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The impact of cortisol reactivity to acute stress on memory: Sex differences in middle-aged people

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Abstract
Stress has been identified as a main factor involved in the cognitive changes that occur during the aging process. This study investigated sex differences in the relationship between the magnitude of the acute stress-induced salivary cortisol response and memory performance among middle-aged people. To this end, 16 men and 16 women (aged 54–72 years) were exposed to the Trier Social Stress Test and a control condition in a crossover design. Afterwards their memory performance was measured using a standardized memory test (Rey's Auditory Verbal Learning Test). Only among women, there was an acute impact of stress on memory performance and a significant relationship between a higher cortisol response to the stressor and poorer memory performance in both the stress and control conditions. Additionally, a poorer memory performance was related to earlier timing of sexual maturation (age at menarche), which was also marginally related to higher cortisol reactivity to stress. These results confirm that sex is a critical factor in the relationship between cortisol and poor memory performance. Furthermore, the findings emphasize a strong link between the individual cortisol response to stress and memory functioning among postmenopausal women.

Keywords: *Cortisol, HPA-axis, memory, middle-aged people, psychosocial stress, sex differences*

Introduction

The aging process is characterized by large individual differences; some older individuals show small cognitive changes over time, whereas others deteriorate dramatically. Stress has been noted as a key factor related to these individual differences, and sex could moderate the relationship between stress and the cognitive decline during aging (McEwen 2002). Women are over-represented in diseases, such as depression or posttraumatic stress disorder (Desai and Jann 2000; Keane et al. 2006), that have a close relationship with both cognitive impairments and the hypothalamus–pituitary–adrenal axis (HPA-axis), the most important system in the control of the stress response (Sapolsky 2000; Young 2009). Moreover, emphasizing the interaction between glucocorticoids and sex hormones, timing of sexual maturation has been related to both HPA-axis activity (Lupien et al.

2009; McCormick and Mathews 2010; Romeo 2010) and risk of depression later in life (Harlow et al. 2004).

HPA-axis activity and its regulation change differently for men and women with old age (Seeman and Robbins 1994; Otte et al. 2005). Elderly persons have a stronger cortisol response to challenge than younger persons, and interestingly this age effect is especially strong among women (Otte et al. 2005). However, studies specifically investigating sex differences in the cortisol response to psychological challenge among elderly people have found mixed results (Seeman and Robbins 1994; Kudielka and Kirschbaum 2005; Kajantie and Phillips 2006). Greater cortisol reactivity in women has been reported in several studies (Seeman et al. 1995, 2001), although more recently other studies have found higher reactivity in older men (Traustadóttir et al. 2003; Kudielka et al. 2004).

Two main brain structures involved in HPA-axis function and regulation are the hippocampus and the

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prefrontal cortex (Patel et al. 2000; Herman et al. 2005) which are both related to several types of memory, such as declarative and working memory (Scoville and Milner 1957; Galloway et al. 2008). A large body of research, usually carried out with young participants, has demonstrated that stress can influence memory processes, although this influence depends on several factors, such as the type and phase of the memory process tested or the emotional valence of the material to be remembered (McEwen 2002; Lupien et al. 2005, 2007; Sandi and Pinelo-Nava 2007). Furthermore, studies using acute administration of synthetic glucocorticoids have described an inverted U-shaped dose-response curve between glucocorticoid concentrations and declarative memory (de Kloet et al. 1999; Domes et al. 2005) or working memory (Lupien et al. 1999).

With stress, many other psychological and physiological changes occur that do not happen with artificial glucocorticoid intake, including mood changes or autonomic activation (Lupien and Schramek 2006). Therefore, standardized laboratory procedures to provoke a consistent stress response have been used to study the effects of stress on memory function, but they have not always yielded consistent results. Most of these studies have been performed with young participants, and they have found impairing effects (Jelicic et al. 2004; Payne et al. 2006; Smeets et al. 2006), no effects (Domes et al. 2004), and even enhancing effects (Smeets et al. 2007; Schwabe et al. 2008) on memory when stress was provoked prior to learning. To our knowledge, few studies have been performed with older people, and the results of these studies are also unclear. When declarative memory was tested after exposure to a stress task, no effects were found in women from 41 to 69 years of age (Bohnen et al. 1990), and from 32 to 68 years of age (Domes et al. 2002). In contrast, Lupien et al. (1997) reported that stress induced a decrease in memory performance in elderly men and women (62–83 years old). These studies examined only women or a mixed-sex group, so that it was not possible to detect sex differences in the impact of stress on memory. However, it has been proposed that sex hormones could moderate glucocorticoid effects on memory (McEwen 2002; Shors 2006; Andreano et al. 2008), and the amygdala, a brain structure with estrogen receptors (Alves and McEwen 1999), has also been associated with the effects of glucocorticoids on memory (de Quervain et al. 2009). Indeed, evidence indicates that sex differences in the relationship between stress and memory may be especially important when studying elderly persons in particular. For example, in a 4-year cross-sectional study, Seeman et al. (1997) found that only elderly women, and not men, with increasing baseline cortisol concentrations over time had poorer declarative memory performance. Furthermore, Wolf et al. (2005) found that elderly women with subjective memory complaints had greater 12-h urinary cortisol concentrations than those without memory complaints, while no such differences were observed among elderly men. Previously, Wolf et al. (1998) reported that the exposure to a laboratory stressor impaired recall more for elderly women than for elderly men.

The focus of the current study was to investigate the moderating role of sex on cortisol responses to an acute psychosocial stressor and its relation with memory performance in middle-aged persons. The participants were exposed to two conditions in a crossover design. In the stress condition, the Trier Social Stress Test (TSST, Kirschbaum et al. 1993) was used. In the control condition, the participants were asked to solve a task designed to induce a similar mental workload and global physical activation to the stress task. In order to investigate the impact on specific processes of memory performance, we employed the Rey Auditory Verbal Learning Test (RAVLT), which provides several memory indicators (Lezak et al. 2004). Based on the results of other studies in aging populations using the TSST (Kudielka and Kirschbaum 2005; Kudielka et al. 2009) we expected a greater cortisol response among men than among women. In addition, we hypothesized that the impact of cortisol reactivity to stress on memory would be different between men and women.

Methods

Participants

The final sample comprised 32 participants (16 men and 16 women) from 54 to 72 years of age (total sample: $M = 62.09$, SEM = 0.85 years; Men: $M = 60.50$, SEM = 1.23 years; Women: $M = 63.69$, SEM = 1.07 years). Most of the participants (91%) had an educational level beyond high school, and their subjective socioeconomic status (Subjective SES scale: Adler et al. 2000) was medium-high. All the men were married, while the women were either married (50%) or widowed (50%). The mean body mass index (BMI) was 26.49, $SEM = 0.54$ (Men: $M = 27.05$, SEM = 0.48; Women: $M = 25.93$, SEM = 0.96). There were no sex differences in age, educational level, SES or BMI (all $p > 0.1$). All the female participants were postmenopausal and had their last menstrual period at least 4 years before. None of these women were receiving estrogen replacement therapy, and none of the men were using anti-androgens or undergoing androgen replacement therapy.

Participants belonged to a study program at the University of Valencia for people older than 50 years of age. For subject recruitment, announcements were posted and informative talks were held in the various departments of the University campus. One hundred and thirteen respondents were interviewed and completed a questionnaire to check whether they met the study prerequisites. In order to avoid the large number of potentially confounding factors that could

interfere with the stress response or with cognitive functioning, we selected a homogeneous healthy sample using highly restrictive criteria. The criteria for exclusion were smoking more than five cigarettes a day; alcohol or other drug abuse; visual or hearing problems, presence of a cardiovascular, endocrine, neurological or psychiatric disease; having been under general anesthesia once or more than once in the past year; and the presence of a stressful life event during the last year. Participants were excluded if they were using any medication directly related to cardiac, emotional, or cognitive function, or one that was able to influence hormonal levels, such as glucocorticoids or β–blockers. In addition, women answered questions concerning reproductive lifetime events (e.g. age at menarche, gynecological problems).

Participants meeting the criteria were contacted by telephone and asked to attend two sessions that took place in a laboratory at the Faculty of Psychology. No payment was made for participation. 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 to consume alcohol since the night before the session. Additionally, they were instructed to drink only water and not eat, smoke, or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, two hours prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct of the study were approved by the University of Valencia Ethics Research Committee. All the participants received verbal and written information about the study and signed an informed consent form.

Procedure

This study employed a within-subject design with two completely randomized and counterbalanced conditions in two separate sessions: a stress condition and a control condition, with about 2 weeks between sessions. The sessions consisted of several phases of equal duration for both conditions (Figure 1). Both sessions took 1 h and 50 min to complete, and they were held between 16.00 and 20.00 h. Each participant started their two sessions at the same hour. Upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked to see whether they had followed the instructions given previously (see Participants).

Stress condition. The participants were subjected to the TSST. The stress task consisted of 5-min of free speech (a simulated job interview) and a 5-min arithmetic task, and it was performed in front of a committee composed of a man and a woman. The participants remained standing at a distance of 1.5 m from the committee. Additionally, a video camera and a microphone were clearly visible. Both the speech and arithmetic tasks were filmed.

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated. Five minutes after the start of this phase, baseline measures were obtained for anxiety (State Anxiety Inventory (STAI-S)) and mood (Positive and Negative Affect Schedule (PANAS)). After the habituation phase, the introduction phase started (duration 5 min). In this phase, the participants were informed about the procedure for the stress task. They received the instructions in front of the committee in the same room where the task took place. Next, the participants had 10 min to prepare for the task. A first saliva sample (0 min pre-stress) was taken 25 min after their arrival at the laboratory, trying to minimize an anticipatory cortisol increase, because elderly people are strongly reactive to a testing environment (Lupien et al. 2007).

Following the preparation phase, the stress task was carried out. Subjects had 20 min to recover after the stress task, and they answered three questionnaires (Situational Appraisal, STAI-S, and PANAS, see
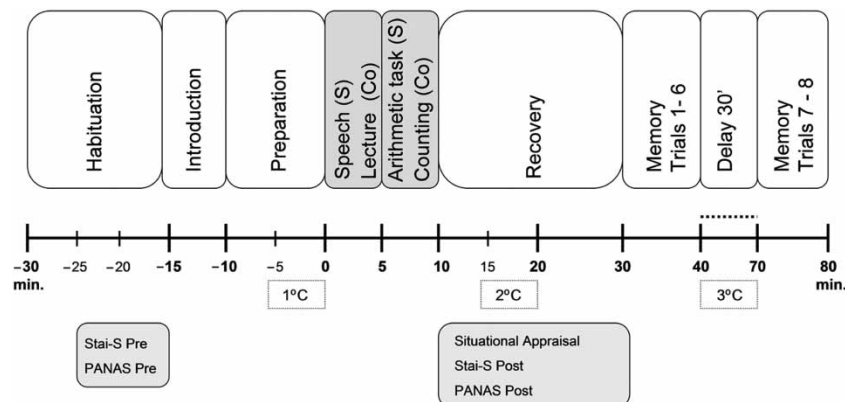

Figure 1. Schedule for the stress (S) and control (Co) conditions. (1°, 2°, 3° C: sequential salivary cortisol sampling; STAI-S, State Anxiety Inventory form S; PANAS, Positive and Negative Affect Schedule).

Questionnaires and scales) and provided a second saliva sample (20 min post-stress). Then each participant performed a standardized memory test which consisted of eight trials (RAVLT, see Questionnaires and scales). The participants completed the first six trials between 30 and 40 min after the TSST. After trial 6, they waited 30 min (delay period) before they continued with the memory test. During this waiting period, they provided a last saliva sample (45 min post-stress). After the delay period, they finished the memory test with trials 7 and 8 and, finally, were debriefed.

Control condition. The control condition was similar to the experimental condition, except that the stressful task was replaced by a control task. This task was designed to be similar to the stress task in mental workload and global physical activity, but without the main components capable of provoking stress, such as evaluative threat and uncontrollability (Dickerson and Kemeny 2004). The control task consisted of 5 min of reading aloud and 5 min of counting without being in front of an audience. In the preparation phase, the participants did not prepare for their task, but instead they read a book with a neutral content. The timing of the saliva samples, the questionnaires used, and the phase durations were the same for the two conditions.

Questionnaires and scales

Situational appraisal. Participants were asked about the stress task according to the five following aspects: stress, difficulty, frustration, effort, and motivation (e.g. *How much effort did the task require?*). The questions used were formulated based on previous studies on this topic (Baggert et al. 1996; Gonzalez-Bono et al. 2002). Subjects responded to each question on a 5-point Likert scale (not at all = 1, to extremely = 5).

Mood. This was evaluated by the Spanish version (Sandín et al. 1999) of the PANAS (Watson et al. 1988). This 20-item questionnaire assesses mood according to two dimensions: Positive affect (PA: e.g. *interested, excited, strong, enthusiastic*), and Negative affect (NA: e.g. *distressed, upset, guilty, scared*) 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). Sandín et al. (1999) reported a high internal consistency for the Spanish version, with a Cronbach's alpha for PA ranging from 0.87 to 0.89 and for NA from 0.89 to 0.91.

Anxiety. To assess state anxiety, the Spanish version of the STAI 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 answers. The Spanish version of the scale has a Cronbach's alpha ranging from 0.90 to 0.93 (Seisdedos 1988).

Memory. To measure declarative memory, the Spanish version of RAVLT was used (Miranda and Valencia 1997). This test has several versions, and for each participant a different version of the RAVLT was used in their second session to avoid learning effects. The order of the two versions was randomized and counterbalanced. The RAVLT is composed of different trials. In the first five trials, the experimenter read aloud a target list of 15 neutral words, and each participant had to repeat as many words as possible in each of the five trials. The performance on these first five trials reflects the rate of learning (trials 1–5: *Learning curve*). After trial 5, the experimenter read aloud an interference list of 15 words and tested the retention of these new words. Following this step, the participants were requested to recall the words from the target list (trial 6: *Recall after interference*); after a delay of 30 min, they had to recall them a second time (trial 7: *Delayed recall*). In trial 8 (*Recognition*), the participants had to recognize the memorized words from a list presented verbally containing 15 new and 15 previously learned words. Trial 8 was divided into two different scores: *Hits*, the number of words correctly recognized as being on the target list; and *False alarms*, the number of words incorrectly recognized as being on the target list.

Cortisol assay

Participants provided three saliva samples by depositing 5 ml of saliva in plastic vials. They took approximately 5 min to fill the vial. The samples were frozen at $-80°C$ until the analyses were done. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Coat-A-Count Cort (DPC, Siemens Medical Solutions Diagnostics). Assay sensitivity was 0.5 ng/ml. For each subject, all the samples were analyzed in the same trial. The within and inter-assay variation coefficients were all below 8%.

Statistical analyses

Student's *t*-tests were used to investigate sex differences in the demographic variables. ANOVAs for repeated measures were used to assess differences in the appraisal of the two tasks, differences in the baseline of the variables measured, and the effects of both the stress and control tasks on mood, anxiety, and salivary cortisol concentrations. We employed condition (stress vs. control) as a within-subject factor. For the changes in cortisol concentrations, we added Time (0, 20, and 45 min) as a within-subject

factor. To assess sex differences, we included sex as a between-subject factor.

The memory test used (RAVLT) provides one score for each trial performed, which consists of the number of correct words recalled in each trial. In trials 1–7, the words from the same target list have to be recalled; for this reason, we performed an ANOVA for repeated measures. We used condition (stress vs. control) and trials (trials 1–7) as within-subject factors and sex as a between-subject factor. To analyze the effects on recognition (trial 8), we used d-prime (d'), which is the difference between the standardized proportion of correct hits and the standardized proportion of false alarms. An ANOVA for repeated measures was performed using d-prime as a dependent variable, condition (stress vs. control) as a within-subject factor, and sex as a between-subject factor.

To assess whether the cortisol response to the stress task was related to memory performance, we correlated the *cortisol reactivity* to stress with the number of words the participants could recall in the RAVLT trials of the stress condition and the control condition. To take into account the individual differences in the cortisol secretory response to stress, as well as in the pattern of cortisol release in a control situation (Lovallo et al. 2010), the *cortisol reactivity* to stress was defined as the difference between the area under the curve with respect to increase (AUCi) in salivary cortisol concentration in the stress condition, and the AUCi in the control condition (in this case 'index of decrease') (Pruessner et al. 2003). We also correlated the age at menarche of the women with the *cortisol reactivity* to stress and memory performance in both conditions. Since a normal distribution could not be expected in a small sample size, Spearman's rank correlation tests were used.

One male participant was removed from the statistical analyses on anxiety, and one female participant was removed for analysis of the memory data, owing to problems in the application of the respective tests. In addition, one multivariate outlier (male participant) was removed on the basis of the $p < 0.001$ criteria for Mahalanobis distance in the cortisol samples.

We checked for order effects (whether the stress or control condition was first) using an ANOVA for repeated measures, which did not reveal any effect of order (all $p > 0.2$). We used Greenhouse-Geisser correction when the requirement of sphericity in the ANOVA for repeated measures was violated. *Post hoc* planned comparisons were performed using the Bonferroni adjustments for the p-values. All p-values reported are two-tailed, and the level of significance was marked at < 0.05. When not otherwise specified, results shown are means $\pm$ SE of means (SEM). We used SPSS 15.0 to perform the statistical analyses.

Results

Psychological responses

Situational appraisal. The stress task was perceived as more stressful ($F(1,29) = 55.242, p < 0.001$), difficult ($F(1,29) = 106.436, p < 0.001$), and frustrating ($F(1,29) = 43.948, p < 0.001$), and as requiring more effort ($F(1,29) = 113.361, p < 0.001$) than the control task. There were no differences in motivation for the stress and control tasks, $F(1,29) = 0.574, p = 0.455$. No interaction was found between sex and condition in any of the variables evaluated (for all $p > 0.3$), although men perceived both tasks as more stressful than women, $F(1,29) = 7.600, p = 0.010$.

Mood and anxiety. There were no baseline differences between the stress and control conditions for mood and for anxiety (all $p > 0.6$). Positive effect was not different after the two tasks, $F(1,29) = 1.234, p = 0.276$, but participants did report a stronger negative mood after the stress task than after the control task (PANAS NA score after the stress task: 14.35 ± 0.94, and after the control task: 11.37 ± 0.44), $F(1,29) = 12.416, p = 0.001$. Furthermore, anxiety scores after the stress task were higher than after the control task (STAI-S score after the stress task: 13.49 ± 1.90, and after the control task: 8.87 ± 0.91), $F(1,28) = 9.903, p = 0.004$. No sex differences were found for mood or anxiety (for all $p > 0.1$).

Salivary cortisol responses

The repeated measures ANOVA with salivary cortisol concentration as the dependent variable showed main effects for condition (stress vs. control): $F(1,29) = 22.389, p < 0.001$; time $(0, +20$ and $+45$ min): $F(1.39,40.21) = 13.879, p < 0.001$; and their interaction: condition $\times$ time: $F(1.29,37.54) = 21.874, p < 0.001$. *Post hoc* analyses showed that baseline salivary concentrations of cortisol were similar for both the stress and control conditions ($p = 0.481$). In the stress condition, cortisol concentration increased after exposure to the TSST ($p < 0.001$), and it remained higher than baseline until 45 min after the onset of the stress task ($p < 0.001$). For the control condition, cortisol concentrations decreased during the consecutive measures, in accordance with the cortisol circadian rhythm.

The factor sex did not reach statistical significance ($F(1,29) = 3.101, p = 0.089$), nor did the three-factor interaction (condition $\times$ time $\times$ sex: $F(1.29,37.54) = 2.767, p = 0.095$). Based on the sex differences observed in the literature and on our own hypothesis, we did *post hoc* planned comparisons that revealed different patterns of cortisol release for both men and women in the stress and control conditions (Figure 2). For men, cortisol concentrations were higher 20 min
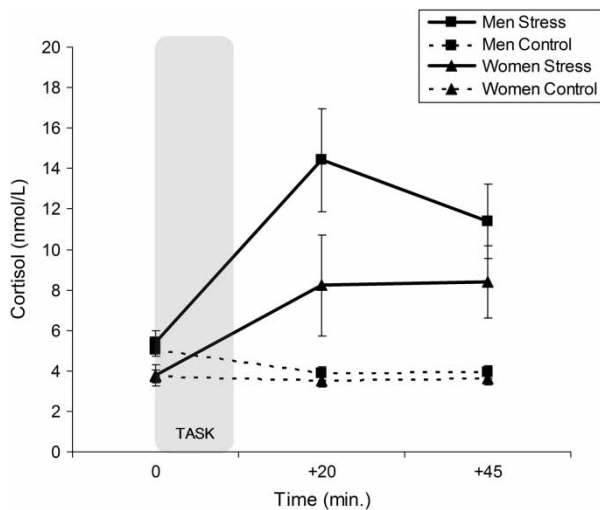
Figure 2.   Salivary cortisol concentrations in the stress (TSST) and control conditions. Cortisol concentrations in the men ($N = 15$) increased sharply ($p = 0.001$) and started to decrease at the end of the session ($p = 0.047$). Cortisol concentration in the women ($N = 16$) was not significantly increased at $+20$ min ($p = 0.138$), but was greater than baseline in the last sample ($p = 0.014$). In the control condition, while cortisol concentrations decreased in the men ($p = 0.001$), concentrations were unchanged in the women ($p > 0.99$).

after the onset of the stress task compared to the baseline ($p = 0.001$). Following this increase, their cortisol concentrations started to decrease ($p = 0.047$), although without reaching baseline in the last saliva sample ($p = 0.002$). In the control condition, cortisol decreased from baseline to the last saliva sample ($p = 0.001$). By contrast, women had a different cortisol release pattern from men. In the stress condition, their cortisol concentrations on average rose from baseline to 20 min after the onset of the stress task, but this increase did not reach statistical significance ($p = 0.138$). However, 45 min after the stress task, their cortisol

concentrations were higher than baseline ($p = 0.014$). In the control condition, the cortisol concentrations of the women did not change for any of the three saliva samples (for all, $p > 0.99$). In addition, men and women differed in their baseline cortisol concentrations. Men had higher baseline cortisol than women in the stress condition ($p = 0.045$) and the control condition ($p = 0.007$).

Memory performance

Stress vs. control condition. The repeated measures ANOVA with memory as the dependent variable revealed the main effect of trials, $F (3.17, 88.66) = 73.461$, $p < 0.001$, and although marginally significant, an interaction between the three factors, condition, trials, and sex, $F (6,168) = 2.070$, $p = 0.059$. *Post hoc* analyses showed that regardless of the condition, there was a positive learning curve across the first five trials. In almost every consecutive trial, more words were remembered ($p < 0.001$), except between trials 3 and 4, ($p = 0.086$). The participants could recall fewer words in the trial performed after the interference list (trial 6) than before the interference list (trial 5), $p < 0.001$. The delay period did not affect the recollection of words, because the participants could recall a similar number of words after the 30-min delay (trial 7) as before the delay period (trial 6), $p = 0.596$.

The interaction between the three factors was further investigated (Figure 3). We found that women recalled more words in trial 1 of the stress condition than in the same trial of the control condition ($p = 0.008$), but they recalled fewer words in trial 6 of the stress condition than in the same trial of the control condition ($p = 0.029$). However, for men there were no differences between the stress and control condition trials ($p > 0.2$).
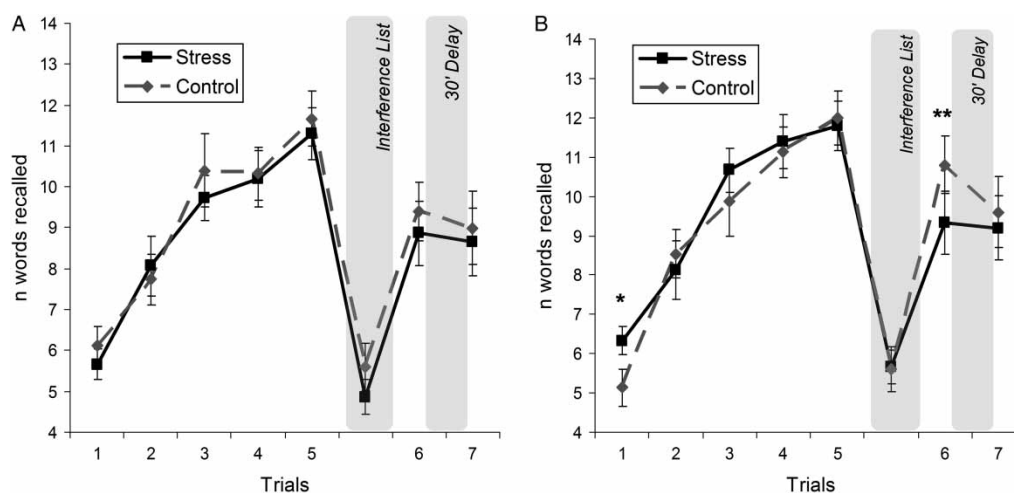

Figure 3.   Number (n) of words recalled by (A) men ($N = 15$) and (B) women ($N = 15$) in each trial of the RAVLT for the stress (TSST) and control conditions. Repeated measures ANOVA showed no differences on memory trials between conditions among men. However, women in the stress condition recalled more words in trial 1 (*$p = 0.008$), but fewer words in trial 6 (**$p = 0.029$), than in the same trials in the control condition. Values are mean $\pm$ SEM.

Table I.   Spearman correlations between cortisol reactivity to the stress task and the number of words recalled in RAVLT trials in the stress and control conditions for men ($N = 15$) and women ($N = 15$).

	Cortisol reactivity to the stress task (TSST)							
	Men				Women			
	Stress		Control		Stress		Control	
RAVLT trials	r	p	r	p	r	p	r	p
Trial 1	−0.02	ns	0.10	ns	−0.44	0.10	−0.50	0.05
Total learning (ΣT1 to T5)	−0.13	ns	0.05	ns	−0.52	0.05	−0.54	0.04
Trial 6	0.20	ns	0.34	ns	−0.60	0.02	−0.25	ns
Trial 7	0.06	ns	0.08	ns	−0.59	0.02	−0.22	ns
Total trials (ΣT1 to T7)	−0.01	ns	−0.02	ns	−0.53	0.04	−0.43	ns
Recognition d-prime	0.07	ns	0.13	ns	−0.33	ns	0.05	ns

Notes: Cortisol reactivity was the difference between the AUCi in the Stress condition, and the AUCi in the Control condition. While in men, the cortisol reactivity was not related to memory performance, among women greater cortisol reactivity was related to poorer memory performance in both conditions.

Finally, the repeated measures ANOVA with recognition (trial 8) as the dependent variable did not show main effects for condition and sex, nor was there an interaction between these two factors (all $p > 0.3$).

Cortisol reactivity to stress and memory performance. The correlations between cortisol reactivity to the stress task and memory performance are shown in Table I. Among men, no significant correlations were found for memory performance in the stress or control condition (for all $p > 0.2$). However, among women, cortisol reactivity to the stress task was negatively correlated with memory performance in the stress condition, and also in the control condition. Hence, the women who reacted to the stress task with large

increases in cortisol concentrations had a worse memory performance in both conditions.

In addition, we found positive correlations between age at menarche ($M = 12.25$, SEM = 0.57 years; *Range:* 9–17 years) and memory performance in the stress condition (trials 1, 6, and 7, total trials and recognition d-Prime: r between 0.535 and 0.602, p between 0.01 and 0.04) and in the control condition (trials 6 and 7, total trials: r between 0.532 and 0.556, p between 0.03 and 0.04) (Figure 4). The correlation between age at menarche and cortisol reactivity was apparently negative, although non-significant ($r = -0.317$, $p = 0.2$). However, after excluding one woman who had an unusually late menarche (17 years), the correlation was marginally significant ($r = -0.484$, $p = 0.06$).
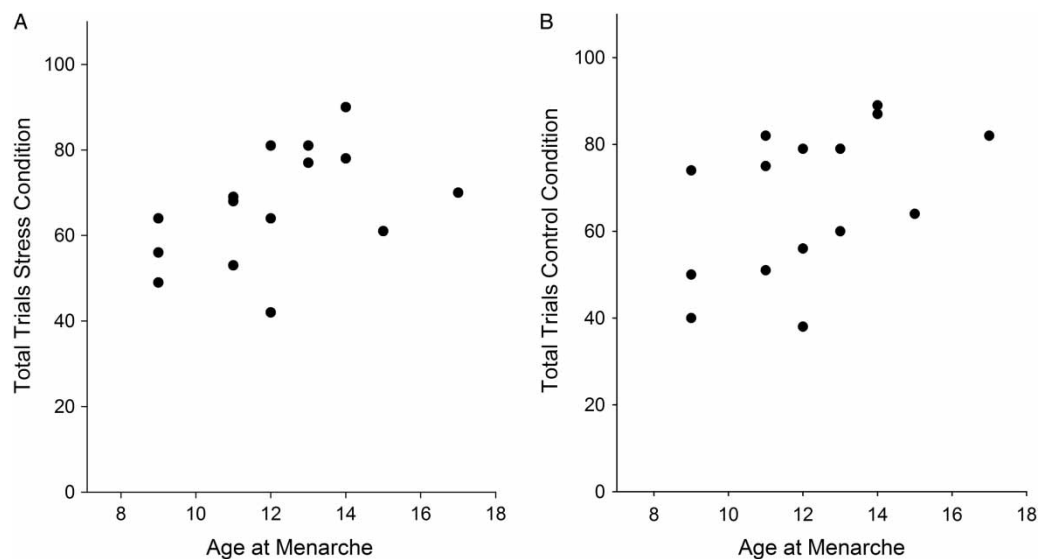

Figure 4.   Scatter plot of timing of sexual maturation (age at menarche) and total trials (ΣT1 to T7) of the RAVLT ($N = 15$). The memory performance was poorer with decreased age at menarche in the stress (A) ($r = 0.535$, $p = 0.04$) and control conditions (B) ($r = 0.532$, $p = 0.04$).

Discussion

This study compared the performance on a declarative memory test when learning occurred after a stress task (TSST) or after a control task in a group of healthy middle-aged men and women. The main findings were threefold. First, the stress had an acute impact on memory processes only among women. Second, and independently of the acute effects of stress on memory, we found a negative relationship between cortisol reactivity to stress and declarative memory performance again only in women. Finally, the timing of the women's sexual maturation (age at menarche) was related to their memory performance, and, as a trend, to their cortisol reactivity to stress.

The psychosocial stress test (TSST) was perceived as stressful, and it provoked psychological changes, because the anxiety and negative mood of both men and women were increased. Moreover, the TSST triggered an increase in cortisol release that was marginally different for men and women. Based on previous studies that reported sex differences in the cortisol response to the TSST and other kinds of stressors (Seeman et al. 1995, 2001; Traustadóttir et al. 2003; Kudielka et al. 2004), we further explored this difference. In our study, the salivary cortisol concentrations of men increased sharply in response to the stress task, but they also started to decrease at the end of the session. The women responded differently, because their cortisol level increased more slowly after the stress task, but they maintained the increased concentrations until the end of the schedule. Additionally, in the control condition, while cortisol concentrations decreased in men, women maintained similar concentrations from the beginning to the last saliva sample. These cortisol differences agree with the notion that elderly women display a more prolonged HPA-axis response to challenge than men because they are more predisposed to the loss of HPA-axis resiliency with age and, therefore, show a decrease in HPA-axis feedback sensitivity (Seeman and Robbins 1994). However, in our study neither men nor women had returned basal concentrations 45 min after the onset of the TSST. Hence, to further test this hypothesis, it will be necessary in future research to examine a longer recovery time.

Concerning our main goal, we found that the stress-induced response had an acute impact on memory performance, but only in women. Most interestingly, among the women the stressor had two different effects. First, in the stress condition women could recall more words in the first trial of the RAVLT than in the control condition. Second, the recall of words was actually impaired when it was tested on trial 6. The first trial is a measure of immediate word span under overload conditions, because the number of words presented (15) greatly exceeds the number a person can retain at once. The score achieved on this first trial has an important attention component (Lezak et al. 2004). By contrast, trial 6 is the first trial that measures recall without the target list being presented immediately before the onset of the trial, and it takes place after the presentation of an interference list. Thus, both effects appear to be within the domain of working memory, even though the cognitive demands of these two trials are very different. According to the original proposal of the Yerkes and Dodson law, a high level of arousal can enhance learning on an easy task but impair learning on a difficult task (Yerkes and Dodson 1908; see also Diamond et al. 2007). Trial 1 requires only the storage of words for a short period of time. Trial 6, however, requires storage and executive processes, because the interference list has to be inhibited, while the target list is recalled. The effect observed in trial 1 was not correlated with the cortisol increase provoked by the stress task. We consider that this effect could be explained by an enhancing effect of stress on attention that improved the number of words retained. However, the effect observed in trial 6 was related to the women's cortisol response to the stress induction. This response coupled with a more complex task could have impaired the executive processes of working memory by worsening the inhibition of the interference list and the retrieval of the target list. When new learned material interferes with the recall of material previously learned, retroactive interference occurs, which has been linked to prefrontal cortex functioning (Dewar et al. 2007). Elderly people seem to be especially vulnerable to this type of interference, because they show a sustained activation of irrelevant stimuli that enter their working memory (Hedden and Park 2001). We found that under high concentrations of cortisol, this failure to inhibit could be heightened in middle-aged women, but not in men. Previous studies also failed to find any acute effect of stress or glucocorticoid administration on working memory among middle-aged men. For example, Wolf et al. (2001) found that cortisol administration decreased the performance on a working memory task (Digit Span) in young men, but not in elderly men.

Furthermore, when we explored the individual differences in cortisol reactivity to stress and its influence on memory, we found that regardless of the condition, only among women was a high cortisol response to the stress associated with a poorer memory performance. The effect in the control condition cannot be explained by the concentrations of cortisol at the moment of the memory testing, because cortisol level was not elevated. These findings coincide with those of Lupien et al. (1997), who found in a sex-mixed group (7 men and 7 women) that high-cortisol responders had poorer memory performance than non-responders both before and after the exposure to stress. These findings contrast, however, with those of Domes et al. (2002), who found better memory

performance in female high-cortisol responders than in low responders. This divergence could be explained by methodological differences, because Domes et al. (2002) also included premenopausal women in their study, and age and menstrual cycle can be important confounding factors in the relationship between cortisol reactivity and memory performance.

Apart from sex differences in HPA-axis feedback sensitivity, other biochemical mechanisms could underlie the sex effects observed in our study. For example, recent research has shown sex differences in the activity of 11 β-hydroxysteroid dehydrogenase type 1 (Vierhapper et al. 2007), an enzyme that reactivates glucocorticoids and modulates tissue exposure to glucocorticoid activity (Holmes and Seckl 2006). Therefore, cortisol concentration may not be the only factor determining the effects of HPA-axis reactivity on memory performance. Furthermore, it has been hypothesized that estrogens could work to contain the HPA-axis and counteract some of the potentially damaging actions of glucocorticoids on nerve cells (McEwen 2002). However, the menopause is characterized by a dramatic reduction in estrogen production, and no such drastic change occurs in men. Indeed, the relationship between sex hormones and the HPA-axis could be more extensive. Timing of sexual maturation is being considered as an important predictor of adult and postmenopausal health (Peeters et al. 1995; Laitinen et al. 2001; Mucci et al. 2001) and it has been related with allostatic load in adulthood (Allsworth et al. 2005). In the current study, early age at menarche was associated with poorer declarative memory performance and, as a trend, with higher cortisol reactivity to stress. Early age at menarche has been associated with early childhood stress (Ellis and Garber, 2000) and according to Lupien et al. (2009), the impact of early adversity when the brain is developing could explain some of the differences observed during aging. However, other explanations are possible, such as life-time exposure to estrogens. To disentangle the ultimate mechanisms of these relationships, more research is clearly warranted.

In the current study, the stressor was applied prior to learning, similarly to other studies performed mainly with young adults (Domes et al. 2004; Jelicic et al. 2004; Payne et al. 2006; Smeets et al. 2006, 2007; Schwabe et al. 2008). Hence, this design does not make it possible to distinguish between the effects of cortisol on the different phases of the memory process. It is possible that the enhancing effects of cortisol on consolidation may have been nullified by the impairing effects on retrieval. Another limitation of our study was the sample size. Trying to avoid as many confounding factors as possible, we were conservative and selected a homogeneous healthy sample for their age. Consequently, the number of participants was considerably reduced. It would be advisable to extend this research to a more general population including various types of aging-related diseases and medication use.

Increased basal cortisol levels over time have been associated with cognitive decline (Lupien et al. 1998). The present study further extends these findings by showing that individual differences in the cortisol reactivity to stress have a strong link to memory performance in later life, and that sex is a critical moderating factor of this relationship.

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References

Adler NE, Epel ES, Castellazzo G, Ickovics JR. 2000. Relationship of subjective and objective social status with psychological and physiological functioning: Preliminary data in healthy, white women. Health Psychol 19(6):586–592.

Allsworth JE, Weitzen S, Boardman LA. 2005. Early age at menarche and allostatic load: Data from the Third Health and Nutrition Examination Survey. Ann Epidemiol 15(6):438–444.

Alves SE, McEwen BS. 1999. Estrogen and brain function: Implications for aging and dementia. Handb Exp Pharmacol 135:315–328.

Andreano JM, Arjomandi H, Cahill L. 2008. Menstrual cycle modulation of the relationship between cortisol and long-term memory. Psychoneuroendocrinology 33(6):874–882.

Baggert HL, Saab PG, Carver CS. 1996. Appraisal, coping, task performance, and cardiovascular responses during the evaluated speaking task. Pers Soc Psychol Bull 22(5):483–494.

Bohnen N, Houx P, Nicolson N, Jolles J. 1990. Cortisol reactivity and cognitive performance in a continuous mental task paradigm. Biol Psychol 31(2):107–116.

de Kloet ER, Oitzl MS, Joëls M. 1999. Stress and cognition: Are corticosteroids good or bad guys? Trends Neurosci 22:422–426.

de Quervain DJ-F, Aerni A, Schelling G, Roozendaal B. 2009. Glucocorticoids and the regulation of memory in health and disease. Front Neuroendocrinol 30:358–370.

Desai HD, Jann MW. 2000. Major depression in women: A review of the literature. J Am Pharm Assoc (Wash) 40(4):525–537.

Dewar MT, Cowan N, Sala SD. 2007. Forgetting due to retroactive interference: A fusion of Müller and Pilzecker's (1900) early insights into everyday forgetting and recent research on anterograde amnesia. Cortex 43(5):616–634.

Diamond DM, Campbell AM, Park CR, Halonen J, Zoladz PR. 2007. The temporal dynamics model of emotional memory processing: A synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. Neural Plast 2007:60803.

Dickerson SS, Kemeny ME. 2004. Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. Psychol Bull 130(3):355–391.

Domes G, Heinrichs M, Reichwald U, Hautzinger M. 2002. Hypothalamic–pituitary–adrenal axis reactivity to psychological stress and memory in middle-aged women: High responders exhibit enhanced declarative memory performance. Psychoneuroendocrinology 27(7):843–853.

Domes G, Heinrichs M, Rimmele U, Reichwald U, Hautzinger M. 2004. Acute stress impairs recognition for positive words–Association with stress-induced cortisol secretion. Stress 7(3):173–181.

Domes G, Rothfischer J, Reichwald U, Hautzinger M. 2005. Inverted-U function between salivary cortisol and retrieval of verbal memory after hydrocortisone treatment. Behav Neurosci 119:512–517.

Ellis BJ, Garber J. 2000. Psychosocial antecedents of variation in girls' pubertal timing: Maternal depression, stepfather presence, and marital and family stress. Child Dev 71(2):485–501.

Galloway EM, Woo NH, Lu B. 2008. Persistent neural activity in the prefrontal cortex: A mechanism by which BDNF regulates working memory? Prog Brain Res 169:251–266.

Gonzalez-Bono E, Moya-Albiol L, Salvador A, Carrillo E, Ricarte J, Gomez-Amor J. 2002. Anticipatory autonomic response to a public speaking task in women: The role of trait anxiety. Biol Psychol 60(1):37–49.

Harlow BL, Cohen LS, Otto MW, Spiegelman D, Cramer DW. 2004. Early life menstrual characteristics and pregnancy experiences among women with and without major depression: The Harvard study of moods and cycles. J Affect Disord 79:167–176.

Hedden T, Park D. 2001. Aging and interference in verbal working memory. Psychol Aging 16(4):666–681.

Herman JP, Ostrander MM, Mueller NK, Figueiredo H. 2005. Limbic system mechanisms of stress regulation: Hypothalamo–pituitary–adrenocortical axis. Prog Neuropsychopharmacol Biol Psychiatry 29(8):1201–1213.

Holmes MC, Seckl JR. 2006. The role of 11β-hydroxysteroid dehydrogenases in the brain. Mol Cell Endocrinol 248(1–2):9–14.

Jelicic M, Geraerts E, Merckelbach H, Guerrieri R. 2004. Acute stress enhances memory for emotional words, but impairs memory for neutral words. Int J Neurosci 114(10):1343–1351.

Kajantie E, Phillips DIW. 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 31(2):151–178.

Keane TM, Marshall AD, Taft CT. 2006. Posttraumatic stress disorder: Etiology, epidemiology, and treatment outcome. Annu Rev Clin Psychol 2(1):161–197.

Kirschbaum C, Pirke K, Hellhammer DH. 1993. The "Trier Social Stress Test": A tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28(1–2):76–81.

Kudielka BM, Kirschbaum C. 2005. Sex differences in HPA axis responses to stress: A review. Biol Psychol 69(1):113–132.

Kudielka BM, Buske-Kirschbaum A, Hellhammer DH, Kirschbaum C. 2004. HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: Impact of age and gender. Psychoneuroendocrinology 29(1):83–98.

Kudielka BM, Hellhammer DH, Wüst S. 2009. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. Psychoneuroendocrinology 34(1):2–18.

Laitinen J, Power C, Jarvelin MR. 2001. Family social class, maternal body mass index, childhood body mass index, and age at menarche as predictors of adult obesity. Am J Clin Nutr 74:287–294.

Lezak MD, Howieson DB, Loring DW, Hannay HJ, Fischer JS. 2004. Neuropsychological assessment. 4th ed., New York: Oxford University Press.

Lovallo WR, Farag NH, Vincent AS. 2010. Use of a resting control day in measuring the cortisol response to mental stress: Diurnal patterns, time of day, and gender effects. Psychoneuroendocrinlogy 35(8):1253–1258.

Lupien SJ, Schramek TE. 2006. The differential effects of stress on memory consolidation and retrieval: A potential involvement of reconsolidation? Theoretical comment on Beckner et al. (2006). Behav Neurosci 120(3):735–738.

Lupien SJ, Gaudreau S, Tchiteya BM, Maheu F, Sharma S, Nair NPV, Hauger RL, McEwen BS, Meaney MJ. 1997. Stress-induced declarative memory impairment in healthy elderly subjects: Relationship to cortisol reactivity. J Clin Endocrinol Metab 82(7):2070–2075.

Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NPV, Thakur M, McEwen BS, Hauger RL, Meaney MJ. 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci 1(1):69–73.

Lupien SJ, Gillin CJ, Hauger RL. 1999. Working memory is more sensitive than declarative memory to the acute effects of corticosteroids: A dose-response study in humans. Behav Neurosci 113:420–430.

Lupien SJ, Fiocco A, Wan N, Maheu F, Lord C, Schramek T, Tu MT. 2005. Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology 30(3):225–242.

Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE. 2007. The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain Cogn 65(3):209–237.

Lupien SJ, McEwen BS, Gunnar MR, Heim C. 2009. Effects of stress through the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 10:434–445.

McCormick CM, Mathews IZ. 2010. Adolescent development, hypothalamic–pituitary–adrenal function, and programming of adult learning and memory. Prog Neuro-Psychopharmacol Biol Psychiatry 34(5):756–765.

McEwen BS. 2002. Sex, stress and the hippocampus: Allostasis, allostatic load and the aging process. Neurobiol Aging 23(5):921–939.

Miranda JP, Valencia RR. 1997. English and Spanish versions of a memory test: Word-length effects versus spoken-duration effects. Hisp J Behav Sci 19(2):171–181.

Mucci LA, Kuper HE, Tamimi R, Lagiou P, Spanos E, Trichopoulos D. 2001. Age at menarche and age at menopause in relation to hepatocellular carcinoma in women. BJOG 108(3):291–294.

Otte C, Hart S, Neylan TC, Marmar CR, Yaffe K, Mohr DC. 2005. A meta-analysis of cortisol response to challenge in human aging: Importance of gender. Psychoneuroendocrinology 30(1):80–91.

Patel PD, Lopez JF, Lyons DM, Burke S, Wallace M, Schatzberg AF. 2000. Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. J Psychiatr Res 34(6):383–392.

Payne JD, Jackson ED, Ryan L, Hoscheidt S, Jacobs WJ, Nadel L. 2006. The impact of stress on neutral and emotional aspects of episodic memory. Memory 14(1):1–16.

Peeters PHM, Verbeek ALM, Krol A, Matthyssen MMM, de Waard F. 1995. Age at menarche and breast cancer risk in nulliparous women. Breast Cancer Res Treat 33(1):55–61.

Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28:916–931.

Romeo RD. 2010. Pubertal maturation and programming of hypothalamic–pituitary–adrenal reactivity. Front Neuroendocrinol 31:232–240.

Sandi C, Pinelo-Nava MT. 2007. Stress and memory: Behavioral effects and neurobiological mechanisms. Neural Plast 2007: 78970.

Sandín B, Chorot P, Lostao L, Joiner TE, Santed MA, Valiente RM. 1999. The PANAS scales of positive and negative affect: Factor analytic validation and cross-cultural convergence. Psicothema 11(1):37–51.

Sapolsky RM. 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 57(10): 925–935.

Schwabe L, Bohringer A, Chatterjee M, Schachinger H. 2008. Effects of pre-learning stress on memory for neutral, positive and negative words: Different roles of cortisol and autonomic arousal. Neurobiol Learn and Mem 90(1):44–53.

Scoville WB, Milner B. 1957. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatr 20:11–21.

Seeman TE, Robbins RJ. 1994. Aging and hypothalamic–pituitary–adrenal response to challenge in humans. Endocr Rev 15(2):233–260.

Seeman TE, Singer B, Charpentier P. 1995. Gender differences in patterns of HPA axis response to challenge: MacArthur studies of successful aging. Psychoneuroendocrinology 20(7):711–725.

Seeman TE, McEwen BS, Singer BH, Albert MS, Rowe JW. 1997. Increase in urinary cortisol excretion and memory declines: MacArthur studies of successful aging. J Clin Endocrinol Metab 82(8):2458–2465.

Seeman TE, Singer B, Wilkinson CW, McEwen B. 2001. Gender differences in age-related changes in HPA axis reactivity. Psychoneuroendocrinology 26(3):225–240.

Seisdedos N. 1988. State-trait anxiety inventory, TEA Ediciones, Madrid.

Shors TJ. 2006. Stressful experience and learning across the lifespan. Annu Rev Psychol 57:55–85.

Smeets T, Jelicic M, Merckelbach H. 2006. The effect of acute stress on memory depends on word valence. Int J Psychophysiol 62(1): 30–37.

Smeets T, Giesbrecht T, Jelicic M, Merckelbach H. 2007. Context-dependent enhancement of declarative memory performance following acute psychosocial stress. Biol Psychol 76(1–2): 116–123.

Spielberger CD, Gorsuch RL, Lushene RE. 1970. Manual for the State-trait anxiety inventory. Palo Alto, CA: Consulting Psychologists Press.

Traustadóttir T, Bosch PR, Matt KS. 2003. Gender differences in cardiovascular and hypothalamic–pituitary–adrenal axis responses to psychological stress in healthy older adult men and women. Stress 6(2):133–140.

Vierhapper H, Heinze G, Nowotny P. 2007. Sex-specific difference in the interconversion of cortisol and cortisone in men and women. Obesity 15(4):820–824.

Watson D, Clark LA, Tellegen A. 1988. Development and validation of brief measures of positive and negative affect: The PANAS scales. J Pers Soc Psychol 54(6):1063–1070.

Wolf OT, Kudielka BM, Hellhammer DH, Hellhammer J, Kirschbaum C. 1998. Opposing effects of DHEA replacement in elderly subjects on declarative memory and attention after exposure to a laboratory stressor. Psychoneuroendocrinology 23(6):617–629.

Wolf OT, Convit A, McHugh PF, Kandil E, Thorn EL, de Santi S, McEwen BS. 2001. Cortisol differentially affects memory in young and elderly men. Behav Neurosci 115(5):1002–1011.

Wolf OT, Dziobek I, McHugh P, Sweat V, de Leon MJ, Javier E, Convit A. 2005. Subjective memory complaints in aging are associated with elevated cortisol levels. Neurobiol Aging 26(10): 1357–1363.

Yerkes RM, Dodson JD. 1908. The relation of strength of stimulus to rapidity of habit formation. J Comp Neurol Psychol 18(5): 459–482.

Young E. 2009. Stress, the HPA axis and depressive illness. In: Squire Larry R, editor. Encyclopedia of neuroscience. Oxford: Academic Press. p 543–548.