No effects of psychosocial stress on memory retrieval in non-treated young students with Generalized Social Phobia

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Generalized Social Phobia (GSP) is a common anxiety disorder that produces clear social life disruptions. There is no consensus on the specific processes involved in its development, but the role of the hypothalamic-pituitary-adrenal (HPA) axis has been suggested. This study analyzed the effects of the cortisol response to the Trier Social Stress Test (TSST) on the memory retrieval of pictures with different emotional valences in 45 non-treated young students with GSP and 50 non-anxious (NA) subjects (mean = 19.35 years, SD = 0.18). No differences were found in the cortisol response of GSP and NA subjects to the TSST and control sessions. In addition, psychosocial stress impaired memory retrieval in both the GSP and NA groups, with no differences between them. Regarding the sex factor, no effects were found in the cortisol response to the TSST. However, during the encoding session, GSP men had higher cortisol levels than GSP women and NA subjects. There was also a significant interaction between sex and stress exposure on memory retrieval. Women recognized more unpleasant and neutral pictures than men; however, under stress, the women’s advantage disappeared, and the men’s performance improved. Sex also interacted with social phobia on positive mood, with GSP women exposed to the TSST showing the lowest positive mood. These results suggest that GSP subjects do not present an HPA axis sensitization to psychosocial stress, and they emphasize the importance of Sex in understanding stress effects on memory.

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1. Introduction

Social Phobia (SP) is a common anxiety disorder with high prevalence during adolescence and youth, characterized by intense fear and/or avoidance of situations where an individual is fearful of a negative social evaluation (American Psychiatric Association, 2000; van Peer et al., 2010); when fears are related to most social situations, the specification of Generalized Social Phobia (GSP) is used. Deficits in the associative learning processes have been proposed as the main impaired underlying mechanism of this condition (Stravynski, 2007), although SP may also be considered a stress-related condition. Several studies have examined the hypothalamic-pituitary-adrenal (HPA) axis reactivity in SP subjects, reporting conflicting findings for cortisol levels that support both sensitization (Condren et al., 2002; Roelofs et al., 2009; van West et al., 2008) and reduced activation of the HPA axis to psychosocial stress (e.g. Shirotsuki et al., 2009) in this population. These studies are heterogeneous, not only in terms of the population under study (e.g. inpatients, community samples, children, and elder individuals), but also in the type of stressor and the nature of the tasks employed. In general, stressful events induce an increase in the hypothalamic production of the corticotropin-releasing hormone (CRH), which stimulates pituitary release of the adrenocorticotropic hormone (ACTH) and, as a consequence, cortisol secretion by the adrenal cortex (Suay and Salvador, 2012). Given the relevance of the reaction to social stress in SP and the inconclusive and sparse reports on the HPA axis response to stress in subjects with SP, more research is needed to understand the acute stress response in this population.

In addition to the cortisol response to psychosocial stress, the relationship between social phobia and emotional reactivity to stress has been extensively studied. At the brain circuitry level, it has been suggested that social phobic subjects display enhanced activation of the prefrontal-limbic circuitry, including the amygdala, anterior cingulate, and orbitofrontal cortex, in response to the anticipation of faces displaying pain (Veit et al., 2002). High levels of
subjective anxiety, but not physiological reactivity, were observed in 12-year-old SP children compared to healthy control subjects (Krämer et al., 2012); by contrast, van West et al. (2008) reported an elevated cortisol response, but no state-anxiety reactivity, in 6–12-year-old children compared to controls, employing the same psychosocial stressor, the Trier Social Stress Test (TSST, Kirschbaum et al., 1993). In older people (Grossman et al., 2001), using a public speaking task and, more recently, in middle-aged people using the TSST (Klustbies et al., 2014), higher subjective anxiety, but not higher cortisol response to the social stressor, has been reported in individuals with social phobia.

Among the main consequences of exposure to stress, cognitive effects, particularly on memory, have attracted considerable attention. Many studies have assessed the acute effects of increased levels of glucocorticoids (GCs) on memory performance, although the precise direction of these effects is dependent on several factors. When the HPA axis is activated, cortisol secretion takes place and crosses the blood–brain barrier, binding to GC receptors located in the hippocampus, amygdala, and prefrontal cortex (Ulrich-Lay and Herman, 2009), which play an essential role in memory. The type of process (acquisition, consolidation or retrieval) and memory type (e.g. declarative, priming, working memory, and so on), as well as the nature of the material (neutral vs emotional) to be recalled, are important factors to be considered. Studies employing declarative memory tasks based on the repetition of a neutral word list have shown that stress (Wolf et al., 2001) or cortisol administration (de Quervain et al., 2000) leads to poorer memory retrieval in humans. However, material with emotional content improves consolidation (Cahill and McGaugh, 1998) and appears to interact with some effects of stress or cortisol administration on memory retrieval (Jelic et al., 2004). Specifically, there is evidence for a negative effect of cortisol on memory retrieval in healthy young adults for negative material, but with no effects on neutral material (de Quervain et al., 2007; Smeets et al., 2007; Marin et al., 2010). However, in other studies, stress and cortisol treatment impaired memory retrieval, especially of emotionally arousing material, regardless of its valence (Buchanan and Tranel, 2008; Smeets et al., 2008; Tullenaar et al., 2008). People with SP have been described as remembering more negative stimuli, both emotional facial expressions (Foa et al., 2000) and specific negative aspects of social events (Hertel et al., 2008). Together, these results point to the anticipation of negative emotional aspects of social events as the trigger for the social fear experienced (Veit et al., 2002). However, how controlled stressful social events affect the memory retrieval of emotionally arousing material in SP subjects remains unknown. Individuals with GSP have been described as having differential working memory and short-term memory capacities compared to healthy controls (Amir and Bomyea, 2011). Specifically, they displayed better performance on negative material and poorer performance on neutral material. This becomes particularly important when studying the effect of stress on delayed memory processes, as these effects may be driven not by the social stress, but by specific deficits in the early acquisition phases. Thus, it is fundamental to measure both immediate and delayed memory in order to properly assess the stress effects on memory retrieval in GSP subjects.

Sex is a fundamental factor when studying the hormonal response to stress, as sex differences in both the cortisol response to stress (Kudielka and Kirschbaum, 2005) and stress effects on learning and memory (Andreano and Cahill, 2009; Wolf, 2006) are often reported. Men tend to show a greater cortisol stress response than women in laboratory studies (Kirschbaum et al., 1999; Kudielka et al., 2009), and they are more affected by stress on declarative memory retrieval (Wolf et al., 2001). There is also evidence that women outperform men on episodic memory tasks of verbal material, faces and pictures (Herlitz et al., 2013; Spalek et al., 2015). When recalling pictures with different emotional valences, women outperformed men on free recall of positive, negative, and neutral pictures, with a particular advantage for positive pictures. However, these sex-related differences disappeared on the recognition task (Spalek et al., 2015). Little is known about whether these sex differences remain after exposure to stress. For instance, in a pre-learning stress study, we found that in the group exposed to the psychosocial stressor, men’s performance on the memory test improved and matched the level displayed by women (Espin et al., 2013). The potential beneficial effects of female sex hormones (Wolf, 2006) and sex differences in the cortisol response to stress (Kudielka and Kirschbaum, 2005) may explain this pattern of results.

To shed light on the different results reported in the literature and examine a period of the life span that is particularly important due to the potential negative effects of social phobia, we conducted a multi-measure study of GSP young people, exposing them to the most widely used social stressor in the aforementioned studies, the TSST, and comparing them with controls. Our aim was to investigate the effects of the stressor on the HPA axis and the subjective state, and find out how stress affects the memory retrieval of pictures with different emotional valences in young GSP and non-anxious (NA) subjects. We expected to find a higher stress-induced cortisol response in GSP subjects during the stressor, and we hypothesized that an elevation in cortisol levels may cause impairing effects on the retrieval process of emotional material (specifically for positive pictures). Finally, we explored whether these stress effects are modulated by sex.

2. Materials and methods

2.1. Participants

The final sample was composed of ninety-five undergraduate students of Psychology (60%) and Computer Engineering (40%), with ages between 18 and 25 years old (mean age: 19.4 years old; S.D. = 0.18), who participated in the study for one class credit. The recruitment of the sample was performed in two steps. In the first step, 675 students filled out the Social Phobia and Anxiety Inventory (SPAI; Turner et al., 1989), but 580 were excluded from the study based on one or more of the following criteria: they scored between 50 and 97 on the SPAI; they displayed a history of alcohol or other drug abuse; they had cardiovascular, endocrine, neurological or psychiatric diseases; they had visual or hearing problems; they had experienced a stressful life event during the past year; they were using any medication directly related to emotional or cognitive function, or one that was able to influence hormonal levels, such as glucocorticoids, oral contraceptives, beta-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, and psychotropic substances. Vitamins, sporadic use of painkillers, and natural therapies were allowed. None of the participants were habitual smokers (more than 10 cigarettes a week). The SPAI has shown good internal consistency coefficients (Cronbach’s alpha = 0.96 for the subscale of Social Phobia) and high test-retest reliability (0.89) in young Spanish adults and college students (Olivares et al., 2010). The remaining 95 students were classified as the GSP group if they obtained a score >97, and as the non-anxious group (NA) if their score was <50 on the Social Phobia subscale, based on available normative data for the Spanish population (Olivares et al., 2010), with the following distribution: GSP group (N = 45) and NA group (N = 50). In the second step, subjects identified as GSP participated in an individual clinical interview with a clinician who was blind to the previous classification. All participants included in the GSP group met all the clinical criteria for Generalized Social Phobia from the Anxiety Disorders interview Schedule (DSM-IV-TR), which confirmed the distribution. There-
fore, two groups were formed: GSP (24 women and 21 men) and NA (24 women and 26 men).

The selected participants were asked to attend two sessions that took place in a laboratory in the Faculty of Psychology at the University of Murcia. Before each session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to: drink only water; not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, 2 h prior to the session; and not brush their teeth at least 1 h prior to the session.

The study was conducted in accordance with the Declaration of Helsinki, and the Ethics Research Committee of the University of Murcia approved the protocol and conduct. All the participants received verbal and written information about the study and signed an informed consent form.

2.2. Questionnaires and measures

2.2.1. Mood

Mood was evaluated by the Spanish version (Sandin et al., 1999) of the Positive and Negative Affect Schedule (PANAS, Watson et al., 1988). This 20-item questionnaire assesses mood according to two dimensions: Positive (PA: interested, excited, strong, enthusiastic, etc.) and Negative affect (NA: distressed, upset, guilty, scared, etc.), with 10 items measuring each. The Spanish version of the scale has a Cronbach’s alpha ranging from 0.87 to 0.89 for PA and from 0.89 to 0.91 for NA.

2.2.2. Anxiety

To assess state anxiety, the Spanish version of the State Anxiety Inventory form S was used (STAI-S, Spielberger et al., 1970). The adapted scale has a Cronbach’s alpha ranging from 0.90 to 0.93 (Seisdedos, 1988).

2.2.3. Salivary cortisol

Saliva was collected using Salivettes (Sarstedt, Rommelsdorf, Germany). In the encoding session, four samples were collected: t – 10, t0, t + 30, t + 40, with reference to the start of the stimuli presentation, while in the stress/control session, six samples were collected: t – 15, t0, t + 5, t + 15, t + 25, t + 45, with reference to the start of the TSST or control task. Salivary cortisol was measured with the commercial radioimmunoassay kit Orion Diagnostica (Espoo, Finland). The saliva samples were stored at −80 °C immediately upon collection until the analyses were performed. The samples were analyzed in the Service of Support Research (SRS) of the University of Murcia. Intra-assay and inter-assay coefficients of variation were less than 10%.

2.3. Procedure

This study employed a between-subjects design, where participants were tested individually in two sessions on consecutive days (i.e. encoding (n = 95) and stress (n = 48)/control (n = 47) sessions). Both sessions were carried out between 1500 h and 1800 h to control for the diurnal cortisol cycle. A figure with the experimental procedure is provided in the Supplemental materials (see Fig. S1 in online version at DOI: 10.1016/j.psyneuen.2016.07.211).

2.3.1. Encoding session

Upon arrival at the laboratory, the participants’ weight and height were measured, and the experimenter checked whether they had followed the instructions given previously (see participants section). Next, the first saliva sample (t – 10) was collected, and the participants filled out the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) and the state anxiety scale from the State-Trait Anxiety Inventory (STAI-S; Spielberger et al., 1970). Then, participants were shown 30 digital color pictures chosen from the International Affective Pictures System (IAPS, Lang et al., 2005). According to the normative data for the Spanish adaptation of the IAPS1 (Vila et al., 2001), 10 unpleasant (valence 1.37–3.77), 10 neutral (valence 4.38–6.56), and 10 pleasant (valence 6.73–8.02) pictures were shown. Five additional neutral pictures were selected to serve as practice. Pictures were displayed with a PC computer and presented for 6 s (with a random inter-trial interval of 10–20 s) on a 40” Samsung screen television (102 cm) located 2.5 m in front of the participants. Three picture-presentation sequences were carefully constructed to avoid showing more than two consecutive pictures of the same valence, and participants were randomly assigned to one of the sequences. While subjects observed the pictures (between 10 and 15 min), they rated each picture on the valence dimension using a pencil and paper version of the Self-Assessment Manikin (SAM) rating system (Hodes et al., 1985). No mention of a memory test was made; thus, the encoding of the pictures was incidental. Immediately before the participants’ exposure to the pictures, the second saliva (t0) sample was collected. After the exposure to the pictures, participants completed a free recall test, during which they wrote down as much information as they could recall about the images for 10 min. After that, the third saliva sample (t + 30) was collected, and the subjects again completed the STAI-S and the PANAS (10 min). Finally, the fourth saliva sample (t + 40) was collected. Then, participants were asked to return at the same time the next day to complete their participation.

2.3.2. Stress or control session

Twenty-four hours later, participants returned to the laboratory. In a different room, a baseline saliva sample (t – 15) was collected, and during a 15 min adaptation period, they filled out the PANAS and STAI-S questionnaires. Next, the participants were randomly assigned to the TSST or control session.

In the stress session, we employed a modified version of the original TSST (Kirschbaum et al., 1993), but with only two experimenters on the committee. In addition, the task preparation phase (5 min) took place in the same room as the speech and arithmetic tasks. As usually occurs on this task, participants stood in front of a committee and a video recorder, and they were informed that the audience would evaluate their performance and that they were not allowed to use their notes. After the instructions for the speech task, the second saliva sample (t0) was collected. Participants were given 5 min to prepare for their speech, and before this preparation period ended, the third saliva sample (t + 5) was collected. After that, participants were asked to stand in front of the committee during the speech task (5 min). Next, the participants performed the mental arithmetic task from the TSST. Then, the fourth (t + 15) saliva sample was collected.

The control session was designed to be as similar as possible to the TSST session without being stressful for the participants, as in previous studies (Espin et al., 2013; Hidalgo et al., 2012). During the preparation phase, the participants read a chapter from a book with neutral content. Next, the control task took place, which consisted of 5 min of reading aloud a fragment of the book and 5 min of counting aloud. The control task was performed in the same room as the TSST, but all stressful elements (video camera, tape recorder, committee and microphone) were removed (Dickerson and Kemeny, 2004). In the control session, the second saliva sample (t0) was col-

1 Pictures selected from the IAPS: unpleasant (1050, 3050, 1300, 3069, 3170, 9040, 3101, 3400, 6313, 9810); neutral (1670, 2190, 2230, 7009, 2320, 5130, 2372, 7080, 2440, 7490); and pleasant (1440, 1710, 2000, (2311, 2660, 4572, 4700, 5480, 8330, 8461).
lected after the instructions, the third (t + 5) before the reading task, and the fourth (t + 15) after the counting task.

After the stress or control tasks, participants returned to the first room and again completed the PANAS and STAI-S questionnaires. Next, the fifth saliva sample (t + 25) was taken, and participants completed a free recall task with the pictures they had seen the previous day (10 min). Subjects were instructed to write down as much information as they could recall about the pictures. Subsequently, the recognition test took place. This consisted of the random presentation of 60 pictures, 30 originally viewed and 30 new pictures (10 negative, 10 neutral and 10 positive) from the IAPS, with comparable valences and arousals and identical memorizing properties to the original stimuli. After each picture, subjects were asked to indicate whether they had seen the pictures in the first session by writing “yes” or “no” on the paper. After the recognition task, a sixth saliva sample (t + 45) was collected.

Memory performance was assessed by two trained judges who were blind to each participant’s group membership. The same criteria were applied to score immediate (free recall phase, during which they wrote down as much information as they could recall about the pictures they had just seen) and delayed free-recall (free recall phase during which they wrote down as much information as they could recall about the pictures that had been presented the previous day). A total score was given for the total number of remembered pictures, and valence scores were obtained considering the valence of the pictures.

According to Buchanan and Tranel (2008), a recall detail was counted as correct if it could be unambiguously linked to a particular picture. In addition, each correctly recalled picture was included in pleasant, unpleasant, and neutral categories; moreover, the number of correct details included in each participant’s description of each picture was counted as unpleasant, pleasant, or neutral. Recognition performance was expressed as the number of recognized pictures according to their valence.

In addition, the memory retention rate was estimated as the percentage ratio between the total number of remembered pictures after the delayed period and the total number of remembered pictures on immediate retrieval.

2.4. Statistical analysis and data management

To investigate Group (GSP vs. NA) and Sex differences on demographic and anthropometric variables (age, BMI, height, and weight), t-tests for independent samples were used. Moreover, two univariate ANOVAs of baseline levels of cortisol on both days were performed.

Raw data for salivary cortisol were log transformed to meet the assumption of normality. The cortisol responses to the IAPS and to the TSST/control were evaluated by using repeated-measures ANOVAs with Group (GSP, NA), Sex (men, women), and Condition (TSST vs Control session) as between-subjects factors, and Time (encoding session: t − 10, t0, t + 20, t + 30 and TSST/control sessions: t − 15, t0, t + 5, t + 15, t + 25, t + 45) as within-subject factors. Six independent repeated-measures ANOVAs (for anxiety, negative and positive mood, in both the encoding and the TSST vs control sessions) were performed with Group, Sex, and Condition as between-subject factors, and time (pre and post-task) as within-subject factor.

Area Under Curve increase (AUCi) in cortisol was calculated for the encoding session (Pruessner et al., 2003) and included as dependent variable in an univariate ANOVA with group and sex as between-subjects factors.

Regarding memory, we first examined whether Group and Sex factors influenced the immediate total recall in the encoding session. A one-way ANOVA was performed with immediate total recall as dependent variable and Group and Sex as between-subjects factors. Furthermore, multivariate analysis of variance (MANOVA) was performed with Group and Sex as between-subjects factors, and Valence (total number of details for pleasant, unpleasant, and neutral pictures) as dependent variables.

One-way ANOVAs were carried out with delayed total recall as dependent variable and Condition (TSST vs Control session), Group, and Sex as between-subjects factors. Then, a MANOVA was performed with Group, Sex, and Condition as between-subjects factors and Valence as the dependent variable. For recognition, a MANOVA was performed with Group, Sex, and Condition as the between-subjects factors, and recognition of pleasant, unpleasant, and neutral pictures as the dependent variables. An ANOVA was carried out with retention rate as dependent variable and Condition, Group and Sex as between-subjects factors. The cortisol Area Under Curve (AUCg) with respect to the ground (Pruessner et al., 2003) was also included as covariate on this analysis.

Post-hoc analysis of ANOVA effects was conducted using the Bonferroni multiple comparison test. Within-subject effects are reported with Greenhouse-Geisser corrections whenever the assumption of sphericity is violated. For all analyses, all p-values reported had a significance level of p < 0.05. Effect-sizes of significant results are reported with the Partial Eta Squared (η²).

3. Results

There were no significant differences between the GSP and NA groups in Age, BMI, positive mood, or baseline levels of cortisol on both days (for all p > 0.05). Significant differences between the two groups were found in negative mood and state anxiety on both days (for all p < 0.02). Significant differences in BMI were found depending on Sex (p ≤ 0.001), with men showing greater BMI scores than women. A summary of the descriptive statistics of the variables under study is displayed in Table 1.

3.1. Cortisol measures

3.1.1. Encoding session

The analyses revealed significant main effects of Time [F (2,08, 189.58) = 27.24; p < 0.001, η² = 0.23]; Group [F (1, 91) = 7.90; p = 0.006, η² = 0.08] and Sex [F (1, 91) = 12.86; p < 0.001, η² = 0.12]. Furthermore, the Time × Group [F (2,08, 189.58) = 3.792; p < 0.02, η² = 0.04], Time × Sex [F(2,08, 189.58) = 5.34; p = 0.005, η² = 0.055] and Group × Sex [F (1, 91) = 4.82; p < 0.03, η² = 0.05] interactions were significant.

Regarding the Time × Group interaction, post hoc analyses showed that GSP subjects had higher cortisol levels than NA subjects at t + 30 and t + 40. In the case of the Time × Sex interaction, men had higher cortisol levels than women in all samples (for all p < 0.004). Regarding the Group × Sex interaction, post hoc analysis showed that GSP men showed higher cortisol levels than NA men (p < 0.001), GSP women (p < 0.001) and NA women (p < 0.001). No differences were found between men and women in the NA group (p = 0.31), or between GSP women and NA women (p = 0.66) (see Fig. 1).

The area under the curve with respect to the increase (AUCi) was calculated as suggested by Pruessner et al. (2003) using the log transformed cortisol levels. The univariate ANOVA with Sex and Group as between-subjects factors revealed a significant effect of Sex [F (1, 91) = 13.44; p < 0.001, η² = 0.13], showing
men (M = 3.63; SD = 18.72) higher cortisol responses than women (M = −8.29; SD = 15.80). No significant effect of Group [F (1, 91) = 3.34; p = 0.07, η² = 0.04] or the Group × Sex interaction were found [F (1, 91) = 0.914; p = 0.37, η² = 0.01].

3.1.2. Stress/control session

The analyses revealed significant main effects of Time [F (2.261, 196.749) = 26.041, p < 0.001, η² = 0.230], Condition [F (1, 917) = 21.974, p < 0.001, η² = 0.202], and Sex [F (1, 917) = 10.324, p = 0.002, η² = 0.106], with men showing higher cortisol levels than women (p = 0.002); however, Group was not significant [F (1, 917) = 2.212, p = 0.14, η² = 0.02]. In addition, a significant effect of the Time×Condition interaction was also found [F (2.261, 196.749) = 64.942, p < 0.001, η² = 0.427]; participants exposed to the TSST exhibited higher levels of cortisol than subjects exposed to the control session at t + 15, t + 25 and t + 45 (for all p < 0.001) (see Fig. 2). Importantly, no significant Time×Condition × Group [F (5, 5485) = 0.743, p = 0.59, η² = 0.008] or Time × Condition × Group × Sex [F (5, 5485) = 0.611, p = 0.69, η² = 0.007] interactions were found.

3.2. Memory measures

3.2.1. Encoding session

First, it is worth noting that no significant differences were found between the standardized ratings of the IAPS pictures and the ratings made by the participants. In fact, correlations between the two ratings were high (Pearson correlations >0.95; p < 0.001). Regarding valence, the results confirmed the a priori classification, so that the negative pictures (M = 1.38, SEM = 0.06) were rated lower than the neutral (M = 4.03, SEM = 0.07) and positive pictures (M = 7.25, SEM = 0.07) for all p < 0.001, and neutral pictures were rated lower than positive pictures (p < 0.001). There were no significant differences between GSP and NA participants in their subjective ratings (for all p > 0.05).
Fig. 1. Means of salivary cortisol levels in the encoding session for GSP (N = 45) and NA subjects (N = 50) at different time points. Error bars represent standard errors of the mean (SEM).

Fig. 2. Means of salivary cortisol levels in the stress (TSST) and control sessions for GSP (N = 45) and NA subjects (N = 50) at different time points. Error bars represent standard errors of the mean (SEM).

GSP and NA groups did not differ in their total picture recall [F(1, 91) = 1.36, p = 0.24, \( \eta^2 = 0.01 \)] or in their recall of neutral [F(1, 91) = 1.44, p = 0.23, \( \eta^2 = 0.01 \)], pleasant [F(1, 91) = 1.21, p = 0.27, \( \eta^2 = 0.01 \)], and unpleasant pictures [F(1, 91) = 0.10, p = 0.74, \( \eta^2 = 0.001 \)].

The Sex factor was significant [F(1, 91) = 6.49, p = 0.012, \( \eta^2 = 0.06 \)], as women recalled significantly more pictures than men. There were also main effects of Sex on the recall of neutral [F(1, 91) = 4.40, p = 0.03, \( \eta^2 = 0.04 \)] and pleasant pictures [F(1, 91) = 6.64, p = 0.01, \( \eta^2 = 0.06 \)], with women showing higher recall than men (see Fig. 3).

3.2.2. Stress/control session

3.2.2.1. Delayed free recall. The Group factor did not show significant effects on picture recall [F(1, 87) = 1.33, p = 0.25, \( \eta^2 = 0.01 \)] or on recall of neutral [F(1, 87) = 0.03, p = 0.85, \( \eta^2 = 0.01 \)], pleasant [F(1, 87) = 1.20, p = 0.27, \( \eta^2 = 0.01 \)], and unpleasant pictures [F(1, 87) = 2.36, p = 0.12, \( \eta^2 = 0.02 \)]. However, Condition [F(1, 87) = 4.652, p = 0.034, \( \eta^2 = 0.05 \)] and Sex [F(1, 87) = 8.752, p = 0.004, \( \eta^2 = 0.09 \)] showed significant effects. Thus, the subjects in the stress session recalled fewer pictures than those in the control session, whereas women recalled significantly more pictures than men. Moreover, sex differences were found for neutral [F(1, 87) = 7.297, p = 0.008, \( \eta^2 = 0.07 \)] and pleasant pictures [F(1, 87) = 8.186, p = 0.005, \( \eta^2 = 0.08 \)], with women showing higher recall than men (see Fig. 3). No significant effects of the Condition x Group [F(1, 87) = 1.06, p = 0.31, \( \eta^2 = 0.01 \)] or the Condition x Group x Sex [all F(1, 87) = 2.62, all p > 0.11, all \( \eta^2 < 0.029 \)] interactions were found.

3.2.2.2. Recognition memory task. Significant effects of the Condition x Sex interaction were found for neutral [F(1, 87) = 4.35, p = 0.04, \( \eta^2 = 0.05 \)] and unpleasant pictures [F(1, 87) = 5.17, p = 0.02, \( \eta^2 = 0.05 \)]. Women recognized more neutral (p = 0.04) and unpleasant pictures (p = 0.037), but not pleasant ones (p = 0.91), than men in the control session (see Fig. 3). However, in the TSST session, no sex differences were found (see Fig. 3).

We only found significant effects of the Condition x Group interaction for pleasant pictures [F(1, 87) = 4.50, p = 0.04, \( \eta^2 = 0.05 \)]. Thus, the NA group recognized more pleasant pictures in the Control session than in the TSST session (p = 0.014) (see Fig. 4), whereas the GSP group did not show significant differences (p = 0.60). No differences between TSST and control session were found for unpleasant and neutral pictures in the NA group or the GSP group (p > 0.2). Moreover, no significant effect of the Condition x Group x Sex interaction was found [all F(1, 87) < 0.251, all p > 0.617, all \( \eta^2 < 0.003 \)].
3.2.2.3. **Retention rate.** Significant effects of Sex [F (1, 87) = 4.29, p = 0.04, η² = 0.04] and the Condition × Sex interaction were found [F (1, 87) = 7.36, p = 0.008, η² = 0.07]. Post hoc analyses showed that women had higher retention rates than men (p = 0.04). In addition, men exposed to the TSST showed a lower retention rate than men in the control session (p < 0.005). Women exposed to the TSST showed higher retention rates than men in the same session (p = 0.001). The Group factor did not show any significant effects (see Fig. 3). No significant effects of the Condition × Group interaction [F (1, 87) = 1.06, p = 0.31, η² = 0.01] or the Condition × Group × Sex interaction [F (1, 87) = 0.001, p = 0.971, η² < 0.001] were found. This pattern of results occurred even when the AUCg was included as a covariate in the univariate model.

### 3.3. **State anxiety and mood measures**

#### 3.3.1. **Encoding session**

##### 3.3.1.1. **State anxiety.** The analyses revealed significant effects of Group [F (1, 91) = 12.25, p = 0.001, η² = 0.11] and the Time × Group interaction [F (1, 91) = 3.81, p = 0.05, η² = 0.04]. Before the task, GSP subjects showed higher levels of state anxiety than NA subjects.
(p < 0.001), but no significant differences were found after the task (p = 0.11) (see Fig. 5).

3.3.1.2. Mood. For negative mood, the analyses showed significant effects of Time [F(1, 91) = 23.58, p < 0.001, η² = 0.20], Group [F(1, 91) = 16.10, p = 0.001, η² = 0.15] and the Time × Group interaction [F(1, 91) = 9.36, p = 0.003, η² = 0.09]. Before the task, GSP subjects showed higher negative mood than NA (p < 0.001). GSP showed higher negative mood at pre-task than at post-task (p = 0.001), whereas in NA no differences were found between pre- and post-task (p = 0.19).

For positive mood, the analyses revealed significant effects of Time [F(1, 91) = 6.33, p = 0.01, η² = 0.06] and the Group × Sex interaction [F(1, 91) = 8.84, p = 0.01, η² = 0.06]. All subjects showed less positive mood after the task than before it. Moreover, GSP women showed less positive mood than GSP men (p = 0.01), NA women (p = 0.008), and NA men (p = 0.04) (see Fig. 5).

3.3.2. Stress/control session

3.3.2.1. State anxiety. We found significant effects of Time [F(1, 87) = 53.949, p < 0.001, η² = 0.383], Group [F(1, 87) = 5.998, p = 0.016, η² = 0.065], Sex [F(1, 87) = 5.390, p = 0.023, η² = 0.058] and Condition [F(1, 87) = 24.972, p < 0.001, η² = 0.223]. For the Time × Sex interaction [F(1, 87) = 6.624, p = 0.012, η² = 0.071], no differences were found before the task (p = 0.384), but women showed higher anxiety than men after it (p = 0.004). Both women and men showed higher anxiety post-task than pre-task (for women, p < 0.001, for men, p = 0.001).

Regarding the Time × Condition interaction [F(1, 87) = 42.136, p < 0.001, η² = 0.326], no differences were found between the TSST and control sessions at baseline (p = 0.175), although higher anxiety appeared after exposure to the TSST, compared to the control session (p < 0.001). After the TSST, anxiety was higher than before it (p < 0.001), whereas no differences were found in the control session (p = 0.548).

Investigating the Sex × Group interaction [F(1, 87) = 3.747, p = 0.05, η² = 0.056], GSP women showed higher anxiety than NA women (p = 0.002), GSP men (p = 0.004), and NA men (p = 0.003).

In the case of the Sex × Condition interaction [F(1, 87) = 7.599, p = 0.007, η² = 0.080], women exposed to the TSST showed higher anxiety than men in the TSST session (p < 0.001) and women in the control session (p < 0.001). Men and women showed higher anxiety after exposure to the TSST compared to pre-task (p < 0.001) (see Fig. 4). Importantly, no significant effects of the Time × Condition × Group interaction [F(1, 87) = 0.202, p = 0.65, η² = 0.002] or the Time × Condition × Group × Sex [F(1, 87) = 0.013, p = 0.91, η² < 0.001] interaction were found.

3.3.2.2. Mood. Significant effects of Time [F(1, 87) = 34.010, p < 0.001, η² = 0.281], Sex [F(1, 87) = 6.032, p = 0.016, η² = 0.065], Condition [F(1, 87) = 13.465, p < 0.001, η² = 0.134] and Group [F(1, 87) = 7.241, p = 0.009, η² = 0.077] were found, with GSP subjects showing higher negative mood than NA subjects.

For the Time × Sex interaction, [F(1, 87) = 9.180, p = 0.003, η² = 0.095], no differences were found in negative mood between men and women at baseline (p = 0.265), but after the task women showed higher negative mood than men (p = 0.003); both women and men showed higher negative mood after the task than before it (for women: p < 0.001, for men: p = 0.05). Regarding the Time × Condition interaction, [F(1, 87) = 36.650, p < 0.001, η² = 0.296], no differences were found in the baseline level (p = 0.378); however, after the task, the subjects in the
TSST session showed higher negative mood than the subjects in the control session (p < 0.001). Significant differences between pre and post-task negative mood were also found in the TSST session (p < 0.001), but not in the control session (p = 0.876). Focusing on the Sex × Condition interaction, [F (1, 87) = 5.561, p = 0.021, \( \eta^2 = 0.060 \)], women in the TSST session showed higher negative mood than men (p < 0.001) and women in the control session (p < 0.001). After exposure to the TSST, women showed higher negative mood than men (p < 0.001), but both men and women had more negative mood after the TSST (p < 0.001 and p = 0.003, respectively) (see Fig. 5). Importantly, no significant effects of the Time × Condition × Group interaction [F (1, 87) = 0.347, p = 0.56, \( \eta^2 = 0.004 \)] or the Time × Condition × Group × Sex [F (1, 87) = 0.237, p = 0.63, \( \eta^2 = 0.003 \)] interaction were found.

For positive mood, we found significant effects of Time [F (1, 87) = 23.474, p < 0.001, \( \eta^2 = 0.212 \)] and Condition [F (1, 87) = 4.289, p = 0.041, \( \eta^2 = 0.047 \)], and subjects in the TSST session showed less positive mood than subjects in the control session (p = 0.015) after the task. Based on the Group × Condition interaction [F (1, 87) = 5.007, p = 0.028, \( \eta^2 = 0.054 \)], GSP exposed to the TSST showed less positive mood than NA (p = 0.032) and GSP controls (p = 0.004). The Sex × Group interaction [F (1, 87) = 8.242, p = 0.005, \( \eta^2 = 0.087 \)] revealed that GSP women showed less positive mood than GSP men (p = 0.010), NA women (p = 0.05), and NA men (p = 0.04). In the case of the Sex × Condition interaction [F (1, 87) = 3.718, p = 0.05, \( \eta^2 = 0.041 \)], women exposed to the TSST showed less positive mood than control women (p = 0.05) and men exposed to the TSST (p = 0.043) (see Fig. 5). Finally, no significant effects of the Time × Condition × Group interaction [F (1, 87) = 1.18, p = 0.28, \( \eta^2 = 0.013 \)] or the Time × Condition × Group × Sex [F (1, 87) = 0.453, p = 0.50, \( \eta^2 = 0.005 \)] interaction were found.

4. Discussion

This study assessed both hormonal and subjective responses to a psychosocial stressor and their effects on the memory retrieval of pictures with different emotional valences in non-treated young people with GSP compared to NA subjects.

As expected, subjects exposed to the TSST showed higher cortisol responses than subjects in the control session. In addition, men had higher cortisol levels than women, as suggested in other studies (Kudielka et al., 2009). However, no differences were found between GSP and NA in their cortisol response to the TSST; our results showed that both groups had a similar hormonal response to psychosocial stress. In fact, the curve of the stress-induced cortisol response for the GSP and NA groups was similar to the expected response to the TSST (see Fig. 2). According to these data, GSP subjects did not present a reduced (e.g. Shirotsuki et al., 2009) or increased (Condren et al., 2002; Roelofs et al., 2009; van West et al., 2008) HPA axis reactivity to psychosocial stress in comparison with the healthy population, which coincides with other studies that failed to find these differences (Martel et al., 1999; Krämer et al., 2012; Klumbies et al., 2014). A normal HPA axis reactivity in the GSP group might be the result of repeated exposure to social stressors in daily life. Students face many social situations every day. Frequent exposure to social situations that cause anxiety or stress could wear down the physiological responses in highly anxious individuals. For example, Schommer et al. (2003) suggested that repeated exposure to the TSST dulled the HPA axis reactivity, and Wüst et al. (2005) reported a decrease in cortisol and ACTH responses after repeated stress exposure. Furthermore, Connor et al. (2007) showed that SAD (social anxiety disorder) patients perceived higher daily stress than the general population. Therefore, a history of repeated exposure to social stressors could have resulted in normal responsiveness of the HPA axis in the GSP subjects. These findings could suggest that in non-phobic people, situational fear is reliably associated with cortisol elevations. The differences observed between the clinical and nonclinical samples may be due to differences in HPA axis reactivity between the two subsamples. Nonetheless, these differences are also consistent with the notion that fearful situations in natural contexts are more potent cortisol releasers than feared objects in laboratory contexts (Alpers et al., 2003). It is also relevant that our sample is composed of young students with high social phobia traits, and so it is possible that illness duration may play a relevant moderator role in the cortisol response to stressful events in GSP, and this should be further tested.

In addition, no sex differences were found in the stress-induced cortisol response of GSP and control subjects to the TSST. Sex differences in HPA axis responses to psychological stress might be different in clinical populations and in healthy subjects (Kudielka and Kirschbaum, 2005); some laboratory studies employing the TSST failed to find any significant differences between increases in plasma cortisol and sex in subjects with social phobia (Condren et al., 2002) or depression and/or anxiety disorders (Young et al., 2004) and healthy controls. Other studies have investigated HPA axis stress responses to acute psychological stress in SP subjects, but they did not analyze sex differences (Roelofs et al., 2009; Shirotsuki et al., 2009; Krämer et al., 2012; Klumbies et al., 2014). Therefore, it seems that both young men and women with GSP show a similar cortisol response to a standardized laboratory stressor. By contrast, in the encoding session, GSP men had higher cortisol levels than GSP women and NA subjects. This occurred in the absence of the stressor, and even before the presentation of the IAPS pictures, which suggests that GSP men may interpret the experimental setting as more threatening or as a stressful situation. In addition, men had higher cortisol levels during the encoding session when subtracting the baseline values, as revealed the AUC analyses, which suggests that, overall, men find the tasks in the encoding session to be more stressful than women do.

We also note that subjects with GSP showed a higher negative psychological state, with higher scores on negative mood and state anxiety, regardless of exposure to stress. In addition, GSP subjects exposed to the TSST showed less positive mood than the NA group. Nevertheless, the scores on positive mood were influenced by exposure to the TSST because perceived positive mood after exposure to the TSST was lower in GSP subjects than in NA. In the case of sex effects, regardless of exposure to the TSST, GSP women showed higher state-anxiety and less positive mood than GSP men and NA subjects in response to the experimental session. According to Roelofs et al. (2009), women show a greater negative psychological state, especially women exposed to the stress session. Taking into account that women had lower cortisol levels than men, men may have reported lower subjective anxiety due to the anxiolytic effect of cortisol, which has been shown to reduce subjective anxiety in a social phobic sample of male subjects in response to a social stressor (Soravia et al., 2006). However, to our knowledge, this is the first study to report a modulating effect of sex on positive mood in GSP. Our findings suggest that these differences may be driven by more subtle sex differences in the subjective response to stress, highlighting the importance of controlling sex when studying the stress response in this population.

Regarding memory performance, GSP and NA did not differ on immediate or delayed free recall. No differences were found between the GSP and NA groups in the delayed free recall of pictures depending on exposure to stress. Nonetheless, the exposure to the TSST seemed to reduce the subjects’ memory performance (both GSP and NA). This result supports previous findings in healthy young individuals in response to a stress task (Smeets et al., 2008; Tollenaar et al., 2008). Furthermore, women showed a higher
immediate and delayed free recall performance than men, which agrees with the notion that women outperform men in recalling verbal material, faces, and pictures on episodic memory tasks (Herlitz et al., 2013; Spalek et al., 2015). In addition, our data coincide with a previous study and support the hypothesis that sex differences in memory performance are dependent on valence category and task (Spalek et al., 2015).

No differences were found between the GSP group exposed to the TSST and those exposed to the control session on the recognition memory task. However, the NA group recognized more pleasant pictures in the control session compared to the NA group exposed to the TSST session. Finally, the women's advantage found on the recognition memory task in the control session was not present in the group exposed to the TSST, where the men's recognition memory of neutral and unpleasant pictures improved to the level of the women. This result is consistent with previous findings by our group using a pre-learning stress induction design (Espin et al., 2013), and with findings showing that stress after learning/encoding can also improve memory consolidation and retrieval (see Roozendaal, 2000 for a review). In addition, stress did not affect the recognition task in GSP subjects, but in the NA group, stress worsened the memory of pleasant pictures compared to the control session, perhaps because several aspects can contribute to the effects of stress on memory when considering stimuli of different valences. One of the most relevant aspects has to do with the timing and content of the stress induction. This means that when stress induction is performed a significant length of time after the acquisition phase (for example, after a 24 h interval), memory formation is impaired (Schwabe et al., 2012). Accordingly, this impairment is enhanced by the dissimilarities between the material to be encoded and the stressor, which explains the facilitator effect of stress induction on memory for negative material (Henckens et al., 2009). Taken together, these results may help to explain our finding that stress impaired the memory of pleasant pictures in the NA subjects exposed to the TSST.

In the present study, we only included young undergraduate students, allowing us to complement results described in the literature on children and middle-aged and elderly individuals. Taking into account the effects of age on the cortisol response to stress in the general population (Kudielka et al., 2009), it is very important to extend this research to include different age groups in the SP population. Furthermore, we included Sex as an important factor that was not taken into account in most of the studies carried out on this topic.

To summarize, our data show that GSP subjects did not present a higher HPA-axis response to psychosocial stress compared to healthy control subjects because their response was similar. However, GSP subjects, and especially GSP women, showed high levels of psychological discomfort. This discordance between subjective and physiological measures was also observed in a recent study with SP subjects (Klumbies et al., 2014). In this regard, the psychophysiological findings of our study support the cognitive models of SP (Clark and Wells, 1995; Rapee and Heimberg, 1997), which propose a biased processing of social information as a central mechanism in the maintenance of this disorder. In addition, no differences were found between GSP and NA subjects on immediate free recall and, 24 h later, on delayed free recall or on the recognition memory task after the TSST. Moreover, our data do not support the notion that phobic subjects selectively remember more negative aspects (Hertel et al., 2008). Thus, we do not find any deterioration or improvement in memory task performance in GSP subjects. We found, as suggested by previous studies, that women outperformed men on a declarative memory task, but our experimental design revealed that, under stress, these differences disappeared, and men and women showed similar performances on emotional memory tasks. At the moment, it is difficult to point to a mechanism that can explain a differential effect of stress and sex on free recall versus recognition memory tasks in social phobia.

In this study, we analyzed social phobia in a non-treated but clinically relevant sample, allowing the pure diagnosis of social phobia. By studying this phenomenon without the influence of medication or comorbidity with other psychiatric illnesses, we were able to disentangle the specific features associated with social fear that have the potential to affect subjective and physiological responses to stress (Foa et al., 2000). Thus, it seems that the specific features of social phobia do not affect the hormonal and subjective response to social stress.

It is worth noting that the HPA-axis displays sexual dimorphism (Kudielka et al., 2009), and this response is dependent on women's menstrual cycle phase (Kirschbaum et al., 1999; Espín et al., 2013); therefore, a limitation of our study was not including women in different phases of their menstrual cycle. The effect of stress on phobic women depending on the menstrual cycle phase has not yet been explored. We have included Sex in our study, but in future studies with a young GSP population it would be appropriate to include women in different phases of their menstrual cycle, in order to better analyze the potential sex differences.

In conclusion, our results indicate that GSP subjects do not present HPA-axis sensitization to psychosocial stress. However, GSP men showed a higher HPA-axis activity during the encoding session. Taking both results together, one could speculate that phobic men appear to interpret situations that are not evaluated as stressful by phobic women and NA subjects as more threatening or more stressful. In addition, relevant sex-related differences on both memory and subjective state measures highlight the need to control sex effects, specifically differences in sex hormones, when studying the stress response. To our knowledge, this study is the first attempt to describe how phobic young men and women respond differently to the presentation of emotional stimuli and to stress induction, and their effects on the retrieval of pictures. Importantly, our findings support the cognitive models of social phobia, with no differences found in HPA-reactivity to psychosocial stress between the GSP and NA groups. Moreover, our study highlights the importance of studying social phobia related mechanisms in subclinical samples that may display a purer manifestation of the symptoms associated with social phobia. Finally, our study shows the importance of controlling gender when studying individuals with social phobia. Further studies should explore the mechanisms underlying these differences, in order to improve our understanding of the manifestation and development of social phobia.

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Conflicts of interest

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

Contributors

All authors contributed significantly to the experimental design and to the manuscript. LE, MM, and VH designed the experiment. LE and MM conducted the experiment and collected the reported data. LE, MM, VH, AS and JGA were enrolled in data analysis and in the manuscript writing process.
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References

Andreason, J.M., Cahill, L., 2005. Sex influences on the neurobiology of learning and memory. Learn. Mem. 12, 248–266.


