to previous studies with infants (Thompson et al., 2015; Thompson & Trevathan, 2008; 2009), we observed that a pattern of stress responses characterized by "up-regulation" followed by "down-regulation" was those which allowed the improvement of WM in women. On the contrary, both male and females increasers and no changers did not manifest any modification in their WM performance. In this line, it was also observed that negative relationship between cortisol and attention and

maintenance in females. Together, these results point out that females' "tend to be friend" pattern of stress response (Taylor et al., 2000), which is related to a HPA down-regulation during stress, permits them to perform better after stress.

Overall, the results shown along this thesis add new information about the role of some antecedents and consequences of psychological and physiological responses to stress. Particularly, we studied stress taking into consideration all the components involved in its responses: psychological, physiological and behavioral. Moreover, the last study sheds light on important sex related differences in stress responses. Indeed, it suggests the need to consider the three patterns of stress responses (increasers, decreasers and no-changers) when stress responses are studied, particularly in relation to sex.

6.2. Limitations and Strengths

In the previous chapters, the specific limitations of each one of the study have already been mentioned. Hence, this section presents the general comments to consider when interpreting the main findings of this thesis. Among them, the most important is the fact that young adult participants were selected though a strict selection criteria. Indeed, participants were cognitively and physically healthy, which makes difficult to generalize these results to people in different age ranges or health situation (i.e. pathological anxiety or depression) in the general population. Moreover, the strict selection criteria prevented to have a bigger sample size. Thus, larger samples are needed in future studies.

Another weakness could be that participants in these studies were medium/high educated people because they were university students. Thus, it might be of interest replicate the studies presented in this thesis using populations with different educational levels.

Along the thesis, we measured physiological indexes several times along the experimental protocol, and a pre-post stress test for cognitive performance. Nevertheless, only an experimental condition has been used. For this reason, future investigation using a control condition might be useful. It will allow having more specific information about the effect of stress on the variables studies in this type of sample.

Despite these limitations, this thesis also has a number of strengths that allow us to be confident about our results. The strongest point in all the studies is the rigor to the sampling selection process. Indeed, we applied numerous and very restrictive inclusion criteria. Thus, if from one side it prevented us to obtaining a larger sample size, it allowed us to create a very homogeneous sample. In this way, we have avoided the effect of several confounding factors, which was especially important when we used physiological variables. In the same way, we were very strict in the collection of the salivary cortisol sample. In order to avoid biased cortisol sample, we asked participant in all three studies to follow some recommendations (i.e. consumption of alcohol not allowed from the night before the session until the end of tests) prior to the experimental session. Before starting the session, participants were asked to confirm that they followed the recommendations.

In order to produce stress, we subjected people to one of the most real life stressful situation for young adults' individuals: a social evaluative stress situation. Moreover, we carried out physiological laboratory analysis in order to provide objective measures of stress, we administered self-reports and obtained data about psychological trait and state, we developed an instrument to the analysis and computation of non verbal behavior, and finally we subjected people to cognitive test. All of it allowed us to study a wide range of stress related components.

6.3. Future directions

The situation of being unemployed, as well as personality traits and sex, might differently affect stress responses over time. Thus, more knowledge might be attained from longitudinal studies on the psychophysiological antecedents and consequences of acute stress. Particularly, longitudinal studies could show more information about whether and how the effect of being unemployed on psychophysiological responses to stress changes over time, and if the role of dispositional optimism on cognitive appraisal, ANS and HPA axis and displacement behaviors vary during ages. Moreover, it might show if males and females from youth to more advanced ages are differently affected by psychophysiological responses to stress on WM.

Further investigations might also include other measures (i.e. electrophysiology) in addition to the neuroendocrine measures used in this thesis. In this way, studies might complement the assessment of the

neuroendocrine activity for the understanding of individuals' social stress responses with cortical activity.

Moreover, it might be important to take into consideration the role of other personality traits (i.e. neuroticism) on cognitive appraisal and on displacement behavior. In the same way, it might be interesting to investigate whether dispositional optimism and cognitive appraisal influence other stress related behaviors (i.e. submissive behavior). This approach would allow scholars to have an integrative and more comprehensive perspective of the stress response.

Finally, further investigations might take into consideration all these variables in order to study social stress in different natural settings, such as real job interviews, academic exams, or congress presentations.

CHAPTER VII

CONCLUSIONS

7. Main conclusions

The main conclusions of this doctoral dissertation are the following:

- Unemployment and looking for a job decrease threat appraisal, and consequently the cardiac responses to stress. It might be due to the effect of chronic stress or to a habituation process.

- Threat appraisal mediates the relationship between to be unemployed looking for a job and sympathetic and parasympathetic cardiac responses to stress.

- Dispositional optimism would promote a more positive experience of stress by decreasing the perception of threat.

- Threat appraisal mediates the relationship between dispositional optimism and displacement behaviors.

- The relationship between threat appraisal and displacement behavior is moderated by HR.

- Threat appraisal and HR responses strongly influence the use of displacement behaviors. Displacement behavior are displayed in both conditions: when HR is low and threat appraisal is high, and when HR is high and threat appraisal is high or low.

- Women decreasers (women that showed a decrease of more than – 1.5 nmol/l in their salivary cortisol concentrations after stress) perform better in attention and maintenance performance after stress.

- Females' responses to stress might be caratherized by a "Tend to be friend" pattern of physiological and behavioural responses. This pattern of responses is associated to a HPA down regulation during stress which it might be involved in the improvement of females' WM performance after stress.

CHAPTER VIII

GENERAL SUMMARY IN SPANISH

8. La respuesta de estrés

La respuesta de estrés está caracterizada por un conjunto de mecanismos psicológicos, biológicos y conductuales involucrados en un amplio rango de funciones adaptativas tanto en humanos como en otras especies animales (Fink, 2010). La respuesta al estrés desencadena una serie de cambios tanto a nivel fisiológico como psicológico y de comportamiento que permiten mantener la homeostasis interna (estado de equilibrio fisiológico de todos los organismos) (Canon, 1929), y favorecen la adaptación individual al estrés (Johnson et al., 1992). Hace medio siglo, Selye (1946) describió la compleja respuesta que se produce en un organismo cuando su homeostasis está amenazada. El autor acuñó el término "*síndrome de adaptación general*" (GAS) para describir la combinación de modificaciones adaptativas que se producen a nivel del sistema nervioso, cardiovascular, endocrino e inmunológico. El GAS está compuesto por tres etapas. La respuesta de estrés empieza con una "reacción de alarma" la cual implica la activación del sistema simpático-adrenomedular (SAM) y el sistema hipotálamo-hipofisario-adrenal (HHA). Durante esta fase, el cuerpo se prepara para las respuestas de lucha o huida. Sin embargo, el sistema inmunológico disminuye su eficacia haciendo a las personas más vulnerables a desarrollar enfermedades. Después de esta primera activación, comienza una "etapa de resistencia", también conocida como la etapa de adaptación. Durante esta fase, las respuestas adaptativas provocadas por las señales neuroendocrinas ayudan a reducir el estrés. Por lo tanto, el cuerpo se adapta al factor de estrés incluso si éste permanece en el tiempo. Finalmente, se

experimenta una "etapa de agotamiento" caracterizada por una disminución gradual de la resistencia al estrés. Al comienzo de esta etapa el cuerpo está terminado su reserva de energía, el sistema inmune deja de trabajar de manera eficiente y se experimenta agotamiento. Sin embargo, una vez pasado el estrés el cuerpo se recupera con el tiempo por completo. Por el contrario, un estrés prolongado en el tiempo o demasiado fuerte puede comprometer el retorno a la homeostasis causa trastornos permanentes en el cuerpo o la mente de los individuos (McEwen, 1998). El desequilibrio de la homeostasis interna ha sido definido por McEwen (1998) como "carga alostática". El autor describe tres tipos de carga alostática: la primera está caracterizada por una activación continua o frecuente de los sistemas implicados en el mantenimiento de la homeostasis interna (o alostasis como fue definido por McEwen, 1998). La segunda está representada por la incapacidad de esos sistemas para desconectarse después del estrés. La tercera está definida por una respuesta inadecuada de los sistemas implicados en el mantenimiento de la alostasis. La carga alostática bloquea la respuesta natural dinámica al estrés agudo, lo cual conduce a una inadecuada reactividad y recuperación de los sistemas fisiológicos implicados en la respuesta al estrés. Todos estos tipos de carga alostática han sido indicados como posible causa de enfermedades físicas y mentales, como la depresión, la ansiedad o el deterioro cognitivo, por mencionar algunas (McEwen, 1998). Sin embargo, aunque el estrés crónico es deletéreo para la salud humana, el estrés a corto plazo es beneficioso y permite a las personas adaptarse en respuesta a las demandas ambientales y cambios que la persona enfrenta cada día (Salvador, 2005). En particular, la respuesta al estrés en personas jóvenes adultas debe ser

considerada como un proceso natural de adaptación y útil para hacer frente a los retos diarios. En personas sanas, las respuestas psicofisiológicas al estrés proporcionan todas las reacciones necesarias para mantener la homeostasis interna (Folkman, 2013).

Los mecanismos involucrados en la respuesta al estrés

Componentes fisiológicos

Los principales sistemas implicados en las respuestas de estrés son el sistema nervioso autónomo (SNA) y el eje HHA. Su alteración como respuesta al estrés implica una modificación de las respuestas cognitivas, emocionales y conductuales (Blascovich y Tomaka, 1996). A pesar de que las respuestas al estrés incluyen otras sustancias, como por ejemplo alfa-amilasa (Nater y Rohleder, 2009), en esta tesis hemos focalizado nuestra atención en las respuestas del eje HHA, y del SNA utilizando parámetros cardiacos que miden sus funciones bajo estrés.

El sistema nervioso autónomo (SNA)

El SNA es el primero y uno de los más importantes sistemas activados en las respuestas al estrés. Cuando alguna persona se enfrenta a una situación de desafío, la información llega a la amígdala, un área cerebral que participa en el procesamiento emocional. Si la situación es percibida como peligrosa, se envían señales de socorro al hipotálamo.

El hipotálamo considerado como un centro de mando efector, se comunica con el resto del cuerpo a través del SNA que, a su vez, controla funciones involuntarias tales como la respiración, la presión arterial y el ritmo cardíaco. El SNA tiene dos componentes: el sistema nervioso simpático (SNS), que prepara el cuerpo para la acción de "lucha o huida" y el sistema

nervioso parasimpático (SNP), cuya función principal es activar la respuesta de "descansar y digerir" y devolver el cuerpo a la homeostasis después de la reacción de lucha o huida (McCorry, 2007).

Tras la señal de la amígdala, el hipotálamo activa el sistema nervioso simpático a través de las glándulas suprarrenales. Estas glándulas responden mediante la liberación de la hormona epinefrina (también conocida como adrenalina) en el torrente sanguíneo. La epinefrina produce una serie de cambios fisiológicos (como el corazón late más rápido de lo normal, la frecuencia del pulso y la presión arterial sube, la persona comienza a respirar más rápidamente). Todos estos cambios ocurren rápidamente. De hecho, la amígdala y el hipotálamo inician la respuesta de estrés incluso antes de que el cerebro haya tenido la oportunidad de procesar completamente lo que está sucediendo (McCorry, 2007).

Los indicadores más comunes que se utilizan para evaluar la labor del SNA y la relación entre el SNS y SNP son la frecuencia cardíaca (FC) y la variabilidad de la frecuencia cardíaca (VFC) (Task Force, 1996). La FC se mide en latidos por minuto. En condiciones normales el incremento de la FC indica un predominio del SNS. La VFC mide los cambios de la frecuencia cardiaca (o variabilidad) entre latidos cardíacos sucesivos y muestra la continua interacción entre el SNS y SNP. La variabilidad entre latidos se mide en milisegundos (ms) y es definido como "intervalo RR". Diferentes factores fisiológicos pueden influir en la VFC como el género, la edad, el ritmo circadiano, o la respiración, entre otros. Los índices de la VFC más comunes se calculan a partir de la media y la desviación estándar del intervalo RR (es decir, rMSSD, SDNN) (Sztajzel, 2004).

A diferencia de la FC y la VFC que son respuestas rápidas que alcanzan los niveles más altos casi de inmediato, las concentraciones de cortisol alcanzan su pico alrededor de 15-30 minutos después de que los estímulos estresante se hayan presentado (Engert et al., 2011). De la misma manera, mientras la FC y la VFC vuelven a sus niveles normales algunos minutos después de que el estrés haya finalizado, el cortisol necesita casi 60 minutos para volver a las concentraciones originales (Ulrich et al., 2004). El estrés crónico y los niveles elevados de cortisol pueden tener efectos negativos sobre la salud (McEwen, 1998). Particularmente, se ha observado que los altos niveles de cortisol interfieren con algunas funciones cognitivas como el aprendizaje y la memoria (de Quervain et al., 2017), y llevan a una reducción de la función inmune, etc. (Lundberg, 2005). Asimismo, los niveles elevados de cortisol aumentan el riesgo de depresión, de enfermedad mental, y se relacionan con una menor esperanza de vida (Staufenbiel et al., 2013).

Componentes psicológicos

Varias teorías han indicado que la manera en la que las personas perciben un evento estresante afecta a las respuestas fisiológicas e influye sobre su comportamiento. Como afirmó Cannon (1932), las emociones negativas son factores clave en el desencadenamiento de respuestas fisiológicas para hacer frente a los retos medioambientales. Particularmente, la forma de evaluar la situación genera una serie de cambios fisiológicos y de comportamiento útiles para lidiar con el estrés (Carver y Connor-Smith, 2010). Por lo tanto, la primera respuesta de estrés es la valoración de éste (Carver y Connor-Smith, 2010; Dickerson y Kemeny, 2004; Schlotz et al.,

2011). Importantes situaciones de estrés (como por ejemplo, estar desempleado o enfermedades crónicas) pueden influir en la forma de valorar la situación y hacerle frente.

La evaluación cognitiva del estrés

En el modelo transaccional de estrés y afrontamiento de Richard Lazarus (1966) se define estrés como una relación entre la persona y el medio ambiente que se aprecia como personalmente significativa. Las personas perciben estrés cuando la situación externa excede sus capacidades de hacerle frente. La teoría hace hincapié en la importancia de dos procesos, la evaluación de los estímulos estresantes (evaluación primaria) y la percepción de la capacidad de hacer frente a ellos (valoración secundaria) como mediadores de la relación existente entre la persona y el medio ambiente.

Durante la evaluación primaria las personas se preguntan ¿me siento seguro? mientras que durante la evaluación secundaria los individuos se preguntan ¿Puedo hacer frente a eso? Por lo tanto, de acuerdo con la valoración primaria las personas evalúan el estimulo como una amenaza o reto para su propio bienestar. La valoración de amenaza se refiere a la evaluación de la situación como potencialmente perjudicial o como una fuente de fracaso. Por el contrario, la evaluación de reto se refiere a la evaluación de la situación como una oportunidad para el crecimiento personal y beneficioso para el bienestar de la persona (Folkman, 1993). La capacidad de afrontamiento se refiere a los pensamientos y acciones que las personas usan para manejar la situación estresante. Por lo tanto, la evaluación del estrés está influenciada por las capacidades de afrontamiento de los individuos tales como recursos psicológicos, sociales, ambientales y

materiales (Lundberg, 2005). Las situaciones que se valoran como altas en relevancia personal y bajas en capacidad de control se perciben como amenazantes; situaciones que se valoran como altas en relevancia personal y altas en capacidad de control se perciben como un reto (Folkman, 1993). Por lo tanto, cuando las personas se enfrentan a una situación desafiante, la valoración cognitiva del estrés se produce desde la diferencia entre la valoración primaria y secundaria (Drach-Zahavy y Erez, 2002). Además de eso, algunas situaciones crónicas importantes o factores tales como algunos rasgos de personalidad pueden influir en la forma de evaluar la situación (David & Suls, 1999; Kirschbaum, et al., 1992; Miller et al, 2007; Ockenfels, et al, 1995).

En esta línea, el modelo biopsicosocial (BPS) de reto y amenaza (Blascovich y Tomaka, 1996) es una de las teorías más completas que pone de relieve cómo la evaluación de la situación da forma a las respuesta al estrés. Más específicamente, dependiendo de si el estrés se evalúa como un reto o amenaza, diferentes respuestas fisiológicas al estrés se desencadenan (Blascovich et al., 1999). Aunque ambas evaluaciones han sido asociadas con respuestas fisiológicas similares, la percepción de la situación como un reto ha mostrado una reactividad autonómica más eficiente y un mejor rendimiento. Por el contrario, la percepción de la situación como amenaza ha sido asociada a una reactividad autonómica ineficiente y un mal desempeño (Blascovich y Tomaka, 1996).

Componente conductual

El comportamiento es considerado como el último resultado secuencial de la respuesta al estrés. Sin embargo, sólo recientemente se ha

prestado más atención hacia este importante factor relacionado con el estrés. Durante varios años, la respuesta al estrés humano se ha considerado, tanto a nivel fisiológico como de comportamiento, caracterizado por la respuesta de "lucha o huida" (Cannon, 1932). Sin embargo, aunque la lucha o huida se ha considerado la respuestas conductuales principales tanto para hombres como para mujeres, recientemente se ha propuesto que las mujeres se diferencian en su respuesta de estrés de los hombres. La respuesta de las mujeres ha sido relacionada con un patrón definido como "cuidar y hacer amistad".

La respuesta de lucha o huida es la respuesta humana más común al estrés. Este patrón de respuesta fue descrito por primera vez por Cannon en 1932 y está caracterizado por la activación del SNS que inerva la médula adrenal, produciendo una cascada hormonal que resulta en la secreción de catecolaminas, principalmente norepinefrina y epinefrina en el torrente sanguíneo. Adicionalmente a sus concomitantes fisiológicos, el binomio lucha o huida, se ha utilizado para describir el comportamiento humano en las respuestas al estrés. Específicamente, el hecho de que un humano (o animal) luche o huya en respuesta a la activación simpática depende de la naturaleza del factor estresante y su valoración. Si los individuos consideran que tienen posibilidades reales de superar el desafío, entonces es probable que respondan con la lucha. En circunstancias en que la amenaza es mayor que sus habilidades de afrontamiento, la huida es más probable.

La respuesta "cuidar y hacer amistad", descrita por Taylor y sus colegas (2000), afirma que para afrentar situaciones de estrés las mujeres son más propensas a usar un comportamiento social de afiliación. De hecho, biológicamente tienden a la tranquilidad y el cuidado de los hijos. Este patrón

de respuesta puede ser eficaz para tratar una amplia gama de amenazas. Por otra parte, de acuerdo con este modelo de respuesta al estrés, las mujeres son más propensas a hacerse amigas de los enemigos (causantes de estrés), o a buscar apoyo social de los miembros de la familia o amigos. Fisiológicamente, contrariamente a la liberación de grandes cantidades de norepinefrina y cortisol en el torrente sanguíneo como en el caso de los hombres, las mujeres responden al estrés mediante la secreción de endorfinas (unas hormonas peptídicas que inhibe la transmisión de señales de dolor y produce una sensación de euforia durante las interacciones sociales) y la oxitocina (una neuro-hormona que regula la interacción social, particularmente la relación materno-infantil y la reproducción sexual).

Durante los últimos años, varios comportamientos has sido estudiados en relación con el estrés (comportamiento afiliativo, sumisivo, asertivo o de desplazamiento, entre otros). Cada uno de ellos ha sido relacionado con diferentes estados emocionales y actitudes sociales (Mohiyeddini et al., 2013a-b, Mohiyeddini & Sample, 2013; Sgoifo et al, 2003, Villada et al., 2014). Además de esto, varios sistemas han sido desarrollados con el fin de detectar y evaluar estos tipos de comportamientos (Mohiyeddini et al., 3013; Troisi, 2000). En particular, se ha encontrado una fuerte relación entre respuesta fisiológica (HR y cortisol) y respuesta emocional (por ejemplo, rasgo y estado de ansiedad) con los comportamientos de desplazamiento. Los comportamientos de desplazamiento se definen como un conjunto de movimientos directos hacia el cuerpo, como frotarse las manos, tocarse la cara con las manos, o rascarse (Mohiyeddini et al, 2013; Pico-Alfonso et al, 2007; Sgoifo et al, 2003, Villada et al., 2014). Sin embargo, poco se sabe

acerca de cómo influyen los diferentes rasgos de personalidad y la evaluación del estrés sobre este tipo de comportamiento.

Objetivo de la tesis

El objetivo general de este trabajo fue evaluar algunos posibles antecedentes y consecuentes de las respuestas psicológicas y fisiológicas al estrés en personas adultas jóvenes sanas. Con ese fin, llevamos a cabo un estudio semi-experimental, en el que sometimos a los individuos a un estresor de laboratorio estandarizado. Además, se utilizó un enfoque integrador: se realizaron análisis fisiológico, se administraron cuestionarios, se llevó a cabo una observación etológica y computación del comportamiento no verbal, y se sometió a los participantes a una prueba cognitiva. En los párrafos que siguen se describe el objetivo principal de cada uno de los estudios.

Estudio 1.

Hoy en día, la alta tasa de desempleo presente en diferentes países de Europa hace que sea más difícil para las personas encontrar un trabajo en un corto período de tiempo. Por esta razón, las personas pueden pasar mucho tiempo (meses, años) en busca de trabajo. Esto implica estar sometido a diferentes procesos de selección y entrevistas de trabajo, es decir, a un estresor agudo de evaluación social. El desempleo, junto con la búsqueda activa de empleo es una de las mayores causas de estrés, especialmente para muchos adultos jóvenes (Knabe & Rätzel, 2011). Se ha demostrado que estar desempleado en busca activa de un empleo tiene consecuencias negativas para la salud psicológica y física (Ali et al., 2016; Urbanos-Garrido y López-Valcárcel, 2014). Aunque varios estudios han sido llevados a cabo con el fin de entender el papel de desempleo sobre la salud (Roelfs et al., 2011;

Wamberg, 2012), se ha prestado poca atención a los mecanismos psicofisiológicos que están en la base de esta relación. Con el fin de explorar esta laguna de investigación, el objetivo de este estudio fue investigar el efecto de estar desempleado (en búsqueda de trabajo) sobre la evaluación cognitiva y las respuestas fisiológicas al estrés agudo, particularmente a una entrevista de trabajo.

Estudio 2.

Hoy en día el estrés está presente en la vida de las personas, especialmente en los jóvenes adultos sometidos constantemente a situaciones sociales evaluativas debido a contextos altamente competitivos tanto a nivel académico como laboral. El evaluación de amenaza es una fundamental y primera reacción que se activa cuando las personas se enfrentan a una situación estresante (Folkman, 2013). La forma en que las personas evalúan el estrés está influenciada por los rasgos de la personalidad (Penley y Tomaka, 2002). Del mismo modo, la evaluación del estrés influye sobre el comportamientos de las personas (Troisi, 2002). El objetivo principal de este estudio fue investigar el papel de un rasgo de la personalidad positiva (optimismo) en la evaluación de la amenaza de estrés. Además de ello, se evaluó el papel mediador de la evaluación de amenaza entre el optimismo y un comportamiento relacionado con el estrés (actividades de desplazamiento). Asimismo, se evaluó el rol moderador de la FC y el cortisol en la relación entre la evaluación de amenazas y las conductas de desplazamiento.

Estudio 3.

Se ha observado que la respuesta al estrés tiene un papel importante en el rendimiento cognitivo (Wolf et al., 2016). Por otra parte, se ha considerado el sexo como un importante factor moderador en la relación entre el estrés y el rendimiento cognitivo, especialmente en la memoria de trabajo (Almela et al., 2011). Sin embargo, los resultados acerca de las diferencias de sexo en la memoria de trabajo son fuertemente contradictorias, y el impacto de la evaluación cognitiva del estrés sobre la memoria de trabajo no ha recibido mucha atención. El objetivo de este estudio fue investigar el rol de las respuestas fisiológicas (FC y cortisol) y la evaluación cognitiva del estrés en rendimiento de la memoria de trabajo en hombres y mujeres.

Metodología

Participantes y muestreo

La muestra utilizada en los tres artículos se recogió durante el curso académico 2012/2013. Los individuos fueron reclutados de una amplia gama de clases en la Universidad de la Universidad de Valencia, en el Servicio Valenciano de Ocupación y Formación (SERVEF), y del Observatorio de Inserción Profesional y Asesoramiento Laboral (OPAL). El proceso de reclutamiento se compuso de dos fases:

Primera fase. Los potenciales participantes fueron evaluados (edad, nivel de educación, etc.) por un psicólogo experto a través de una entrevista telefónica.

Segunda fase. A los sujetos que pasaron la primera fase de reclutamiento se les pidió que rellenarán un cuestionario general con los criterios de inclusión más específicos y enviarlo de vuelta por correo

electrónico (para mayor información sobre los criterios de inclusión ver la sección "Participantes" en los tres estudios). En total, se evaluaron 162 individuos.

A los sujetos que cumplían todos los criterios se les pidió asistir a una sesión experimental individual que tuvo lugar en un laboratorio de la Facultad de Psicología.

La muestra final de los tres estudios se compone de 82 jóvenes adultos sanos (37 hombres y 45 mujeres) de entre 20 y 39 años de edad (media ± SEM: Edad = 24.98± 0.55). Los participantes eran estudiantes que estaban cursando el último año de licenciatura (32,1%), graduados (58%), y postgraduados (9,9%) provenientes de una amplia gama de especialidades en la Universidad de Valencia. El 6,9% de ellos consideraba su situación económica media/alta. Todos los participantes eran voluntarios, sin embargo, se les ofreció recibir una retroalimentación por parte de un entrevistador experto sobre cómo mejorar su rendimiento en una entrevista de trabajo. Se le proporcionaba la retroalimentación al final de la sesión experimental. Más información acerca de las características específicas de la muestra se ha proporcionado en cada una de las secciones "participantes" incluidas en los artículos que componen esta tesis.

Diseño experimental

Las sesiones experimentales se llevaron a cabo durante el curso académico 2012/2013. Tenían una duración de aproximadamente 90 minutos, y tuvieron lugar entre las 16:00 y las 19.00 horas de lunes a viernes evitando festividades civiles o religiosas.

La sesión experimental se componía por diferentes fases secuenciales (ver Figura 2.):

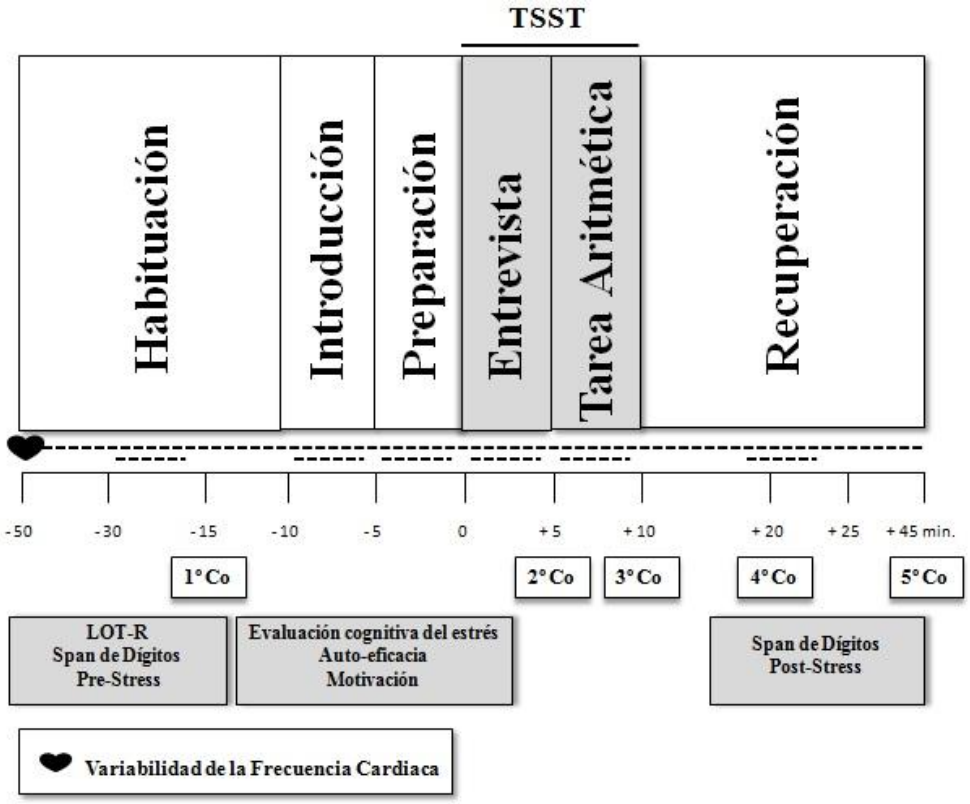

Figura 2. Protocolo general

Habituación

La sesión comenzaba con una fase de habituación que duraba 40 minutos. Durante este tiempo, los participantes tenían que empezar a rellenar un cuestionario general que incluía una batería de test y cuestionarios para medir rasgos de personalidad, estados psicológicos y rendimiento cognitivo. En particular, los cuestionarios que los participantes tenían que rellenar durante la fase de habituación fueron: the *Life Orientation Test* (LOT-R, Scheier et al., 1994) y el *Trait Anxiety Inventory* (STAI-T) (Spielberg et al., 1970). Por otra parte, se midió el rendimiento cognitivo pre-estrés a través de un test de Span de Dígitos con números. Paralelamente, se medían las variables fisiológicas: la VFC se registró continuamente durante todo el

procedimiento experimental, mientras que cinco tomas de cortisol en saliva se recogieron a lo largo de la sesión. La primera muestra de cortisol fue tomada durante la fase de habituación (-15 min.).

Introducción

Durante esta fase, el entrevistador introducía a los participantes las tareas estresantes del *Trier Social Stress Test* (TSST): una entrevista de trabajo y una tarea aritmética. Inmediatamente después de que la entrevista de trabajo había sido introducida, los participantes tenían que rellenar la escala *Primary Appraisal Secondary Appraisal* (PASA, GAAB, Rohleder et al., 2005) y una escala general de motivación y auto-eficacia que seguían las recomendaciones de Bandura (1997) (Costa et al., 2016; van der Meij et al, 2010).

Preparación y TSST

Para producir estrés los participantes fueron sometidos a una versión modificada de la prueba de *Trier Social Stress Test* (TSST, Kirschbaum et al., 1993). Las modificaciones respecto a la versión original fueron: (i) todas las fases se llevaron a cabo en la misma habitación, mientras que en el protocolo original se utilizaron dos habitaciones separadas (una habitación para la fase de introducción y TSST, y otra habitación para el resto de las fases); (ii) el comité fue compuesto por una sola persona (mujer) que se introducía como un experto en recursos humanos, mientras que en el protocolo original el comité estaba compuesto por dos personas (un hombre y una mujer).

El TSST consta de cinco minutos de presentación oral (entrevista de trabajo), seguido por 5 minutos de tarea aritmética. Durante el TSST los

participantes estaban invitados a permanecer de pie a una distancia de 1,5 metros del entrevistador. Una videocámara, un micrófono y un monitor donde los sujetos podían ver sus actuaciones eran claramente visibles para el sujeto. Tanto el discurso y como la tareas aritméticas fueron grabadas en vídeo.

Antes de de empezar la entrevista de trabajo se realizaba una fase de preparación. Durante esta fase, se pedía a los participantes que escribieran sus principales ideas sobre lo que les hubiese gustado decir durante la entrevista de trabajo. Sin embargo, no podían utilizar estas notas durante la tarea. La fase de preparación duraba 5 minutos.

Entrevista de trabajo

El objetivo principal del participante era convencer al entrevistador experto que él/ella era el mejor candidato para su "trabajo soñado". El experto introducía la entrevista diciendo que el curriculum profesional de la persona había sido evaluado positivamente, pero que sin embargo, para una evaluación final se necesitaban conocer las características y competencias personales que el entrevistado tenía para desempeñar esa posición. La tarea duraba 5 minutos. Al entrevistador se le permitía hacer preguntas sólo si los sujetos eran incapaces de hablar durante toda la duración de la fase. Al finalizar la tarea, se tomaba la segunda muestra de cortisol (+5 min.).

Tarea aritmética

Los sujetos tenían que realizar un cálculo aritmético durante 5 minutos frente al evaluador. Al final de la tarea aritmética, se tomaba la tercera muestra de cortisol (+10 min.).

Recuperación

La fase de recuperación tenía una duración 35 minutos. El objetivo principal de esta fase era medir variables fisiológicas y cognitivas post-estrés. Por lo tanto, a lo largo de esta fase se tomó la cuarta medida de cortisol (+20 min.). Por otra parte, el rendimiento cognitivo post-estrés se evalúo con el test Span de dígitos con números. Al final de esta fase, la quinta muestra de cortisol fue tomada (+45 min.), y el peso y la altura fueron medidos con el fin de calcular el índice de masa corporal de los participantes (IMC).

Medidas

A continuación, se describen la batería de cuestionarios, tests cognitivos y variables de comportamiento utilizados a lo largo de los tres artículos presentados en la presente tesis.

Variables fisiológicas

Frecuencia cardíaca y Variabilidad de la frecuencia cardíaco

Los datos de la frecuencia cardíaca (FC) y la variabilidad de la frecuencia cardiaca (VFC) se registraron continuamente durante toda la sesión utilizando a través de un reloj Polar RS800CX © (Polar CIC, EE.UU.), que consiste en un cinturón de pecho para la detección y la transmisión de los latidos del corazón, y un reloj para la recogida y almacenamiento de los datos. El cinturón de pecho se coloca en el plexo solar y transmite información sobre la FC y VFC al receptor (reloj Polar). Los datos recogidos por el reloj Polar fueron descargados y almacenados en el programa *Polar ProTrainer5TM* y analizados a través del programa informático Kubios (Biomedical Signal Analysis Group, Universidad de Kuopio, Finlandia).

Los parámetros fisiológicos que se utilizaron a lo largo de la tesis fueron FC y VFC. En particular, en el primer artículo se utilizaron como parámetros el promedio de la FC medida en latidos por minuto (lpm) como un indicador de la activación simpática, y el parámetro rMSSD en milisegundos como indicador de la activación del sistema parasimpático. En los artículos segundo y tercero se utilizó el promedio de la FC (bpm).

Respuesta neuroendocrina

La actividad del eje hipotálamo-pituitario-adrenal (HHA) fue medida a través del análisis de cinco muestras de cortisol en saliva tomadas a través de la sesión. Los participantes proporcionaban las muestras de saliva usando Salivettes (Sarstedt, Nümbrecht, Alemania). Se les instó a los participantes a mantener el algodón en la boca durante exactamente 2 minutos, sin masticarlo. Las muestras se centrifugaron a 3000 rpm durante 5 min. Los análisis se realizaron en la Unidad Central de Investigación (Unidad Central de Investigación) de la Facultad de Medicina en la Universidad de Valencia (España).

Las medidas de cortisol fueron usadas para investigar las hipótesis del segundo y tercer articulo. Por el contrario, no se usaron muestras de cortisol en el primer artículo.

Variables psicológicas

Ansiedad rasgo

La ansiedad rasgo ha sido evaluada a través de la versión española (Seisdedos, 1988) del *Trait Anxiety Inventory* (STAI-T, Spielberger et al, 1970). El inventario se compone por 20 ítems (por ejemplo, “me siento

relajado", "me siento molesto") con una escala Likert de 4 puntos que va desde 0 (nada) a 3 (extremadamente).

Auto-eficacia

La auto-eficacia ha sido evaluada a través de dos preguntas basadas en las recomendaciones de Bandura (1997): (i) ¿Seria usted capaz de realizar con éxito esta tarea? (Ii) ¿Cuánta confianza tiene usted en que va a realizar con éxito esta tarea? La respuestas venían puntuadas con una estaca desde de 1 (nada) a 100 (mucho). El promedio de estas dos preguntas permitió obtener la puntuación de auto-eficacia (Costa et al, 2016; Van der Meij et al., 2010).

Motivación

La motivación ha sido evaluada mediante la pregunta (también esta vez con una escala desde 1 a 100): ¿Qué importancia tiene para usted llevar a cabo con éxito esta tarea?

La ansiedad rasgo, la auto-eficacia y motivación se utilizaron en los análisis preliminares del primer artículo para verificar sus posibles efectos en las variables de estudio.

Evaluación cognitiva del estrés

La valoración cognitiva del estrés ha sido evaluada con las escala PASA (Gaab et al., 2005). Esta escala se utilizó para medir la evaluación cognitiva de las pruebas del TSST. La escala PASA se compone de dos subescalas: "valoración primaria", que incluye dos subescalas específicas "Evaluación de Amenaza" ("no me siento amenazado por la situación") y 'Evaluación de Reto' ("la situación no es un reto para mí"), y la subescala de "valoración secundaria", que incluye dos subescalas específicas: "uto-concepto de la propia competencia" ("sé lo que puedo hacer en esta

situación") y "Expectativa de control" ("depende de mí si los jueces me evaluarán positivamente"). Cada subescala tiene cuatro ítems a los que se contesta con una escala tipo Likert de 6 puntos que va desde "muy en desacuerdo" a "totalmente de acuerdo".

La subescala de "Evaluación de Amenaza" ha sido usada con el fin de responder a las hipótesis específicas del estudio primero y segundo, mientras que en el tercer estudio se evaluaron las cuatro subescalas específicas que componen la valoración cognitiva del estrés (Lazarus y Folkman, 1984). Además de esto, la "evaluación terciaria" (también llamado el "índice global de evaluación cognitiva de estrés") ha sido calculada utilizando la fórmula propuesta por Gaab y colegas (2005).

Optimismo (LOT-R; Scheier et al., 1994; Otero et al., 1998)

LOT-R está compuesta por 10 ítems y dos subescalas: optimismo y pesimismo. Tres ítems miden el optimismo (p.e. "En tiempos de incertidumbre, normalmente espero lo mejor"), y otros tres ítems miden el pesimismo (p.e. "Si algo puede salir mal para mí, saldrá"); Los ítems restantes son distractores. Los ítems se contestan a través de una escala tipo Likert de 5 puntos, desde 0 (muy en desacuerdo) a 4 (muy de acuerdo).

El optimismo se utilizó para verificar la hipótesis del segundo artículo. Los ítems de pesimismo se invirtieron para obtener una medida unidimensional del optimismo.

Desempeño cognitivo

Memoria de trabajo: Span de dígitos

El Span de dígitos es un subtest del *Wechsler Memory Scale III* (Wechsler, 1997). La administración de este test ha sido realizado antes (fase

de habituación) y después (fase de recuperación) del TSST. El Span de dígitos ha sido usado en el tercer estudio con el fin de evaluar sus hipótesis específicas.

Comportamiento no verbal

Comportamiento de desplazamiento

Los comportamientos de desplazamiento han sido analizados usando una versión modificada del Ethological Coding System for Interviews (ECSI) (Troisi, 2002). Se analizaron dos subcategorías principales de comportamiento: 1. Los comportamientos de desplazamiento definidos por los movimientos de las manos; 2. Comportamientos de desplazamiento definidos por los movimientos corporales. Cada comportamiento de desplazamiento se ha evaluado como presente = 1 o no presente = 0, con una frecuencia de 20 segundos. Cada video tenía una duración de 5 minutos (entrevista de trabajo). La puntuación total indicaba la frecuencia de los comportamientos de desplazamiento en cada categoría. Con el fin de tener una variable única de comportamiento de desplazamiento se utilizó la media de las puntuaciones de las dos subcategorias. La variable comportamientos de desplazamiento ha sido usada para evaluar las hipótesis del segundo estudio.

Variables individuales

Ser desempleado en búsqueda de trabajo

La variable “ser desempleado en búsqueda de trabajo” ha sido utilizada para comparar dos grupos: desempleados en búsqueda de trabajo y personas inactivas (estudiantes). (Para obtener más información sobre cómo se evaluó esta variable véase "Participantes" en la página 68).

Esta variable ha sido utilizada para verificar las hipótesis del primer artículo.

Sexo

La variable sexo ha sido utilizada para comparar hombres y mujeres en el rendimiento cognitivo en el tercer articulo. Además, se tuvo en cuenta una posible influencia del ciclo menstrual en los resultados (véase página 68 para más información sobre la medición de la misma).

Análisis estadísticos

Análisis preliminares

En los tres estudios la distribución normal y la homogeneidad de la varianza han sido analizadas usando las pruebas Kolmogorov-Smirnov y Levene. Estos análisis revelaron desviaciones significativas en los valores de la FC, rMSSD y Cortisol. Por lo tanto, los datos fueron transformados en raíz cuadrada en el primer estudio, mientras que fueron transformados en logaritmos en el segundo y tercer estudio (Bartlett, 1936; Bartlett y Kendall, 1946).

Los *outliers* (valores atípicos) han sido evaluados en los tres estudios empíricos. Generalmente se consideran valores atípicos aquellos datos que están muy lejos de la norma para una variable o población (Jarrell, 1994). En la presente tesis han sido utilizados dos procedimientos distintos de valoración de valores atípicos: en el primer estudio han sido evaluados los valores atípicos en base a Mahalanobis D2 (De Maesschalck et al., 2000). En el segundo y tercer estudio han sido considerados valores atípicos aquellos cuyos índices diferían más de 3 D.T. de la media total de la muestra (Seo, 2006).

Para realizar el análisis de correlación y regresión con variables fisiológicas, en el primer estudio ha sido calculada la variable *areas under the total curve with respect to the ground* (AUCg) para FC y rMSSD usando la fórmula especificada en Pruessner y colegas (2003). En el segundo y tercer estudio, han sido calculados los cambios (Delta (Δ)) de la FC y Cortisol, restando los valores basales (FC: habituación, cortisol: -15 min) de los índices más altos (FC: entrevista de trabajo y cortisol: +20 min).

Además, se investigaron los posibles efectos de las variables demográficas, antropométricas y/o psicológicas sobre las principales variables estudiadas. Para ello se realizó una serie de análisis de varianza (ANOVAs) y correlaciones de Pearson (para más detalles sobre estos análisis preliminares véanse las secciones "Análisis estadísticos" en cada uno de los tres estudios).

En los tres estudios se evaluó el efecto del TSST sobre la FC, rMSSD y/o cortisol durante la sesión experimental realizando análisis ANOVAs o Análisis de Covarianza (ANCOVAs) para medidas repetidas. En todos los casos, cuando se realizaron ANOVAS o ANCOVAs, se utilizó el procedimiento de Greenhouse-Geisser cuando no se cumplió el requisito de esfericidad. Las comparaciones post hoc han sido realizadas usando los ajustes de Bonferroni para los valores *p*. Todos los valores *p* reportados son a dos colas, y el nivel de significación $p < 0.05$. Todos los análisis se realizaron con SPSS 20.0.

Análisis estadístico específico para los tres estudios

Los análisis estadísticos específicos realizados para verificar las hipótesis de cada uno de los tres estudios se indican a continuación.

Estudio 1.

Con el fin de crear la variable "desempleado en búsqueda de trabajo", ha sido calculada una variable *dummy* (desempleados en búsqueda de trabajo = 1, y desempleados que no buscan trabajo = 0). Además, con el fin de verificar el papel mediador de la evaluación cognitiva del estrés entre ser desempleado en búsqueda de trabajo y la FC, se llevó a cabo un análisis de mediación usando el modelo 4 del programa PROCESS (Preacher and Hayes, 2004).

Estudio 2.

Con el fin de verificar el papel mediador de la percepción de amenaza entre el optimismo y el comportamiento de desplazamiento, y el rol moderador de la respuesta fisiológica (FC y cortisol) en la relación entre la evaluación de amenaza y el comportamiento de desplazamiento, dos análisis fueron llevados a cabo: una análisis de mediación usando el modelo 4 del programa PROCESS, y una mediación moderada usando el modelo 14 del programa PROCESS (Preacher y Hayes, 2004).

Estudio 3.

Con el fin de verificar diferencias de sexo en los diversos factores que componen la evaluación cognitiva del estrés, se realizó un análisis multivariado de la varianza (MANOVA). Una ANCOVA para medidas repetidas fue llevada a cabo para evaluar las diferencias de sexo en cortisol, HR y memoria de trabajo (Span de dígitos-*Forward y* Span de dígitos-*Backward*). Además, se realizaron análisis de regresión jerárquica para investigar el papel del cortisol y FC y la evaluación cognitiva del estrés cognitivo sobre la memoria de trabajo. Finalmente, la muestra se dividió en

Increasers, Decreasers and no-changers (véase página 140). Con el fin de verificar las posibles diferencias entre estos tres grupos sobre cortisol, HR, y evaluación cognitiva del estrés, y sobre la memoria de trabajo, se han realizado ANCOVAs para medidas repetidas.

Discusión General

El estrés es un sentimiento muy común que los individuos experimentan en diferentes momentos de su propia vida. Éste desencadena una serie de respuestas psicofisiológicas que permiten a los sujetos enfrentarse a distintas situaciones. En primer lugar, las personas valoran el estresor como un reto o una amenaza para su propio bienestar (Blascovich & Mendes, 2000). En segundo lugar, según su evaluación, el organismo reacciona al estrés activando una amplia gama de funciones fisiológicas (p.e. SNA y eje HHA). Estas respuestas fisiológicas son necesarias no sólo para afrontar la situación estresante, sino para ayudar al cuerpo a restablecer la homeostasis interna desequilibrada del estrés (Folkman, 2013).

Las respuestas psicológicas y fisiológicas al estrés han sido ampliamente estudiadas (Chida & Hamer, 2008), sin embargo, queda mucho por investigar. A lo largo de esta tesis, se han presentado nuevas evidencias sobre algunos factores importantes relacionados con las respuestas psicológicas y fisiológicas al estrés. Concretamente, se ha estudiado cómo ser desempleado en busca activa de empleo y el optimismo afectan las respuestas psicológicas y fisiológicas al estrés (es decir, la evaluación cognitiva del estrés, la variabilidad de la frecuencia cardíaca y el cortisol). Además, se han estudiado también las consecuencias de las respuestas psicológicas y fisiológicas al estrés agudo en la memoria de trabajo y las conductas de

desplazamiento. Para ello, sometimos a las personas a una sesión experimental concretada con el TSST. Se utilizó un enfoque integrador de estudio que incluía la administración de varios cuestionarios, la medición de las variables fisiológicas a lo largo de todo el protocolo experimental, pruebas cognitivas, y la realización de un análisis etológico de conducta de las personas. Este enfoque nos ha permitido obtener resultados de gran relevancia a diferentes niveles: psicológico, fisiológico, cognitivo y de conducta.

Estar desempleado en búsqueda activa de trabajo y el optimismo como antecedentes de las respuestas psicológicas y fisiológicas al estrés.

Con el fin de comprender mejor las diferencias individuales que podría afectar las respuestas psicológicas y fisiológicas al estrés, los primeros dos estudios han investigado dos antecedentes de estrés. El primer factor individual que influye en las respuestas psicofisiológicas al estrés estudiado a lo largo de esta tesis ha sido una de las situaciones de estrés más comunes entre los jóvenes adultos en Europa: estar desempleado en busca de empleo (estudio 1). El estudio del desempleo y del estrés producido por estar en búsqueda activa de trabajo es particularmente importante dadas las diferentes evidencias empíricas que han señalado como esta situación está relacionada con una negativa salud física y mental (Ali et al., 2016, Brownet al., 2003, Koziel et al., 2010, Gallo et al., 2004; Sullivan & von Wachter, 2009; Robles et al., 2011; Urbanos-Garrido & Lopez-Valcárcel, 2014; Wanberg, 2012). Además de ello, el miedo a un posible desempleo futuro ha sido descrito como una fuente de estrés en sí mismo y perjudicial para el bienestar de las personas, particularmente en países con altas tasas de desempleo donde la

búsqueda de trabajo puede durar mucho tiempo (meses, años) (Knabe & Rätzel, 2011). Aunque varios estudios han centrado su atención en el efecto del desempleo sobre la salud, ningún estudio ha tenido en cuenta cuales son las repercusiones de estar desempleado en búsqueda activa de trabajo sobre la respuesta psicofisiológica al estrés. La comprensión de cómo esta particular situación estresante afecta a la respuesta de estrés podría proporcionar información útil a la prevención de las consecuencias negativas para la salud a través de un apoyo psicológico adecuado a los desempleados que buscan activamente trabajo.

El primer estudio ha demostrado que los desempleados en búsqueda activa de trabajo tienen una respuesta cardíaca más baja, junto con una menor evaluación de la amenaza cognitiva, en comparación con los desempleados inactivos (estudiantes). Asimismo, observamos un papel mediador de la evaluación cognitiva de amenaza en la relación entre estar desempleado en búsqueda activa de trabajo y la respuesta cardíaca al estrés. Estos resultados pueden ser interpretados de diferentes formas. En primer lugar, según algunos estudios, las personas sometidas a una situación estresante prolongada ven reducida la forma en que consideran la situación estresante como amenazante (Quigley et al., 2002, Tomaka et al., 1993), y en consecuencia, esta evaluación de amenaza lleva a una respuesta fisiológicas más baja (Thayer et al., 2012). En particular, una respuesta psicofisiológica limitada podría deberse a una respuesta de carga alostática en la que una hipoactividad inicial conduciría en el tiempo a una reducción excesiva de la respuesta psicofisiológica. Sin embargo, la reducción de esta respuesta podría resultar en una excesiva actividad de otros sistemas alostáticos tales como las

citoquinas inflamatorias (McEwen, 1998). En el primer estudio, el 71% de los participantes estaban desempleados en búsqueda activa de trabajo entre diez meses y un año. Aunque, no es posible afirmar que se encontraban en situación de estrés crónico, podría ser posible que la condición de desempleo estuviera afectando la evaluación cognitiva de estrés de las personas que, a su vez, afectaba a la respuesta cardíaca. Otra explicación de los resultados obtenidos podría ser que los desempleados en búsqueda activa de trabajo mostraban en sus respuestas psicofisiológicas un proceso de habituación. De hecho, según estudios previos, las personas expuestas a una situación estresante prolongada podrían aprender con el tiempo nuevas estrategias de afrontamiento, percibiendo los estímulos como menos amenazantes (Cacioppo et al., 1990, Kesley et al., 1999, 2000, 2004, Schommer et al., 2003). Igualmente, los desempleados en búsqueda activa de trabajo simplemente podrían haber aprendido a auto-regular sus emociones durante la entrevista de trabajo y por esta razón la evalúan de amenaza y consecuentemente la respuesta fisiológica era reducida con respecto a los desempleados que no estaban buscando trabajo.

Además, nuestros resultados también pueden verse afectados por otras variables que no se han tenido en cuenta en este estudio (p.e. la motivación para buscar activamente trabajo). De acuerdo con algunos estudios, dado que la probabilidad de encontrar trabajo en una región con una alta tasa de desempleo es bastante baja, las personas pierden su motivación para lograrlo (McKee-Ryan et al, 2005; Latack et al, 1995). Por lo tanto, menos motivación podría implicar menor evaluación de amenaza (p.e. los desempleados en búsqueda de trabajo creen que no van a obtener el trabajo a causa de la alta

demanda de candidatos, por lo tanto la entrevista no los preocupa) y consecuentemente una limitada respuestas cardíaca al estrés. En general, este estudio pone de relieve el importante papel del desempleo y la búsqueda de trabajo en la respuesta psicofisiológica al estrés. Sin embargo, investigaciones futuras podrían analizar el efecto de otras variables (p.e. motivación, estrategias de afrontamiento) sobre la relación entre clarificar la respuesta psicofisiológica al estrés y el estar desempleado en búsqueda de trabajo.

Un segundo factor que influye en las respuestas individuales al estrés psicológico estudiado a lo largo de esta tesis ha sido el optimismo (estudio 2). En particular, ha sido evaluado el papel del optimismo sobre la valoración de amenaza, lo cual ha añadido nueva información relevante sobre el tema. De hecho, hasta donde sabemos, solamente dos estudios han investigado la relación entre el optimismo y la evaluación de amenaza. Por otra parte, estos estudios han sido llevado a cabo con personas adultas (Endrighi et al, 2011; Puig-Pérez et al, 2017). Los resultados han demostrado que las personas que obtuvieron una puntuación alta en el optimismo percibían la tarea estresante como menos amenazante. Este resultado confirma la relación positiva entre el optimismo y evaluación cognitiva del estrés encontrado en un estudio anterior (Endrighi et al, 2011; Puig-Pérez et al, 2017). De hecho, las personas optimistas sienten poseer las estrategias de afrontamiento necesarias para hacer frente a la situación estresante (Nes & Segerstrom, 2006), lo cual podría ser la razón por la cual las personas optimistas perciben como menos amenazadoras las tareas estresantes presentadas en la sesión experimental.

Conductas de desplazamiento y memoria de trabajo como consecuencia de la respuesta psicológica y fisiológica al estrés, y el papel moderador de la FC.

A lo largo de la tesis se ha evaluado el papel de la respuesta psicológica y fisiológica al estrés sobre la conducta de desplazamiento y la memoria de trabajo.

La conducta de desplazamiento ha sido definida como una serie de actividades relacionadas con el estrés, tales como conductas repetitivas perpetradas hacia el proprio cuerpo (rascarse, flotar las manos, tocarse el pelo, etc) (Troisi, 2002; McFarland, 1966). Aunque algunos estudios han investigado su relación con las respuestas psicológicas y fisiológicas al estrés (p.e. Mohiyeddini et al, 2013 a-b; Mohuyeddini et al., 2013; Pico-Alfonso et al, 2007; Troisi, 2002; Villada et al, 2014), estudios anteriores no han tenido en consideración el papel mediador de la evaluación cognitiva de amenaza entre el optimismo y las conductas de desplazamiento. Por otra parte, en los últimos años el interés acerca de los rasgos de la personalidad y su relación con la respuesta psicofisiológica del estrés y las conductas de desplazamiento ha ido aumentando. Este hecho podría proporcionar información relevante para los profesionales como psicólogos, psiquiatras, expertos en selección de personal, etc., sobre cómo captar, a partir del comportamiento no verbal, la personalidad de la persona y sus estrategias de manejo del estrés. Los resultados de este estudio han demostrado que la evaluación de amenaza media totalmente la relación entre el optimismo y las conductas de desplazamiento. De acuerdo con lo que hemos afirmado en el párrafo anterior, el optimismo influye positivamente sobre la evaluación de amenaza.

Sin embargo, no influye directamente sobre las conductas de desplazamiento. Según algunos estudios, las conductas de desplazamiento están altamente relacionadas con el estrés y particularmente tienen un papel en el re-equilibrio de la homeostasis interna del cuerpo cuando ésta ha sido comprometida por el estrés. De hecho, las personas con menor activación cardiovascular después del estrés fueron los que también mostraron más conductas de desplazamiento durante el estrés (Troisi 2002; Villada et al, 2014). Por lo tanto, las conductas de desplazamiento necesitan altos niveles de estrés (evaluación de amenaza o respuesta fisiológica) para ser manifestadas. Así que, de acuerdo con todo lo explicado anteriormente, el optimismo influye sobre la evaluación de amenaza que a su vez influye sobre las conductas de desplazamiento.

No sólo la evaluación de la amenaza tiene un impacto sobre las conductas de desplazamiento. De hecho, nuestro resultado ha demostrado que la FC tiene un papel moderador entre la evaluación cognitiva de amenaza y las conductas de desplazamiento, cuando la evaluación cognitiva de amenaza es un factor mediador entre el optimismo y las conductas de desplazamiento. En nuestro estudio se observó que cuando la FC es baja, sólo las personas con alta evaluación cognitiva de amenaza manifiestan conductas de desplazamiento, mientras que cuando la FC es alta, tanto las personas con baja como con alta evaluación cognitiva de amenaza muestran conductas de desplazamiento. Este resultado confirma que los comportamientos de desplazamiento están relacionados con el estrés, tanto a nivel de evaluación cognitiva como de respuesta fisiológica. De hecho, sólo cuando la evaluación cognitiva de amenaza es alta (incluso con baja FC) o la FC es alta (incluso

con baja evaluación cognitiva de amenaza) las conducta de desplazamiento se manifiestan. Como se discutió anteriormente, la razón de ello podría ser que en ambos casos cuando el estrés psicológico o fisiológico es alto, las conductas de desplazamiento son necesarias para re-equilibrar el funcionamiento interno del cuerpo desequilibrado por la respuesta de estrés.

Otro factor consecuente a la respuesta psicológica y fisiológica al estrés estudiado en esta tesis ha sido el rendimiento de la memoria de trabajo considerando su dos componentes "atención y span de memoria" y "función ejecutiva" (D'Esposito, 2007). La relación entre la respuesta fisiológica al estrés y la memoria de trabajo ha sido estudiada evaluando también las posible diferencias entre hombres y mujeres. De hecho, durante los últimos años la memoria de trabajo ha sido ampliamente estudiada (Cornelisse et al., 2011, Duncko et al., 2009, Gärtner et al., 2014, Luethi et al., 2009, Oei et al., 2006). Sin embargo, los resultados son muy contradictorios particularmente en referencia a las diferencias de sexo (Elzinga & Roelofs, 2005; Staphil et al., 2009; Terfhr et al., 2011; Hoffman & al'Absi, 2004, Kuhlmann et al., 2005, Smeets et al., 2006, Stauble et al., 2013). Los resultados obtenidos en esta tesis han puesto de evidencia que las mujeres manifestaban una mayor atención y span de memoria después del TSST, mientras que los hombres no mostraban modificaciones en el rendimiento de la memoria de trabajo antes y después del estrés. Además de ello, siguiendo la perspectiva de autorregulación de Thompson y colegas (2015), la muestra se dividió en *increasers, decreasers* y *no changers,* y se evaluó el rendimiento de la memoria de trabajo comparando estos tres tipos de respuestas al estrés. De acuerdo con estudios realizados anteriormente con bebés (Thompson et al.,

2015; Thompson & Trevathan, 2008; 2009), observamos que el patrón de respuestas de estrés caracterizado por "incremento" seguido de "decremento" durante la tarea estresante permite a las mujeres mejorar su rendimiento en “atención y span de memoria”. Por el contrario, tanto los hombres como las mujeres que simplemente obtuvieron un incremento de la respuesta fisiológica no manifestaron ningún cambio en el rendimiento de la memoria de trabajo. En el estudio también se observó una relación negativa entre el cortisol y la función “atención y span de memoria” en mujeres. Por lo tanto, los bajos niveles de cortisol parecen permitir a las mujeres mejorar su rendimiento. En conjunto, estos resultados podrían interpretarse en el sentido de que el patrón de respuesta al estrés "cuidar y hacer amistad" (Taylor et al., 2000), que está relacionado con una regulación negativa de la HHA durante el estrés, permite a las mujeres tener un mejor desempeño en la memoria de trabajo después del estrés.

En general, los resultados mostrados a lo largo de esta tesis aportan nueva información sobre el papel de diferentes antecedentes (estar desempleado en búsqueda activa de trabajo y optimismo) y consecuentes (conductas de desplazamiento y memoria de trabajo) de las respuestas psicológicas y fisiológicas al estrés. Particularmente, estudiamos el estrés teniendo en cuenta todos los componentes involucrados en su respuesta: psicológica, fisiológica y conductual. Además, el último estudio enfatiza la importancia de considerar las diferencias de sexo en las respuestas al estrés. De hecho, sugiere la necesidad de tener en consideración los tres patrones de respuestas al estrés (*increasers, decreasers, and no-changers*) cuando se

estudia la respuesta al estrés, particularmente en relación con las diferencias de sexo.

Limitaciones y fortalezas

En los capítulos anteriores se han mencionado las limitaciones específicas de cada uno de los estudios. Por lo tanto, esta sección presenta las limitaciones generales a tener en consideración al interpretar los principales resultados de esta tesis. Entre ellos, la limitación más importante, aunque su fortaleza, está en los estrictos criterios de inclusión usados para la selección de la muestra. De hecho, los participantes eran jóvenes adultos cognitiva y físicamente sanos, lo que dificulta la generalización de estos resultados a personas de diferentes rangos de edad o situación de salud (p.e. ansiedad patológica o depresión). Además, los estrictos criterios de selección dificultaron enormemente tener una muestra de mayor tamaño. Por lo tanto, futuras investigaciones serán necesarias para evaluar los resultados de estos estudios con muestras más grandes y heterogéneas.

Otra debilidad es que los participantes en estos estudios fueron personas de educación media/alta, puesto que eran estudiantes universitarios. Futuras investigaciones deberían replicar los estudios presentados en esta tesis utilizando poblaciones con diferentes niveles educativos.

A lo largo de la tesis, se midieron los índices fisiológicos de cortisol y VFC varias veces a lo largo del protocolo experimental, y el desempeño en memoria de trabajo se midió con una prueba pre-post estrés. Sin embargo, otra limitación de esta tesis es que en los tres estudios sólo se ha utilizado una condición experimental y no una condición control. Por esta razón, serian

necesarias investigaciones que utilicen una condición de control para replicar nuestros estudios. Esto permitiría tener información más específica sobre el efecto del estrés en las variables estudiadas en los tres artículos.

A pesar de estas limitaciones, la presente tesis también tiene una serie de fortalezas. Una de las más importantes en todos los estudios es el rigor en el proceso de selección del muestreo. De hecho, aplicamos numerosos y muy restrictivos criterios de inclusión. Por lo tanto, si por un lado nos impidió obtener un tamaño de muestra mayor, por otro lado nos permitió crear una muestra muy homogénea. De esta manera, hemos evitado el efecto de varios factores que podían afectar nuestros resultados, lo cual fue especialmente importante cuando se utilizaron variables fisiológicas. De la misma manera, fuimos muy estrictos en la recolección de la muestra de cortisol en saliva. Con el fin de evitar una muestra sesgada de cortisol, pedimos a los participantes en los tres estudios que siguieran algunas recomendaciones antes de la sesión experimental (p.e. no consumir alcohol durante la noche anterior a la sesión hasta el final de las pruebas). Antes de comenzar la sesión, se pidió a los participantes que confirmaran que siguieron las recomendaciones.

Con el fin de producir estrés, hemos sometido a las personas a una de las situaciones de vida real más estresante para los jóvenes adultos: una situación de evaluación social. Además, se realizaron análisis fisiológicos de laboratorio para proporcionar medidas objetivas de estrés, se administraron cuestionarios y se obtuvieron datos sobre rasgos de personalidad y estado psicológico, se desarrolló un instrumento para el análisis y computación del comportamiento no verbal de desplazamiento, y finalmente se sometió a las

personas a una prueba cognitiva. Todo ello nos permitió estudiar una amplia gama de componentes relacionados con el estrés usando un enfoque integrador.

Direcciones futuras

Estudios longitudinales podría proporcionar información relevante sobre cómo se modifica en el tiempo la relación entre variables como el estar desempleado en búsqueda activa de trabajo, el optimismo y las respuestas psicológicas y fisiológicas al estrés.

Otras investigaciones podrían incluir otras medidas (p.e. electrofisiología) además de las medidas neuroendocrinas utilizadas en esta tesis. De esta manera, futuros estudios podrían complementar la evaluación de la actividad neuroendocrina con actividad cortical en el estudio de las relaciones exploradas en los tres estudios.

Asimismo, podría ser importante tener en cuenta el papel de otros rasgos de personalidad (p.e. neuroticismo) en la evaluación cognitiva del estrés y en las conductas de desplazamiento. De la misma manera, podría ser interesante investigar si el optimismo y la evaluación cognitiva del estrés influyen en otras conductas relacionadas con el estrés (p.e. conductas de sumisión).

Finalmente, futuras investigaciones podrían replicar los estudios en entornos naturales, como entrevistas de trabajo reales, exámenes académicos o presentaciones en congresos.

Principales conclusiones

Las principales conclusiones de esta tesis doctoral son las siguientes:

- Estar desempleado en búsqueda de trabajo produce una disminución de la evaluación cognitiva de la amenaza y en consecuencia de la respuesta cardíaca al estrés. Esta disminución en la respuesta psicológica y fisiológica puede ser debida al efecto del estrés crónico o a un proceso de habituación.

- La evaluación cognitiva de amenaza media la relación entre estar desempleado buscando un trabajo y las respuestas cardíacas simpáticas y parasimpáticas al estrés.

- El optimismo podría promueve una experiencia más positiva de estrés disminuyendo la percepción de amenaza.

- La evaluación cognitiva de amenaza media la relación entre el optimismo y las conductas de desplazamiento.

- La relación entre la evaluación cognitiva de amenaza y las conductas de desplazamiento es moderada por la FC.

- La evaluación cognitiva de amenazas y la respuesta cardiaca influyen sobre el uso de las conductas de desplazamiento. Las conductas de desplazamiento se observan en ambas condiciones: cuando la FC es baja y la evaluación de la amenaza es alta, y cuando la FC es alta y la evaluación de la amenaza es alta o baja.

- Las mujeres *decreasers* (mujeres que mostraron una disminución de más de - 1,5 nmol / l en sus concentraciones salivales de cortisol después del

estrés) desempeñan mejor en “atención y span de memoria” después del estrés.

- El patrón de respuestas al estrés "cuidar y hacer amistad" que está asociado a una regulación negativa de la HHA durante el estrés, está implicado en la mejora del rendimiento de la memoria de trabajo en las mujeres después del estrés.

FUNDING SOURCES

This thesis has been supported by the Spanish Education and Science Ministry (PSI2010/21343, PSI2013/46889) and Generalitat Valenciana (GRISOLIAP/2011/082, PROMETEOII2015/020).

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