Acute pre-learning stress and declarative memory: impact of sex, cortisol response and menstrual cycle phase

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Abstract

This study explores the influence of pre-learning stress on performance on declarative memory tasks in healthy young adults in relation to sex and menstrual cycle phase. The sample was composed of 119 students (32 men and 87 women) from 18 to 25 years of age. The women were tested in different hormonal stages (30 in follicular phase, 34 in luteal phase, and 23 using oral contraceptives). The participants were exposed to the Trier Social Stress Test (TSST) or a control condition. Afterwards, their memory performance was measured using a standardized memory test (Rey's Auditory Verbal Learning Test). In the control condition, all groups of women recalled more words than men, but these differences disappeared in the group exposed to TSST because men's performance on the memory test improved, but only to the level of women. In addition, our data suggest that in women the relationship between cortisol and memory can be modulated by sex hormone concentrations in men compared to women (Kajantie and Phillips, 2006; Kirschbaum et al., 1999; Lupien and McEwen, 1997). Some studies have shown that stress-induced cortisol increase was negatively related to declarative memory performance when stress was applied prior to learning (Kirschbaum et al., 1996; Wolf et al., 2001b). In contrast, Nater et al. (2007) found the opposite result: high cortisol responders to stress actually had better recall on declarative memory performance than low cortisol responders. Along the same lines, Joels et al. (2006) proposed that cortisol released around the time of learning facilitates ongoing learning processes and, thus, would predict memory-enhancing effects of stress experienced shortly before learning.

Introduction

The relationship between stress and declarative memory has been widely studied, although with contradictory results. Several studies have indicated that declarative memory can be impaired when subjects are exposed to stress before learning (Payne et al., 2006; Smeets et al., 2006), while others have found no effect (Elzinga et al., 2005; Wolf et al., 2001b) or even an enhancing effect of stress on declarative memory performance (Domes et al., 2002; Nater et al., 2007; Schwabe et al., 2008). This discrepancy has been explained by diverse factors, such as the memory phase under investigation (acquisition, consolidation or retrieval) and the time of testing (morning vs afternoon), among others (Het et al., 2005).

There is a body of literature suggesting that the release of cortisol is mainly involved in the effects of acute stress on memory performance (de Kloet et al., 1999; Het et al., 2005; Lupien and McEwen, 1997). Some studies have shown that stress-induced cortisol increase was negatively related to declarative memory performance when stress was applied prior to learning (Kirschbaum et al., 1996; Wolf et al., 2001b). In contrast, Nater et al. (2007) found the opposite result: high cortisol responders to stress actually had better recall on declarative memory performance than low cortisol responders. Along the same lines, Joels et al. (2006) proposed that cortisol released around the
was no such association for women tested in the luteal phase (Wolf et al., 2001b). The reason for this sex difference is unclear, although there has been speculation about the potential beneficial effects of female sex hormones (Wolf, 2006) and about sex differences in the cortisol response to stress (Kudielka and Kirschbaum, 2005). In a more recent study, only women using oral contraceptives were included in order to avoid the menstrual cycle effect. The results of this study showed that there were no differences between men and women in cortisol response, and no significant effect of sex was found on free recall (Schwabe et al., 2008). Both studies included men and women, but without taking into account the effect of the different phases of the menstrual cycle, a factor that should be considered when studying the impact of sex on the cortisol response to acute stress (Bouma et al., 2009; Hidalgo et al., 2012; Kirschbaum et al., 1996, 1999; Kudielka and Kirschbaum, 2005). Moreover, because the effect of cortisol on memory may differ depending on the levels of estrogen and progesterone circulating in different phases in the menstrual cycle, findings showing no relationship between stress hormones and memory in women may have resulted from combining women in hormonally distinct phases into a single group (Andreano et al., 2008).

The current study was designed to examine the effects of pre-learning stress on declarative memory performance, and we hypothesized a memory-enhancing effect of stress applied shortly before learning (Joels et al., 2006). In order to investigate the impact of stress on specific processes of memory performance, we employed the Rey Auditory Verbal Learning Test (RAVLT; Miranda and Valencia, 1997), consisting of different trials, was used for previous estimation and estimate the ovulation point, Basal Body Temperature (BBT) was recorded daily during two complete menstrual cycles by means of sublingual temperature, taken for 5 min before getting up. To analyze the BBT, the method of the “smoothed curve” (SMC) was used, as described by McCarthy and Rockette (1983, 1986).

The subjects were referred a few days before the experiment, so that they could be given a series of instructions to follow to participate in the study. The instructions were to abstain from excessive physical activity within 48 h of the experiment, any sports activities within 24 h, intake of alcohol and caffeine within 18 h, and eating 60 min before the study, and not sleep less than usual (7–8 h). Naturally cycling women were trained in the daily recording of their basal body temperature (BBT), and they were given a chart and a thermometer for this purpose. Participants were not evaluated during stressful periods (such as exam periods).

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

**Questionnaires and scales**

**Mood**

This was evaluated by the Spanish version (Sandín et al., 1999) of the PANAS (Positive and Negative Affect Schedule; Watson et al., 1988). This 20-item questionnaire assesses mood according to two dimensions: positive affect (PA: interested, excited, strong, enthusiastic, etc.) and negative affect (NA: distressed, upset, guilty, scared, etc.), with 10 items measuring each state. Participants were asked to complete the questionnaire based on how they felt at that particular moment. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely).

**Anxiety**

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

**Memory**

To measure declarative memory, the Spanish version of the RAVLT (Miranda and Valencia, 1997), consisting of different trials, was used regularly...
after exposure to the TSST. The RAVLT was administered according to its original standards: fifteen neutral words (list A) were read aloud by the examiner before each trial, followed by the subject’s free recall (A1–A5), five times consecutively; each participant had to say as many words as possible in each of the five trials. The performance on these first five trials showed the rate of learning (trials 1 to 5: learning curve). After the fifth recall, the examiner read an interference list (trial 6: list B) of 15 new words aloud, and then tested the free recall of this new list. Immediately after that, the participants were asked to recall the words from list A without the examiner reading them (trial 7: recall after interference). After a period of 30 min, participants had to recall list A again (trial 8: delayed recall).

Procedure

Experimental sessions were run in the laboratory at the university between 2 pm and 5 pm, when basal cortisol levels are low and stable (the sequence is presented schematically, see Fig. 1). Participants were tested individually. After arrival at the laboratory, the participants were asked by the experimenter whether they had followed the instructions given in the days preceding the study, and their weight and height were measured.

This study employed a between-subjects design, where participants were tested in a single session. On arrival at the laboratory, subjects were randomly assigned to either the TSST or control condition. Fifty-seven participants were exposed to the TSST, while the other sixty-two were assigned to a control condition.

TSST condition

As a psychosocial stress protocol, the TSST was employed according to the description provided by Kirschbaum et al. (1993). This test consists of a 10-min preparation phase that includes instructions for the speech, 5 min of free speech (a simulated job interview), and a 5 min mental arithmetic task in front of a committee composed of a man and a woman. The participants remained standing at a distance of 1.5 m from the committee. During the speech, each participant had to convince the committee that he/she was the perfect applicant for a vacant position (his or her ‘dream job’). Furthermore, it was announced that the participant’s performance would be recorded on a video-cassette-recorder in order to later analyze the interview and the nonverbal behavior. If the participant finished his/her speech in less than 5 min, the members of the committee asked standardized questions. Then the participants completed an arithmetic task for 5 min, and it was also videotaped. The entire procedure, including the introduction to the free speech and the preparation phase, took approximately 20 min.

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated, and baseline measures were obtained for cortisol, anxiety (STAI-S) and mood (PANAS). After the habituation phase, at time 0 they were taken to a second room (room B) and introduced to the task they would have to perform next. They received the instructions in front of the committee, and they were told that, after a preparation period, they should introduce themselves to the committee, give the speech, and do a second task. Next, the participants returned to the first room, and they had 10 min to prepare for the speech at hand.

Once the preparation phase was over, the speech and arithmetic tasks were carried out. Subjects had 15 min to recover after the tasks, and they then answered the questionnaires (STAI-S and PANAS). Subsequently, each participant performed a standardized memory test (Rey Auditory Verbal Learning Test, RAVLT), which consisted of eight trials. The participants completed the first seven trials between 15 and 25 min after the TSST had ended. After trial 7, they waited 30 min (delay period) before they continued with the memory test. After the delay period, they finished the memory test by performing trial 8 of the memory test.

Control condition

The control task was designed to be as similar as possible to the TSST without being stressful for the participants (Dickerson and Kemeny, 2004). During the 10 min preparation phase, the participants read a chapter from a book with neutral content. Next, the preparation phase was followed by 5 min of reading aloud and an arithmetic task, which consisted of counting by one for 5 min. The task was performed in the same room as the TSST, but all stressful elements were removed prior to starting it (video camera, tape recorder, committee and microphone).

Saliva sampling and biochemical analyses

The participants provided four saliva samples by depositing 5 ml of saliva in plastic vials. They took approximately 5 min to fill the vial. The samples were obtained over a 65 min period at four assessment points: t-10 (baseline), t + 5, t + 30 and t + 50 min, with reference to the start of the stressor or control task. The uncentrifuged
saliva samples were stored at −80 °C immediately upon collection, until the analyses were performed. To reduce sources of variability, all four samples taken from each participant were analyzed in the same assay. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Coat-A-Count Cort (DPC, Siemens Medical Solutions Diagnostics). Assay sensitivity was 0.5 ng/ml. Cortisol levels were expressed in nmol/l, with coefficients of intra- and inter-assay variations of less than 10%.

Data analysis

Data were checked for normal distribution and homogeneity of variance using the Kolmogorov–Smirnov and Levene tests before the statistical procedures were applied. As none of the cortisol data had a normal distribution, they were square-root-transformed values. All statistical analyses are described in detail in the Results section, with each section starting with the analysis performed. We used Greenhouse–Geisser correction when the assumption of sphericity in the ANOVA for repeated measures was not met. All post hoc comparisons were performed using the Bonferroni adjustments for multiple comparisons for the p-values. In the case of significant results, all p-values reported had a significance level < 0.05. As a measure of the effect size, we report Partial Eta Squared (η²_p).

Results

Demographic and anthropometric variables

To evaluate potential differences in demographic and anthropometric variables between the TSST vs control conditions, Student’s t-tests were conducted (see Table 1). The results showed that there were no significant differences between the two conditions on age, height, weight or body mass index (BMI).

Mood and anxiety

Three different repeated-measures ANOVAs were conducted, each focusing on one dependent measure of subjective stress (PANAS-PA, PANAS-NA, STAI) with two between-subject factors, stress condition (TSST vs control) and hormone group (luteal vs follicular vs men vs OC users), and time (pre vs post stress) as the within-subject factor. The ANOVA for PANAS-PA showed only a significant main effect for time (F (1,111) = 40.67; p < 0.001; η²_p = 0.26), with a significant post-task decrease in PA (pre vs post-task: p < 0.001). The ANOVA for PANAS-NA showed a significant main effect for stress condition (F (1, 111) = 6.39; p = 0.01; η²_p = 0.05) and for the interaction: time × stress condition (F (1, 111) = 37.16; p < 0.001; η²_p = 0.25). Concerning the time × stress condition interaction, post hoc analyses showed significant differences between the two conditions in t + 30 and t + 50, with the subjects exposed to the TSST showing greater cortisol concentrations than the subjects of the control condition (for both comparisons p < 0.001). Besides, in the exposure to TSST condition, higher cortisol concentrations were found in t + 30 with respect to other times (for all p < 0.001), and t + 50 with respect to t-10 (p = 0.008) and t + 5 (p = 0.01). Investigating the time × hormone group interaction, and considering each hormone group separately, only luteal women showed significant differences in t + 30 with respect to other times (for all p < 0.001), and men in t + 30 with respect to t + 50 (p = 0.005) (see Fig. 2).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Student’s test for descriptive characteristics of the sample for age, height, weight and body mass index (BMI) for TSST vs control conditions. The values represent mean and standard error of the mean (S.E.M.).</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 119</td>
<td>Stress condition</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>TSST</td>
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<tr>
<td>Height (m)</td>
<td>Control</td>
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<td></td>
<td>TSST</td>
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<tr>
<td>Weight (kg)</td>
<td>Control</td>
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<td></td>
<td>TSST</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>Control</td>
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<tr>
<td></td>
<td>TSST</td>
</tr>
</tbody>
</table>

Salivary cortisol

A repeated-measures ANOVA was conducted with time (t-10 vs t = 5 vs t + 30 vs t + 50) as within-subject factor and stress condition (2) and hormone group (4) as between-subject factors. The results showed significant main effects for time (F (3, 333) = 14.88; p < 0.001; η²_p = 0.11), stress condition (F (1, 111) = 24.88; p < 0.001; η²_p = 0.18), and the interactions: time × stress condition: (F (3, 333) = 25.28; p < 0.001; η²_p = 0.18) and time × hormone group (F (9, 333) = 3.05; p = 0.02; η²_p = 0.07). Concerning the time × stress condition interaction, post hoc analyses showed significant differences between the two conditions in t + 30 and t + 50, with the subjects exposed to the TSST showing greater cortisol concentrations than the subjects of the control condition (for both comparisons p < 0.001). Besides, in the exposure to TSST condition, higher cortisol concentrations were found in t + 30 with respect to other times (for all p < 0.001), and t + 50 with respect to t-10 (p = 0.008) and t + 5 (p = 0.01). Investigating the time × hormone group interaction, and considering each hormone group separately, only luteal women showed significant differences in t + 30 with respect to other times (for all p < 0.001), and men in t + 30 with respect to t + 50 (p = 0.005) (see Fig. 2).
Based on the above results, in order to statistically control the existence of a possible interaction between individual differences in baseline levels and cortisol response to acute stress, a repeated-measures ANOVA using only the two time points (t-10: baseline cortisol level and t + 30: peak cortisol level) was conducted with stress condition (2) and hormone group (4) as between-subject factors, and time (2) as within-subject factor.

The analyses showed a significant main effect for time (F(1,111) = 28.51; p < 0.001; η²_p = 0.20), stress condition (F(1,111) = 34.25; p < 0.001; η²_p = 0.23) and the interactions: time × stress condition (F(1,111) = 39.54; p < 0.001; η²_p = 0.26) and time × hormone group (F(3,111) = 4.45; p = 0.005; η²_p = 0.11). First, examining the time × stress condition interaction, subjects exposed to the TSST showed significant differences between t + 30 and t-10, p < 0.001, with higher cortisol response in t + 30. However, the subjects in the control condition did not show significant differences between t + 30 and t-10 (p = 0.49). Significant differences between the two conditions were found in t + 30 (p < 0.001), with higher cortisol responses to stress. Second, investigating the time × hormone group interaction, and considering each hormone group separately, luteal women were the only group that showed significant differences between t + 30 and t-10 (p < 0.001), with higher levels in t + 30. Moreover, only luteal women showed significant differences with follicular women in t + 30 (p = 0.05), and no significant differences with the other hormone groups (p = 0.27 for men and p = 0.34 for OC users).

Memory performance

The effect of pre-learning stress on memory performance was measured with a repeated-measures ANOVA with stress condition (TSST vs control) and hormone group (luteal vs follicular vs men vs OC users) as between-subject factors, and each RAVLT Trial (trials 1–8) as a within-subject factor.

The ANOVA revealed the main effect of trial (F(7,777) = 298.79; p ≤ 0.001; η²_p = 0.72), and the following interactions were also significant: trial × stress condition (F(7,777) = 1.96; p = 0.05; η²_p = 0.01) and stress condition × hormone group (F(3,111) = 2.97; p = 0.03; η²_p = 0.07). Decomposing the trial × stress condition interaction, post hoc analyses showed that there were greater recall after the interference list (trial 7), and greater delayed recall (trial 8) in the TSST condition than in the control condition (for both p < 0.01). We explored the stress condition × hormone group interaction, and we found that men exposed to the TSST recalled more words than men in the control condition (p = 0.001). In each group of women, there were no differences between the TSST and control conditions (p > 0.20). Finally, in the control condition, all groups of women showed better recall than men (for all p < 0.001) (see Fig. 3).

To examine whether the stress-induced mood and anxiety responses could affect memory performance, we conducted Pearson's bivariate correlation analyses of the relationships between memory and post-task measures of state anxiety (STAI-S), negative affect (PANAS-NA) and positive affect (PANAS-PA). The results showed that there were no significant correlations between anxiety, mood and memory.

Additionally, linear regression analyses were performed to test the relationships between stress condition (TSST vs control), baseline levels (t-10) and cortisol levels after the task (t + 30) with memory performance, using stress condition, t-10 and t + 30 as the predictor variables, and the memory performance trials (mean value of trials 1–5, trial 6, trial 7 and trial 8) as the dependent variables, for each level of hormone group variable (see Table 2). Luteal women and men showed a positive relationship between stress condition and the learning curve measures (trials 1–5), immediate recall (trial 7) and delayed recall (trial 8) on the RAVLT (for all p < .05). In addition, only luteal women had a negative association between t + 30 (peak cortisol level) and trials 1–5, trial 7 and trial 8 of the RAVLT (for all p < .05).

Discussion

This study compared the performance of healthy young men and women tested in different phases of the menstrual cycle on a declarative memory test when learning occurred after a stress task. The main finding was that in the control condition all the groups of women had a better performance on the RAVLT (i.e. they recalled more words) than the men. However, these sex differences disappeared in the group exposed to the TSST because psychosocial stress improved the performance of men to match that of women.

The TSST was perceived as stressful because it increased the anxiety and negative mood of the participants. These results coincide with those from other studies that observed an increase in negative mood after exposure to the TSST (Buchanan and Tranel, 2008; Schoofs and Wolf, 2011). Our results also indicate that this increase in negative mood and anxiety was not associated with changes in memory performance. In the present study, no gender differences or effects of cycle phase were found for anxiety or negative mood in response to the TSST.

In the subjects submitted to the TSST, increased levels of cortisol were found in their saliva in comparison with the subjects in the control condition. This increase was reached its maximum level 30 min after the onset of the task, followed by a gradual decrease up to 50 min later. However, in the control condition there was a
progressive decrease in cortisol concentrations from the beginning of this task. There were no group differences in cortisol concentrations after exposure to the TSST, probably because of the large individual differences in cortisol responses to stress, a prominent and well-documented phenomenon in psychoneuroendocrine studies (Mason, 1968). Although the condition × hormone group interaction was not statistically significant, Fig. 2 shows a clear trend of increased cortisol response to TSST in luteal women compared to the other groups. Further analysis of the data showed that significant differences between groups only appeared after the task (t + 30), with luteal women showing the highest cortisol levels. Physiological differences in estrogen levels probably contribute to explaining many of the differences in stress responsiveness associated with luteal women, as estrogen concentrations are high during this phase (Kajantie and Phillips, 2006). However, estrogen levels are also known to be high some days of the follicular phase, and the luteal and follicular phases differ most on progesterone levels. In this sense, it has recently been suggested that high progesterone levels are associated with higher cortisol levels (Andreano et al., 2008). Even so, these authors only included women in their study, and a different stress task was used; therefore, our data are not comparable with theirs. In any case, our findings support the notion that exposure to psychosocial stress in the laboratory did not impair word-list recall when the stress was applied prior to learning, compared to non-stressed subjects (Domes et al., 2002; Hidalgo et al., 2012; Schwabe et al., 2008; Wolf et al., 2001b).

This discrepancy in results could be explained by the memory test used (recall of a neutral word list after a brief delay), which might be less sensitive to cortisol-induced effects than previously used working or declarative memory tests (Kirschbaum et al., 1996; Wolf et al., 2001b). Moreover, some studies that have found a worse performance due to the increase in glucocorticoid levels have used exogenous administration of cortisol (de Quervain et al., 2003; Wolf et al., 2001a). As pointed out by Tollenaar et al. (2008), there are discrepancies between findings from pharmacological and psychosocial stress studies that may be related to the level of cortisol, as cortisol levels obtained in stress studies are generally much lower than the levels found after exogenous administration of cortisol. Consequently, more pronounced stress-induced cortisol increases may be required to find learning memory impairments immediately after stress exposure. However, stress not only leads to an endogenous release of cortisol, but it also invokes a whole host of other, distinct hormonal and physiological changes, and changes in these other systems might also be responsible for the differences between stress and exogenous cortisol administration.

A further regression analysis of our data showed that in luteal women there was a negative relationship between memory and peak cortisol level. As seen above, it was precisely this group of luteal women who showed a tendency toward higher cortisol response to the TSST. This result could confirm findings from other studies suggesting that in women the relationship between cortisol and memory can be modulated by sex hormone levels (Andreano et al., 2008; Kuhlmann and Wolf, 2005; Wolf et al., 2001b). In our study, this relationship could be observed during the luteal phase, when progesterone and estradiol are high, but not during the follicular phase, with comparatively lower levels of these hormones. This relationship could also be explained by the fact that the different concentrations of cortisol may have a non-linear effect on memory, as indicated by the model of the inverted U-shaped dose response function of glucocorticoids in the memory process (Conrad et al., 1999; Lupien and McEwen, 1997; Roozendaal, 2000).

In addition, some authors have concluded that studies performed in the afternoon on average yielded an effect size that was smaller than, and in the opposite direction to, the effect size found by studies performed in the morning (Het et al., 2005). Therefore, it remains to be seen whether the effect of stress in improving memory in men

<table>
<thead>
<tr>
<th>RAVLT</th>
<th>Luteal (N = 34)</th>
<th>Follicular (N = 30)</th>
<th>Men (N = 32)</th>
<th>OC users (N = 23)</th>
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<td></td>
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<td>p</td>
<td>Predictor</td>
<td>Beta</td>
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<td>t-10</td>
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<td>0.03*</td>
<td>t + 30</td>
<td>-0.12</td>
</tr>
<tr>
<td>Trial 6</td>
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<td>0.80</td>
<td>Stress</td>
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</tr>
<tr>
<td>(Interference list)</td>
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<td>0.93</td>
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</tr>
<tr>
<td>Trial 7</td>
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<td>0.82</td>
<td>t + 30</td>
<td>0.07</td>
</tr>
<tr>
<td>(Recall after interference)</td>
<td>0.45</td>
<td>0.03*</td>
<td>Stress</td>
<td>0.10</td>
</tr>
<tr>
<td>Trial 8</td>
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<td>0.04*</td>
<td>t + 30</td>
<td>-0.13</td>
</tr>
<tr>
<td>(Delayed recall)</td>
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<td>0.80</td>
<td>t-10</td>
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</tr>
<tr>
<td></td>
<td>-0.49</td>
<td>0.03*</td>
<td>t + 30</td>
<td>-0.16</td>
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can be generalized to other times of the day. Future studies should consider this issue.

From the findings of this study, we conclude that pre-learning stress reduces sex differences found in a control situation when performing a declarative memory task, since men’s performance on the memory test is improved, but only to the level of women.

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