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

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HIGHLIGHTS

- Females performed better on attention and maintenance after stress task
- Cortisol negatively affects females' attention and maintenance performance
- Only females' cortisol decreasers performed better after stress task
- Sex and cognitive stress appraisal have a mediating role in working memory performance
- Cognitive stress appraisal affects working memory performance in females

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ABSTRACT

Sex is considered a moderating factor in the relationship between stress and cognitive performance. However, sex differences and the impact of cognitive stress appraisal on working memory performance have not received much attention. The aim of this study was to investigate the role of physiological responses (heart rate and salivary cortisol) and cognitive stress appraisal in Working Memory (WM) performance in males and females. For this purpose, we subjected a comparable number of healthy young adult males ($N = 37$) and females ($N = 45$) to a modified version of the Trier Social Stress Test (TSST), and we evaluated WM performance before and after the stress task. Females performed better on attention and maintenance after the TSST, but males did not. Moreover, we found that attention and maintenance performance presented a negative relationship with cortisol reactivity in females, but not in males. Nevertheless, we observed that only the females who showed a cortisol decrease after the TSST performed better after the stress task, whereas females and males who showed an increase or no change in cortisol levels, and males who showed a cortisol decrease, did not change their performance over time. In females, we also found that the global indexes of cognitive stress appraisal and cognitive threat appraisal were negatively related to attention and maintenance performance, whereas the Self-concept of Own Competence was positively related to it. However, these relationships were not found in males.

1. Introduction

Acute stress triggers physiological and psychological responses that affect cognitive performance, especially memory performance [36]. 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) [51]. Studies in humans indicate that cortisol affects memory through the GC receptors located especially in the prefrontal cortex (PFC), hippocampus, and amygdala (see [73]). Therefore, these brain regions have been considered the focal point of the stress effect and, consequently, related to memory performance [28,45].

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) [14]. To date, most studies on the effects of stress on WM have found a negative effect [17,20,24,35,43,53,54,62,74,75], although some studies have shown a positive effect [11,17,59,71], or even no effect [25,32,57].

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Recently, sex has been considered an important moderating factor in the relationship between stress and WM (e.g. [3,52,72]). 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 [43,53,54,74,75] and females [52]. However, other studies have found that acute stress positively affects the executive component of WM only in males [11,52,71], and some have shown no effect or no sex-related differences in the executive component of WM [25,32,57]. On the other hand, the attention and maintenance component has been found to be positively affected by acute stress in males [57,59], but no effects and sex-related differences have also been observed ([20,25,32]. 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 et al. [61] 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 [39], and its release has been shown to be higher in females than males [27]. 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 [61]. 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 [16]. 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 [22]. Some findings have associated the cognitive threat appraisal with impaired cognitive performance (e.g., [8,41]). Moreover, another study showed a tendency where an increase in the cognitive threat appraisal led to WM performance impairment [19]. Furthermore, even though coping strategies have been positively related to WM (i.e. [48,58]), 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 [46]. 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 [6].

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.

2. Methods

2.1. 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, 2 h 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.

2.2. Procedure

The study involved an individual session that lasted approximately 90 min and took place between about 16.00 and 19.00 h. The experimental sessions were composed of different phases (see Fig. 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, [30]). 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 min they were left alone

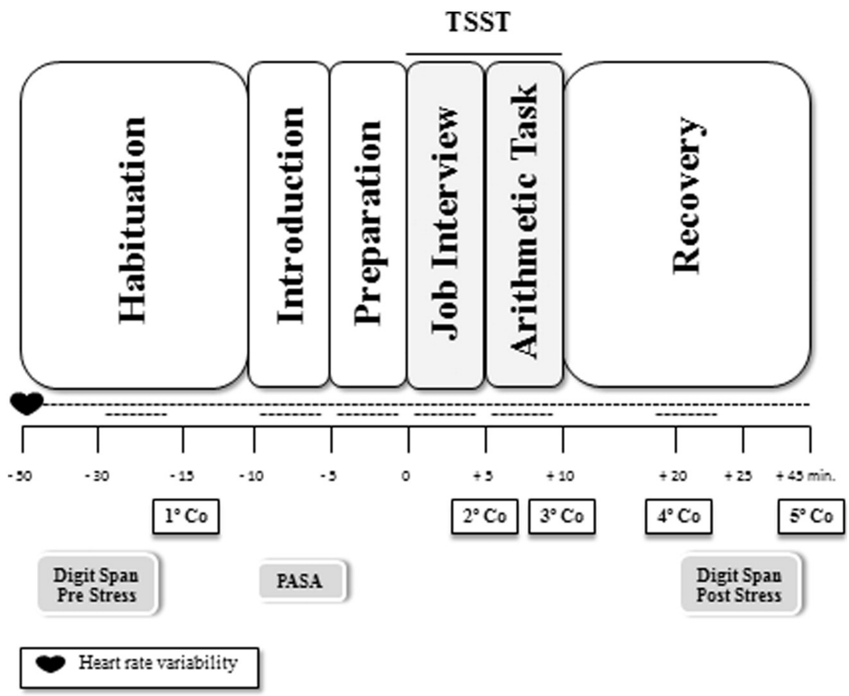

Fig. 1. Timeline of the TSST. Dotted lines depict the time of HR collection. Salivary cortisol samples = 1°Co, 2°Co, 3°Co, 4°Co. PASA, Primary appraisal and Secondary appraisal.

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 min 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 min of free speech (job interview), followed by a 5- min 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 m 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.

2.3. Measures

2.3.1. Demographic and anthropometric measures

To assess possible differences between groups, we collected data such as age, BMI, and subjective socioeconomic status (SES) [2].

2.3.2. 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 [47].

2.3.3. 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 [60], we analyzed HR in periods of 5 min. Whereas the job interview and arithmetic task phases lasted 5 min each, the habituation, preparation, and recovery phases lasted longer than 5 min; for this reason, we chose the central 5 min 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.

2.3.4. Cognitive stress appraisal

Cognitive stress appraisal was evaluated with the PASA [23]. This scale was employed to assess cognitive appraisal processes before performing the TSST, based on transactional stress theory [33]. 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 [33], the “Tertiary appraisal” scale (also called the “global index of cognitive stress appraisal”) was calculated using the formula proposed by Gaab et al. [23]. 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.

2.3.5. WM test: Digit Span subtest

The Digit Span subtest of the Wechsler Memory Scale III [70] 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 [34].

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.[1] In females, age had a positive relationship with the DS-Backward ($r = 0.409$, $p = 0.005$) and a tendency toward a positive relationship with the DS-Forward ($r = 0.271$, $p = 0.06$) (see Table 1). In males, BMI had a positive relationship with the post-stress DS-Forward ($r = 0.327$, $p = 0.04$). 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., [1,12,21,68]). 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 >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 $p < 0.05$. 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 $F(1.75) = 18.968$, $p > 0.001$. 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 ($p = 0.66$), 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.

4. Results[2]

4.1. Preliminary analysis

There were no sex differences in age (males: $M = 25.81$, $SEM \pm 0.9$; females: $M = 24.31$, $SEM \pm 0.6$; $F(1,80) = 1.831$, $p = 0.18$) or SES (males: $M = 5.89$, $SEM \pm 0.1$; females: $M = 6.28$, $SEM \pm 0.1$; $F(1,80) = 2.540$, $p = 0.11$), but males had a higher BMI than females (males: $M = 25.13$, $SEM \pm 0.6$; females: $M = 22.27$, $SEM \pm 0.4$, $F(1,80) = 14.265$, $p = 0.001$) (see Table 1).

4.2. Cognitive appraisal

The MANOVA did not find significant Sex differences in Cognitive Threat Appraisal (males: $M = 3.02$, $SEM \pm 0.1$; females: $M = 2.93$, $SEM \pm 0.1$; $F(1,80) = 0.238$, $p = 0.62$), Challenge Appraisal (males: $M = 3.57$, $SEM \pm 0.1$; females: $M = 3.40$, $SEM \pm 0.1$; $F(1,80) = 1.201$, $p = 0.27$), Self-concept of Own Competence (males: $M = 3.94$, $SEM \pm 0.1$; females: $M = 3.97$, $SEM \pm 0.1$; $F(1,80) = 0.029$, $p = 0.86$), Control Expectancy (males: $M = 4.45$, $SEM \pm 0.1$; females: $M = 4.45$, $SEM \pm 0.1$; $F(1,80) = 0.003$, $p = 0.95$), or the Tertiary PASA index (males: $M = -0.90$, $SEM \pm 0.1$; females: $M = -1.01$, $SEM \pm 0.1$; $F(1.80) = 0.276$, $p = 0.60$) (see Table 1).

4.3. Salivary cortisol

The repeated-measures ANCOVA with salivary cortisol concentrations as the dependent variable showed a main effect of Sex ($F(1,73) = 17.324$, $p < 0.001$). Overall, males had higher cortisol concentrations than females. Moreover, the main effect of Time ($F(1.786,130.362) = 4.563$, $p = 0.01$) was also significant. Thus, cortisol concentrations increased immediately after the job interview (−15 min sample vs. +5 min sample, $p = 0.01$) and continued to increase until reaching peak levels 20 min after the onset of the stress task (−15 min sample vs. +20 min sample, $p = 0.005$). Afterwards, cortisol levels decreased, reaching baseline levels in the last saliva sample (−15 min sample vs. +45 min sample, $p > 0.99$) (see Fig. 2). Moreover, the Time and Age interaction was also significant ($F(1.786,130.362) = 5.876$, $p = 0.005$). The interaction between Time and Sex and the main effect of Age and BMI were not significant (all p's > 0.38).

4.4. Heart rate

The repeated measures ANCOVA with HR as the dependent variable showed a main effect of Sex ($F(1,72) = 6.284$, $p = 0.01$). Overall,

[1] 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, $U = 17.550$, $p = 0.72$), and there were no significant correlations between the “number of cigarettes smoked” and cortisol release ($r = 0.079$, $p = 0.79$).

[2] 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 > 0.15).

Table 1
Descriptive statistics and correlations among the study variables.

Variable	M	SD	1	2	3	4	5	6	7	8	9	10	11
Males													
1. Age	25.81	5.56	–										
2. BMI	25.13	3.68	0.07	–									
3. ΔCortisol	0.37	0.71	0.20	−0.13	–								
4. AHR	−1.37	1.54	−0.08	0.16	0.11	–							
5. DS-Forward post	6.27	1.09	0.20	0.32*	−0.18	−0.30	–						
6. DS-Backward post	4.91	1.13	0.05	0.17	−0.04	−0.38	0.53**	–					
7. Threat stress appraisal	3.02	0.79	−0.10	−0.15	−0.09	0.10	0.10	0.03	–				
8. Challenge stress appraisal	3.57	0.77	−0.08	−0.24	−0.36	−0.23	0.27	0.30	0.25	–			
9. Self-concept of Own Competence	3.94	0.93	−0.03	0.01	0.07	−0.09	0.13	0.03	−0.55**	0.10	–		
10. Control Expectancy	4.45	0.77	−0.22	0.07	−0.04	−0.02	0.16	0.01	0.01	0.57**	0.40**	–	
11. Tertiary appraisal	−0.90	0.95	−0.02	−0.20	−0.19	−0.03	0.01	0.11	0.79**	0.22	−0.85**	−0.37**	–
Female													
1. Age	24.31	4.47	–										
2. BMI	22.27	3.17	0.15	–									
3. ΔCortisol	0.15	0.51	0.29*	−0.01	–								
4. AHR	−0.77	1.44	0.19	0.08	−0.03	–							
5. DS-Forward post	6.28	1.17	0.27	−0.38	−0.32*	−0.12	–						
6. DS-Backward post	4.91	1.18	0.40**	0.09	−0.01	−0.08	0.57**	–					
7. Threat stress appraisal	2.93	0.80	0.05	−0.13	0.22	0.14	−0.40**	0.02	–				
8. Challenge stress appraisal	3.40	0.62	0.19	−0.01	0.21	0.01	−0.01	0.03	0.46**	–			
9. Self- concept of Own Competence	3.97	0.76	0.15	−0.09	−0.08	0.27	0.40	0.06	−0.35*	0.01	–		
10. Control Expectancy	4.45	0.70	−0.21	−0.09	−0.06	0.07	0.13	0.20	- 0.11	0.16	0.17	–	
11. Tertiary appraisal	−1.01	0.91	0.08	0.04	0.20	−0.13	−0.39**	−0.10	0.76**	49**	−0.67**	−0.41**	–

females had higher HR than males. Moreover, the main effect of Time (F(2.998,215.888) = 6.972, $p < 0.001$) was also significant. HR increased immediately after the habituation phase (habituation vs. preparation phase, $p < 0.001$), and it continued to increase until reaching its peak rate during the job interview phase (habituation vs. job interview phase, $p < 0.001$). Then, HR decreased, reaching lower levels than in the habituation phase (habituation vs. recovery phase, $p = 0.01$) (see Fig. 2). Moreover, the Time and Age interaction was also significant (F(2.998,215.888) = 3.475, $p = 0.01$). The interaction between Time and Sex and the main effect of Age and BMI were not significant (all p's > 0.08).

4.5. WM test: Digit Span subset

4.5.1. 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 > 0.55), whereas Age was (F(1,78) = 5.152, $p = 0.02$). However, a significant interaction was found between Time and Sex (F(1,78) = 4.144, $p = 0.04$). Post hoc analysis revealed that females had better performance after the task than before it ($p = 0.04$), but this did not occur in males ($p = 0.31$). Moreover, there were no sex differences before the stress task (pre-stress task, $p = 0.27$) or after the stress task (post-stress task, $p = 0.46$) (see Fig. 3). However, the Time and Age interaction and the Time and BMI interaction were not statistically significant (all p's > 0.41).

4.5.2. 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 > 0.09) (See Fig. 3).

4.6. Relationship between cognitive stress appraisal and WM performance

None of the associations studied for males were significant (all p's > 0.073), except the relationship with BMI (β = −0.397, p = 0.03). In females, regression analysis showed a significant negative relationship between the Tertiary PASA and the DS-Forward (β = −0.380, $p = 0.009$), whereas there was no significant relationship with the DS-Backward (β = −0.094, $p = 0.51$). Moreover, a significant negative relationship was also found between Cognitive Threat appraisal and the DS-Forward (β = −0.390, $p = 0.007$), whereas a positive linear relationship was observed between Self-concept of Own Competence and the DS-Forward (β = 0.391, $p = 0.008$). Furthermore, in females, there were no significant associations between Cognitive Threat and

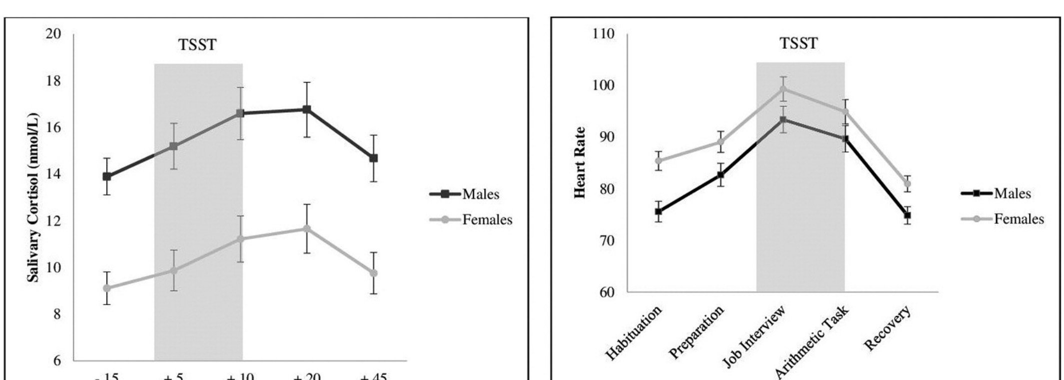

Fig. 2. Salivary Cortisol concentrations (i) and heart rate (ii) for males and females during TSST.

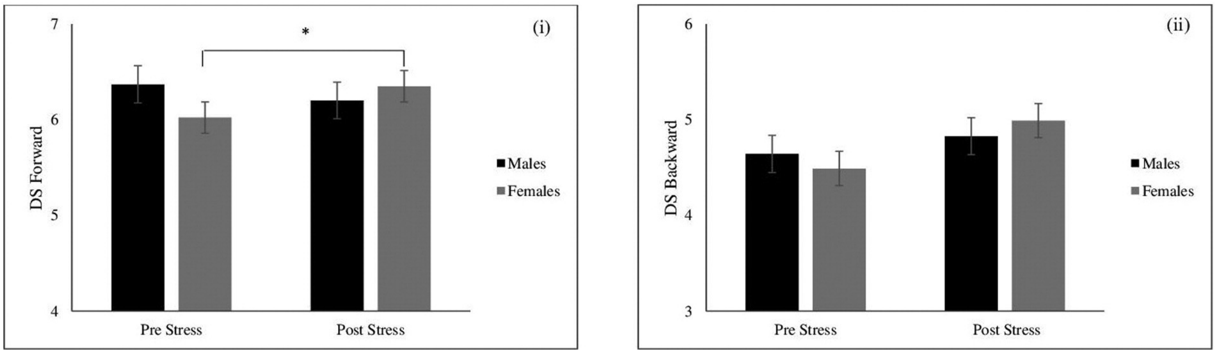

Fig. 3. Performance on Digit Span Forward (DS-Forward) (i) and Digit Span Backward (DS-Backward) (ii) for males and females before and after TSST.

Challenge Appraisal, Self-concept of Own Competence, Control Expectancy, Age or BMI and the DS-Forward and DS-Backward (all p's > 0.18), except between Age and the DS-Backward ($\beta = 0.433$, $p = 0.009$).

4.7. 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 > 0.25). However, we found a significant relationship between BMI and the DS-Forward ($\beta = 0.334$, $p = 0.05$). In females, regression analysis showed a significant negative relationship between Δcortisol and the DS-Forward ($\beta = -0.335$, $p = 0.03$), and between Age and the DS-Forward ($\beta = -0.383$, $p = 0.01$). 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 > 0.21). Furthermore, we did not observe any significant relationships between BMI, the basal level of cortisol and HR, and the DS-Forward (all p's > 0.50).

4.8. Differences between increasers, decreasers, and no-changers on cortisol, HR, and cognitive stress appraisal, and on the DS-forward

Thompson et al. [66]) 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 [66] 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 et al. [42], 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 et al. [61]), 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 ($F(2.218, 153.030) = 4.953$, $p = 0.006$) and a main effect of Sex ($F(1,69) = 10.039$, $p = 0.002$) and Group ($F(1,69) = 10.220$, $p = 0.000$). Moreover, we observed a marginally significant Time and Sex and Group interaction ($F(2.218,153.030) = 2.770$, $p = 0.08$) (Fig. 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 > 0.61). 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 > 0.65). However, we observed a significant interaction between Time and Age ($F(2.893,193.805) = 4.502$, $p = 0.000$) and Sex and Group ($F(1.67) = 3.581$, $p = 0.03$), where female increasers have higher HR than male increasers ($p < 0.001$).[3]

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 > 0.94).

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 ($p = 0.003$), Time and Group ($p = 0.01$), and Time and Sex and Group ($F(1,73) = 4.191$, $p = 0.01$). Post hoc analysis of the Time and Sex interaction revealed that females ($p < 0.001$) performed better after the TSST, but males did not (all p's > 0.45). Post hoc analysis of the Time and Group interaction revealed that decreasers ($p = 0.003$) 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 ($p < 0.001$), whereas female and male increasers and no-changers and male decreasers did not change their performance after the stressful task (all p's > 0.25) (see Fig. 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

[3] 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 ($p = 0.33$): 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) ($N = 8$)).

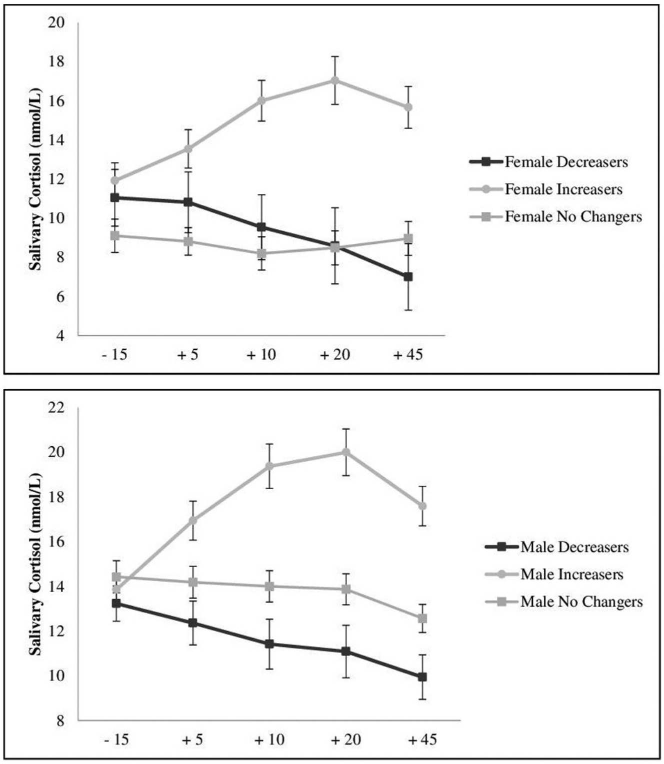

Fig. 4. Salivary cortisol concentrations during TSST for female and male increasers, decreasers and no changers.

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

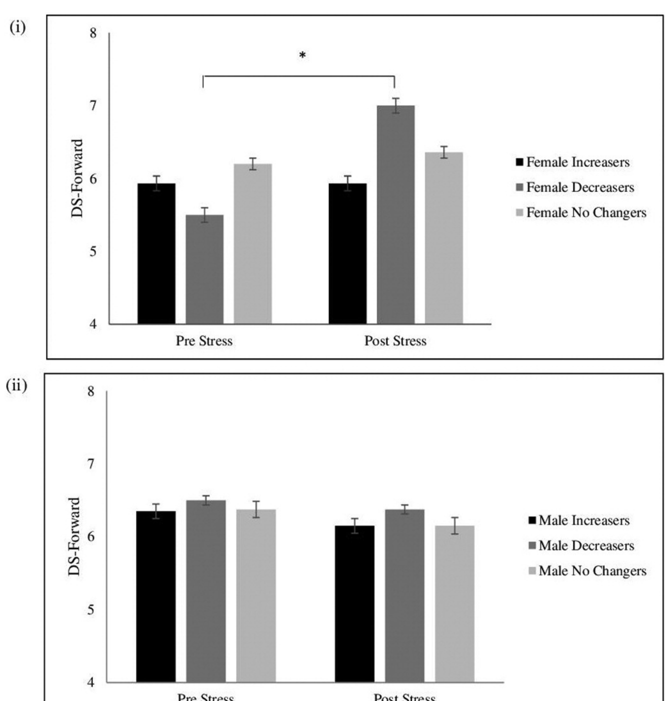

Fig. 5. Performance on Digit Span Forward for female (i) and male (ii) increasers, decreasers and no changers.

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 [64,65], and Thompson et al. [66]) 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 [66], 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" [66]. This response pattern was found in a study by Blair et al. [7], 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 et al. [66] state, decreasers, and in this case female decreasers, may operate with an optimal ratio of mineralocorticoid receptors/glucocorticoid receptors (MR/GR) [13] 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 [20], 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 et al. [61] 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 [25,57,52], or with a study that found a decrease, although not statistically significant, in WM performance after a stress task in females [52]. These different results might be due to the fact that these studies included fewer females than our study did (e.g., [25]: females = 15; [57]: females = 30; [52]: 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. [52] 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 [20,25, 32]. 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 [34] and, consequently, less possibility of showing a practice effect. Only one study found an impairing effect of stress on WM in females [52]. However, this study was conducted in the morning, a time period related to high cortisol concentration and high inter-individual variance [15], when the cortisol concentration could affect WM performance more than in the afternoon [37].

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 et al. [19], 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, [31]). This study and others [38,63], 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. [48,58]). Oxytocin has been related to a higher self-concept of one's own competences in both sexes [10]. 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.

Conflicts of interest

The authors state that there are no conflicts of interest associated with the research.

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