Memory function after stress : the effects of acute stress and cortisol on memory and the inhibition of emotional distraction

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Psychosocial stress impairs working memory at high loads: an association with cortisol levels and memory retrieval

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Abstract

Stress and cortisol are known to impair memory retrieval of well-consolidated declarative material. The effects of cortisol on memory retrieval may in particular be due to glucocorticoid receptors in the hippocampus and prefrontal cortex (PFC). Therefore, effects of stress and cortisol should be observable in both hippocampal-dependent declarative memory retrieval and PFC-dependent working memory (WM). In the present study, it was tested whether psychosocial stress would impair both WM and memory retrieval in 20 young healthy men. In addition, the association between cortisol levels and cognitive performance was assessed. It was found that stress impaired WM at high loads, but not at low loads in a Sternberg paradigm. High cortisol levels at the time of testing were associated with slow WM performance at high loads, and with impaired recall of moderately emotional, but not of highly emotional paragraphs. Furthermore, performance at high WM loads was associated with memory retrieval. These data extend previous results of pharmacological studies in finding WM impairments after acute stress at high workloads and cortisol-related retrieval impairments.

Introduction

Acute high levels of glucocorticoids (GCs, cortisol in humans) affect memory and cognition (Lupien & McEwen, 1997; Wolf, 2003). Cortisol or stress have been found to influence various forms of memory differently (Lupien et al., 1999; Vedhara, Hyde, Gilchrist, Tytherleigh, & Plummer, 2000) and in addition, affect each memory phase differentially (Roozendaal, 2000; 2002). Cortisol elevations immediately after learning, have been shown to enhance declarative memory consolidation, specifically of material with emotionally arousing content (Buchanan & Lovallo, 2001; Cahill et al., 2003; Kuhlmann & Wolf, 2006a). Conversely, the association between pre-retrieval stress or high cortisol levels and impaired memory retrieval has been reported consistently (de Quervain et al., 2000; 2003). Here too, emotionally arousing and negatively valenced material appears to be more affected by high cortisol levels at the time of retrieval testing than neutral, non arousing stimuli (Buchanan et al., 2006; Kuhlmann et al., 2005a; 2005b).

The effects of cortisol on declarative memory retrieval have mainly been attributed to the actions of glucocorticoid (GC) receptors in the hippocampus (McGaugh & Roozendaal, 2002)(Roozendaal, 2002) and the prefrontal cortex (PFC) (Lupien & Lepage, 2001). Therefore, effects of cortisol on prefrontal-dependent memory, like working memory (WM), should be observable. Indeed, some studies found that acute elevations of exogenous glucocorticoids impaired WM, without affecting declarative memory (Lupien et al., 1999; Wolf et al., 2001a). Lupien and colleagues (1999) infused hydrocortisone (40, 300 or 600 μg/dL/Kg) or placebo in young healthy men and assessed WM using a item-recognition task (Sternberg, 1966) that consisted of trials with low to high comparison loads. They found that WM was affected at high comparison loads, indicated by slower reactions times for high- as compared with low comparison loads. Cortisol was not associated with impaired declarative memory. However, both learning and retrieval took place after infusion of hydrocortisone, which made it difficult to draw conclusions with regard to cortisol effects on retrieval specifically, and in comparison with WM.

Although declarative memory encoding and consolidation is known to be dependent on the hippocampus, *retrieval* of declarative memory is also mediated by the PFC (Buckner & Wheeler, 2001; Simons & Spiers, 2003; Ranganath,

Johnson, & D'Esposito, 2003). Also, although WM tasks are known to depend on prefrontal brain areas, there is evidence from studies using magnetic encephalograms (MEG) (Campo et al., 2005) and functional magnetic resonance imaging (fMRI) (Karlsgodt, Shirinyan, Van Erp, Cohen, & Cannon, 2005; Ranganath & D'Esposito, 2001) that the medial temporal lobe (MTL) is activated during WM tasks. Moreover, activity in the dorsolateral PFC has been also found during memory retrieval and WM, possibly reflecting monitoring (Cabeza et al., 2002; Nyberg et al., 2003) or selection of task-relevant information (Sakai & Passingham, 2004). So far, one imaging ($H_2{}^{15}O$ -positron emission tomography) study has shown cortisol-induced decreased blood flow in the MTL associated with impaired performance on a delayed recall task (De Quervain et al., 2003). A recent fMRI-study, showed cortisol-induced decreased brain activation in both the PFC and hippocampus during declarative memory retrieval (Oei et al., 2007), which suggests that stress effects on retrieval may partly be caused by cortisol effects on prefrontal functioning.

Studies in which cortisol levels are elevated by psychosocial stress have seldom tested both WM and declarative memory retrieval. WM was tested in at least two psychosocial stress studies with the Wais-R subtest Digit Span (DS): One reported impairing effects on memory retrieval associated with cortisol levels, but no impairing effects on WM (Kuhlmann et al., 2005b), the other did not assess memory retrieval and reported impairment on DS-Forwards during stress (Elzinga & Roelofs, 2005). However, DS Forwards is considered to be a measure of attention, whereas DS Backwards a test of WM (Ackerman, Beier, & Boyle, 2002). Furthermore, DS has been shown to be selectively preserved following frontal and hippocampal lesions in humans (Cave & Squire, 1992; Daffner et al., 2000). Clearly, stress effects on WM in healthy individuals should be replicated with the use of more sensitive WM tasks.

The goal of the present study was to test whether high cortisol levels impair both WM and declarative memory retrieval in young healthy men and to assess the association between these two measures. In addition, it was examined whether cortisol differentially affects retrieval of material with different arousal properties.

Method

Participants

A total of 20 healthy male first-year psychology students participated in this study. All participants were informed about the study and gave written consent

before participation and received obligatory course marks. Participants were screened before inclusion. Criteria for inclusion were: a body mass index (BMI = kg/m²) between 19 and 25, a healthy medical and psychiatric history, determined by a brief version of the Amsterdam Biographical Interview (ABV; Wilde, 1963) and the Dutch version of the Symptom Checklist-90 (SCL-90; (Arrindell & Ettema, 1986). Exclusion criteria included use of medication or psychotropic drugs within three months prior to the test sessions, blood pressure over 140/90 mmHg, diabetes mellitus, current and past psychiatric problems, and the use of remedies containing corticosteroids. The study was approved by the ethical committee of the department of psychology of the University of Amsterdam. Characteristics of the sample were as follows (mean ± *SD*): Age, 21.86 ± 3.89 years; BMI, 21.44 ± 1.57 kg/m²; SCL-90, 115.24 ± 20.88, which falls in the 'normal range' scoring 'average' using normative ratings for a healthy population. No significant differences were found between groups with different order of stress for age (F [1, 19] = 0.004; p =.95; BMI F[1, 19] = 3.02; p =.10; SCL-90 (F [1, 19] = 1.07; p =.31).

Design

Testing was done in a randomized crossover design on two consecutive ("retrieval") days at 09.30 AM, to ensure high basal endogenous cortisol levels. Although absolute cortisol rises in response to stress do not differ between AM and PM phase (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004), the AM phase was chosen so that cortisol rises would more likely occupy glucocorticoid receptors (see Het et al., 2005; Lupien & Lepage, 2001; Maheu et al., 2005a). The same Sternberg-based WM task as by Lupien and colleagues (1999) was used. All participants encoded paragraphs one day earlier and were randomly assigned to stress order (stress on retrieval day 1, or day 2). Psychosocial stress was induced to elevate cortisol levels.

Memory tasks

Working memory.

WM was measured using the same item-recognition task (Sternberg, 1966) as used and described extensively by Lupien and others (1999). Similar tasks have been reported to significantly activate the dorsolateral PFC in neuroimaging studies (e.g., Veltman, Rombouts, & Dolan, 2003).The WM processing load is manipulated by varying the numbers of uppercase letters (1 to 4 targets) that have to be held in memory for later recognition, and by varying the number of

letters (1 to 4 displayed) presented in the recognition display after a short delay (750-ms), which leads to a load of 2 to 16 comparisons. Participants had to press a 'yes' button indicating they had recognized a target (present-target trials), or a 'no' button, when no target letter was recognized (absent-target trials). Only one target letter was present in the present-target trials. To ensure the task was not too easy, we randomly delivered blocks with differing loads instead of steadily increasing comparison loads. To avoid boredom, we decreased the amount of trials from 300 to 240 (16 trials per each of 15 conditions). The stimulus software (WESP) that was used, was developed at the department of psychology of the University of Amsterdam and it randomizes and presents stimuli, and records reaction times and errors.

Declarative memory.

The Wechsler Memory Scale-Revised Logical Memory test (Wechsler, 1981) was used. This Paragraph recall test was used as a valid and sensitive measure of declarative memory that has proved to be sensitive to cortisol effects in previous studies (Elzinga, Bakker, & Bremner, 2005). According to the WMS-LM method, 4 paragraphs were constructed, containing 21 pieces of information, matched for difficulty. However, the emotionality of the content of two paragraphs was reduced (e.g., a story about a fire alarm was changed into a story about a fire drill) whereas two paragraphs were 'emotionalized' (e.g., a student was beaten to death on his way to his final exams, instead of only beaten). Recall percentage was computed as '(delayed recall/immediate recall) x 100'. In an exit-interview, participants rated the subjective emotional content of the paragraphs on a 9-points Likert scale ranging from 1 (*not emotional at all*) to 9 (*extremely emotional*). A Wilcoxon *t*-test for paired samples showed that participants rated the 'moderately emotional´ paragraphs ($M \pm SE$, 2.2 ± 0.31) as significantly less emotional than the ´highly emotional´ paragraphs ($M \pm SE$, 3.9 ± 0.41) ($z = -2.85$, N – ties = 16, $p = .002$, one-tailed). These means were similar to mean arousal ratings of 'moderately emotional' and 'highly emotional' words used by Buchanan and colleagues (2006).

Psychosocial stress protocol

To induce psychosocial stress, the Trier Social Stress Test (TSST) was employed (Kirschbaum, Pirke, & Hellhammer, 1993). In male participants, the TSST protocol has consistently been shown to raise cortisol levels, in both saliva and blood. This laboratory stressor consists of a 10-min period in anticipation of a 5-min free speech and a 5-min arithmetic task in front of a selection

committee. The TSST protocol was followed, with the exception of the arithmetic task, which was exchanged by a "3-back only" task, to make the stressor even more difficult. A set of 100 randomly generated digits were presented aurally in a fixed order by the computer. Participants had to indicate whether each aurally-presented digit was similar to or different from the digit presented three digits back, by saying out loud "yes" to a target and "no" to a non-target. The task consisted of 30% targets. One committee member responded to incorrect answers by saying out loud "incorrect", while another member kept up participant's performance by means of a clearly visible scoreboard. When "incorrect", the scoreboard was ostentatiously put back to zero.

Cardiovascular measures

Systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), and heart rate (HR, bpm) were recorded using a Finapres blood pressure monitor (Finapres 2300, Ohmeda, Englewood, CO). The Finapres enables non-invasive continuous beat-to-beat monitoring of the finger arterial pressure waveform using a finger cuff applied to the middle phalanx of the middle finger (see also Imholz, Wieling, van Montfrans, & Wesseling, 1998).

Cortisol

Cortisol was assessed with saliva samples using Salivettes (Sarstedt, Germany). Saliva sampling is a stress-free method to assess unbound cortisol (Kirschbaum & Hellhammer, 1994). Saliva samples were centrifuged and thereafter stored at –70 °C until assayed. Free cortisol concentration in saliva was analyzed with a time-resolved immunoassay with fluorometric detection (Dressendorfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). Inter- and intra-assay coefficients of variance were below 12% and 10% respectively. All saliva analyses were conducted at Prof. Kirschbaum's laboratory (http://biopsychologie.tu-dresden.de).

Procedure

Participants were invited to the laboratory on three consecutive days: an acquisition-day, a retrieval day with psychosocial stressor (stress), and a retrieval day without stress (control). Participants were randomly assigned to TSST on retrieval day 1 or TSST on retrieval day 2. On the acquisition-day, participants learned four paragraphs for immediate recall. Paragraph delivery was counterbalanced. On the first retrieval day, the appointment was scheduled at 09.00 h. Participants had to refrain from food intake, sugar- or caffeine

containing drinks, and physical exercise at least 1,5 hr before testing. Immediately after arrival, the first saliva sample was taken. The experimenter explained that all instructions and tasks would be provided on a computer screen and showed the appropriate response keys. She then went to an adjacent room and started the computerized protocol (using the VSRRP98 software package developed at the department of Psychology, University of Amsterdam). Participants received all further instructions, questionnaires and tasks via the computer screen and provided all their responses by means of the response keys, except for the instruction and responses with regard to the 3-back only task. HR and blood pressure were recorded continuously using a Finapres 15 min before, during, and 10 min after the TSST. Participants were instructed to minimize all movement during the physiological recordings. After adaptation to the Finapres a 15-min baseline period followed in which participants watched a documentary about salt men in Tibet. After the TSST a 10-min recovery period followed in which participants watched the second segment of the documentary. Hereafter the Finapres fingercuf was removed. Saliva samples were collected immediately after the baseline period, just before the anticipation of the stressor (t1), before the free speech (t2), immediately after the 3-back task (t3), 10 min after the cessation of the TSST when peak levels are expected (t4) and WM testing starts (09.55hr), and finally, 30 min after the stress challenge at the end of declarative memory testing (t5). WM was tested immediately after the recovery period (t4). Hereafter, delayed recall of two paragraphs (one with highly emotional and one with moderately emotional content) was administered. On the day without stress, participants filled in questionnaires until the WM task and the other two paragraphs (one highly emotional, the other moderately emotional) were administered at exactly the same time as after the stress procedure. Saliva was sampled at exactly the same time points (t4, t5). Paragraph recall was balanced across retrieval days and across participants to avoid any non-random bias. Finally, participants filled in an exit-interview, in which was asked to assess the paragraphs and to indicate their impression and sentiments about the members of the selection committee.

Statistics

Data were analyzed using repeated measures ANOVAs. Data were checked for the sphericity assumption, and Greenhouse-Geisser corrections were applied when this assumption was not met. Follow-up analysis of ANOVA effects was done with *t*-tests. Pearson's Product Moment Correlations between cortisol level

and memory performance were computed. The data were analysed using SPSS for Windows, version 11.5.

Results

Order Effects

An ANOVA for RTs was performed with Order (stress on the first retrieval day vs. stress on the second retrieval day) as between-subjects factor, and Condition (stress vs. control), Target type (present vs. absent) and comparison load (2 vs. 3 vs. 4 vs. 6 vs. 8 vs. 9 vs. 12 vs. 16) as within-subjects factors. The ANOVA for RTs showed a main effect of Order, $F(1, 18) = 5.11$, $p = .036$, and a significant interaction-effect of Condition by Order, $F(1, 18) = 11.22$, $p = .004$, indicating that learning under stress had a significantly negative effect on later (stress-free) performance, whereas stress-free learning appeared to facilitate later performance when stressed (see Figure 1). In contrast, a repeated measures ANOVA with Order (stress on retrieval day 1 vs. stress on retrieval day 2) as between-subjects factor, and Condition (stress vs. control) and Arousal (low, high) as within-subjects factors performed on delayed recall of the paragraphs revealed no significant effect of Order, $F(1, 18) = 0.00$, $p = .99$), and no significant effect of Condition by Order, $F(1, 18) = 2.18$, $p = .16$.

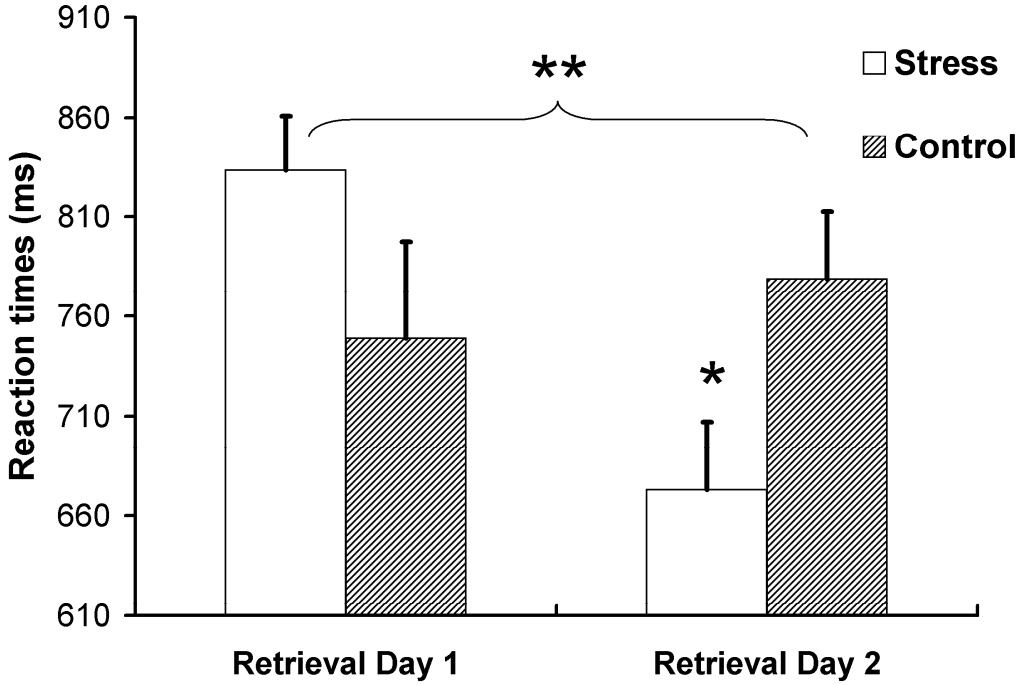
To be able to answer our original research questions, we chose to discard all data from the second retrieval day, because the WM data were free from carry-over effects only on the first retrieval day. Therefore, further analyses are performed on the data from retrieval day 1.

Stress

A repeated measures ANOVA with Time (t1 to t5) as within-subjects factor, showed that free cortisol levels in saliva increased significantly in response to exposure to the stress challenge (see Figure 2), with a significant effect of Time, $F(1.54; 13.83) = 5.74$, $p = .02$ (Greenhouse-Geisser corrected, $\varepsilon = 0.38$). Then, a repeated measures ANOVA with Condition (stress vs. control) as between-subjects factor, and time of cognitive testing (t4, t5) as within-subjects factor was performed. Here, a significant effect of Condition was found, $F(1, 18) = 6.59$, $p < .02$, a significant within-subjects effect of Time, and Time by Condition (Fs > 10, ps $< .005$), which were indicative of a decrease in cortisol level as time passed in the stress group. Independent t-tests showed that cortisol level just before the

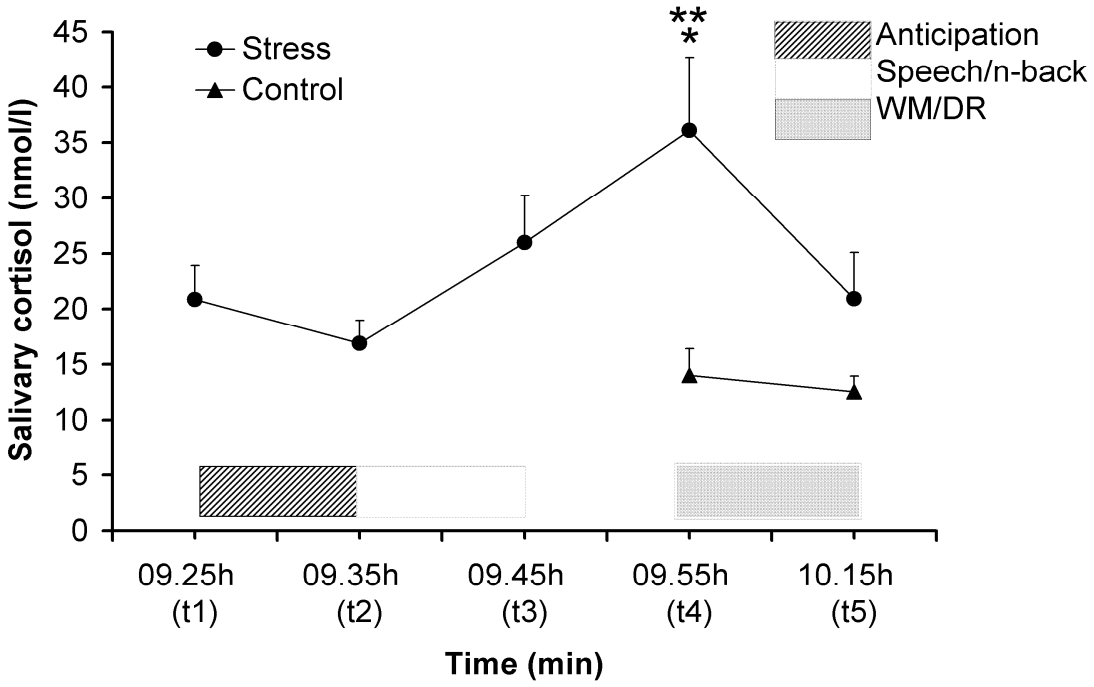
WM task (t4) was higher in the stress group ($M \pm SE$: 34.4 ± 6.6 nmol/L), than in the control group ($M \pm SE$: 14 ± 2.4 nmol/L), $t_{11.37} = 2.89$, $p = .01$, whereas immediately after the declarative tests (t5) the difference between cortisol level in the stress group ($M \pm SE$: 20.5 ± 4.2 nmol/L) and the control group ($M \pm SE$: 12.5 ±1.4 nmol/L) was only a trend, $t_{10.97} = 1.83$, $p = .09$ (equal variances not assumed for both *t*-tests). Baseline cortisol levels of the groups (stress on day 1, $M \pm SE$: 20.8 ± 3.0 nmol/L; stress on day 2, $M \pm SE$: 17.1± 1.7 nmol/L) did not differ significantly, $t_{14.21} = 0.17$ (equal variances not assumed).

Figure 1



Note. **Reaction times (Mean and *SE*) of the groups in the WM task on two consecutive days. Stress on the first day significantly weakened the carry-over effect that was visible on the second day. The group that had stress on the first retrieval day, was control group on the second retrieval day, and vice versa.**
*** Faster WM performance in the stress group compared to the control group, $p < .05$**
**** Significant Condition by Order interaction, $p < .005$**

Figure 2



Note. **Salivary cortisol concentrations (means and SE) in the stress group at five time points (t1–t5) anticipating and responding to the TSST. Salivary cortisol levels of the control (no stress) group at two time points (t4, t5), when cognitive testing was done, are shown for comparison.**
*** Significant difference between cortisol levels at t4 and t1 in the stress group, p <.05.**
**** Significant difference between stress- and control group at t4, p <.05.**

Separate repeated measures ANOVAs for SBP, DBP and HR, with Time (anticipation through end of TSST) as within-subjects factor showed significant elevations of these physiological measures during stress, for SBP, $F(3, 27) = 176.61$, p <.0005; DBP, $F(1,4;12,8) = 50.79$, p <.0005; HR, $F(1,2; 10,6) = 17.38$, p =.001 (see Table 1). Planned comparisons between mean recovery and baseline of these measures were conducted using paired t-tests, which showed that HR had returned back to baseline, $t_9 = -1.64$, $p > .10$, in contrast to blood pressure, SBP, $t_9 = -7.3$, p <.0005; or DBP, $t_9 = -3.6$, p <.01. Additional post-hoc t-tests showed that both of these measures had dropped significantly during the 10 min of recovery compared to mean stress levels during the TSST, SBP, $t_9 = 6.96$; DBP, $t_9 = 8.35$ (ps <.0005).

Table 1. **Blood pressure and heart rate before, during and after psychosocial stress (*N* = 10)**

		SBp	DBp	HR
	Time	M (SD)	M (SD)	M (SD)
Baseline	09.10h	139.40 (14.39)	87.86 (12.21)	68.78 (12.93)
	09.15h	139.84 (13.48)	86.19 (11.45)	68.58 (12.31)
	09.20h	138.14 (13.02)	84.54 (10.67)	67.92 (11.72)
Anticipation	09.25h	150.03 (17.39)	89.84 (13.16)	72.87 (13.76)
	09.30h	150.88 (17.37)	90.48 (14.18)	74.01 (12.87)
Spreech	09.35h	195.23 (18.94)	117.66 (19.96)	89.96 (14.78)
3-back	09.40h	189.31 (24.77)	113.07 (22.59)	82.02 (17.43)
Recovery	09.45h	167.14 (24.99)	101.23 (21.38)	69.91 (10.80)
	09.50h	163.89 (25.90)	99.13 (20.36)	69.09 (10.84)

Note. **Values represent means and standard deviations (SD). SBP = systolic blood pressure (mmHg); DBP = diastolic blood pressure (mmHg); HR = heart rate (bpm, beats per minute).**

Memory Performance

Working memory

First we inspected the data on errors. WM data of one participant from the stress group were excluded from this analysis, because of extreme numbers of detection errors and missing values due to no response within the maximum time (> 25%). A repeated measures ANOVA was performed with Condition (stress vs. control) as between-subjects factor and Error Type (present vs. absent) as within-subjects factors. No main effect was found for Condition, $F(1, 17) = 0.25$, $p = .63$, and conform expectations, a significant main effect for Error Type was found, reflecting more errors on present trials than on absent trials, $F(1, 17) = 73.83$; $p < .0005$. No interaction-effect was found between Condition and Error Type, $F(1, 17) = 0.07$, $p > .70$, or between Condition and Load, $F(7, 119) = 1.59$, $p > .10$. There was however a near significant triple interaction of Condition by Load by Error Type, $F(7, 119) = 2.06$, $p = .05$, with more errors

on present trials at high comparison loads in the stress group ($M \pm SD$: 2.72 ± 1.28) compared to the control group ($M \pm SD$: 1.93 ± 1.03).

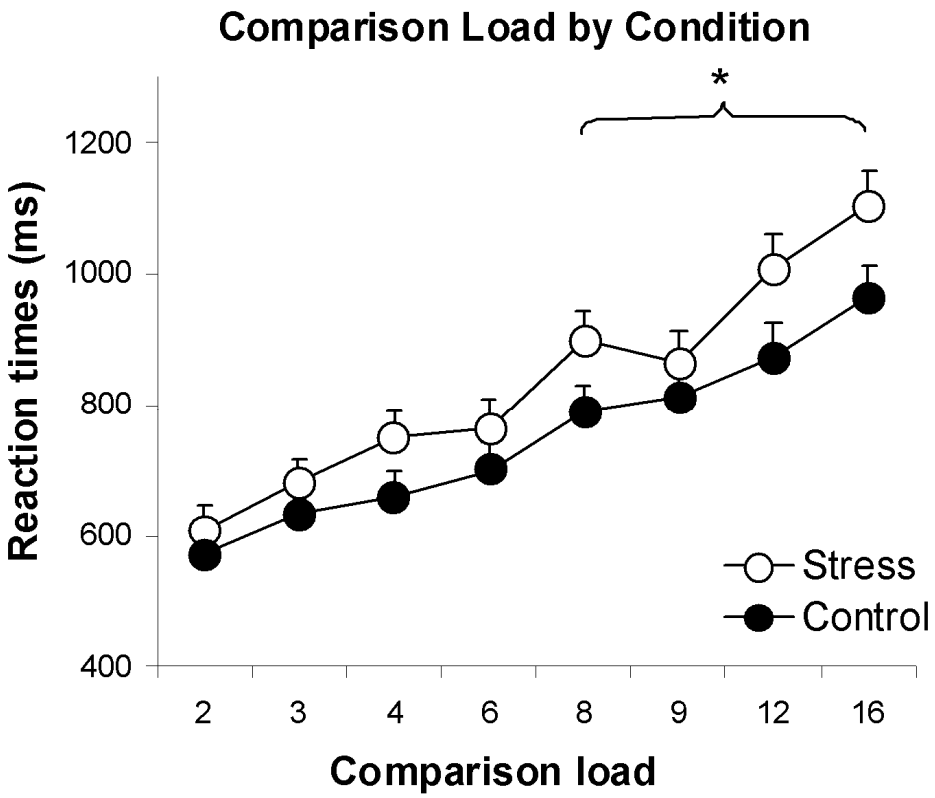
Then, we performed a repeated measures ANOVA on RTs, to see if condition affected WM on different loads. There was no between-subjects effect of Condition, $F(1, 17) = 2.22$, $p = .15$. A significant effect was found for Type, which reflected faster RTs for present trials than for absent trials, $F(1, 17) = 28.22$, $p < .0005$. A main effect for Comparison Load was found, $F(3.38;57.52) = 153.41$; $p < .0005$, showing that higher comparison loads led to a linear increase of RTs. A significant Condition by Comparison Load interaction was found, $F(3.38;57.52) = 2.73$, $p = .046$, with slower RTs in the Stress group at higher comparison loads irrespective of type (see Fig 3). Additional one-tailed *t*-tests showed that the difference between stress and control group on high comparison loads was significant, for load 8 ($t_{14.72} = 1.82$, $p = .04$), load 12 ($t_{14.34} = 1.93$, $p = .04$) and load 16 ($t_{10.22} = 2.06$, $p = .03$) (other loads ps > .10). Post-hoc effect sizes were calculated using *r* (Field, 2005, p.294) which showed that these effects were large, $r = 0.43$, $r = 0.45$, and $r = 0.54$, for load 8, 12, and 16 respectively, indicating that the differences found between stress and control group were not likely due to a type I statistical error. Moreover, the increase in errors with higher loads in the stress group, was not a consequence of a speed-accuracy trade-off, since Pearson's correlations showed that longer RTs of present trials at averaged high loads were positively associated with mean errors in the stress group ($r = 0.65$, $p = .06$), but not in the control group ($r = 0.09$, $p = .82$).

Correlation cortisol and working memory

To see whether cortisol levels at the time of WM testing were associated with WM performance, Pearson's correlations were calculated between cortisol level (t4) and averaged RTs at low loads (2, 3, 4, 6) and high loads (load 8, 12, 16). No significant association was found at low loads ($r = 0.08$, $p = .37$, $n = 19$) or at high load ($r = 0.21$, $p = .20$, $n = 19$, both ps one-tailed). When examining cortisol levels at the time of WM testing, two outliers were detected in the stress group with exceptionally high cortisol levels (> 60 nmol/L). Without these outliers, no significant correlation was found at low loads ($r = 0.33$, $p = .10$, one-tailed), but at high loads, higher cortisol levels were significantly associated with slower RTs ($r = 0.48$, $p = .025$, one-tailed)[6].

[6] To allow for a better comparison with previous work by others, cognitive performance was associated with cortisol levels calculated with the area under the curve method (AUC_g: see

Figure 3



***Note*. RTs (Mean and *SE*) of the two groups (stress and control, *N* = 19) in the WM task as a function of comparison load. At high comparison loads the stress group was significantly slower than the control group. Notice that the RTs at comparison load 9 are faster, similar to the data of Lupien et al. (1999), which is probably because load 9 has fewer events, compared to other loads.**
*** *p* <.05 (one-tailed)**

Declarative memory retrieval

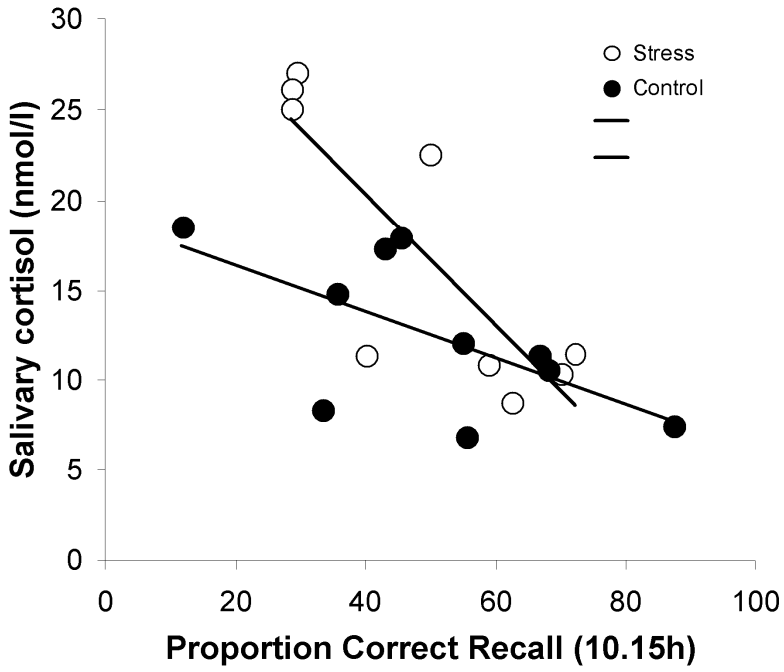
The ANOVA performed on delayed recall of highly emotional (*M* ± *SE*, stress: 48.26 ± 9.14%, and control: 56.65 ± 7.62%) and moderately emotional (M ± *SE*, stress: 48.55 ± 5.34%; control, 50.20 ± 6.74%) paragraphs revealed no main effect of Condition, or Arousal (high, low), and no interaction of Condition by Arousal (all *p*s > .50).

Pruessner *et al.*, 2003, for details on this measure) and with delta increase, which could only be provided of the stress group only (n=9). Cortisol level (in logAUCg) and low loads, r = -.62, p =.04, high loads, r = -.59, p =.04. Delta increase and low loads. r = -.14, p =.36; high loads, r = -.13, r =.37 (all one-tailed).

Correlation cortisol levels and memory retrieval

Pearson's correlations were calculated between cortisol level (t5) and paragraph recall. For the moderately emotional paragraph, a significant negative correlation was found (r = -.44, p =.02, one-tailed, n = 20). When inspecting the scatterplot, one outlier was observed, with extremely high cortisol level (> 50 nmol/L). After removing the outlier the correlation was r = -.67, p =.001 (one-tailed, n = 19), indicating that the higher the cortisol levels, the lower the score on moderately emotional paragraph recall (see Figure 4). No such association was found between cortisol level and recall of the highly emotional paragraphs (r =.17, p > .23, one-tailed)[7].

Figure 4



Note. **The association between the proportion correct recall of the moderately emotional paragraph and cortisol level at the time of testing. Higher cortisol levels were associated with less recall. In the stress group (n = 9), salivary cortisol concentration explained 69% of the variance in moderately emotional declarative memory recall (entire sample: R^2 = 43.5%).**

[7] Cortisol level (log cortisol AUCg) in the stress group (n = 10) correlated significantly with retrieval of the moderately emotional paragraph, r = -.64, p =.02 (one-tailed), but not with the highly emotional paragraph, r =.05, p =.44 (one-tailed). Paragraph recall did not correlate significantly with cortisol when delta increase (t5 – t1) was used as a measure (both ps > .30, one-tailed).

Working memory by memory retrieval

Pearson's correlations were calculated between WM performance at high comparison loads and moderately emotional paragraph recall. A significant negative correlation was found between moderately emotional paragraph recall and high load (8, 12 and 16) ($r = -.57$, $p = .01$, two-tailed).

Discussion

The present study showed that psychosocial stress impaired WM performance at high but not low WM loads. High cortisol levels were associated with slower WM performance at high loads. In addition, a negative association between cortisol levels and delayed recall of moderately emotional material was found. Recall performance of the moderately emotional paragraphs was also associated with WM performance. No such association was found for highly emotional paragraphs. Together, the results of the present study extend the findings of pharmacological studies in finding WM impairments after acute stress, with moderate cortisol elevations.

The impairing effects of stress on WM performance at only high loads are consistent with the findings of Lupien and others (1999). Here too, RTs were slower at high loads in the stress group only. However, the relative increase of cortisol levels in their study (mean ± 90 nmol/L) differed to a great extent with levels found in our study (mean ± 12 nmol/L). In our study comparison loads were randomized to increase the difficulty of the WM task. This may have led to these highly similar results. In addition, in the present study, stress led to the tendency to erroneously indicate present targets at high loads as not previously encountered. These errors were associated with slower RTs. This bias toward rejection was specific for present-target trials. There were no significantly less false hits on the Absent-target trials, so there was no indication of conservative responding in general. This tendency for more errors further corroborates the impairment in WM at high loads after acute stress.

An explanation that has been given in other studies for finding WM deficits (Elzinga & Roelofs, 2005) or declarative memory retrieval deficits (Kuhlmann et al., 2005b) is stronger adrenergic activation due to the psychosocial stress. Rat studies have shown that corticosterone interacts with adrenergic mechanisms in the amygdala and hippocampus in causing retrieval impairments (Roozendaal, Hahn, Nathan, de Quervain, & McGaugh, 2004b). In humans, Elzinga and

Roelofs (2005) did not find WM impairments (DS forwards) 30 min after the TSST had finished and sympathetic activation had subsided but only *during* the psychosocial stress (although the stress context was removed only 15 min before WM testing). We started WM testing 10 minutes after the psychosocial stressor. Unfortunately we were not able to proceed with the continuous measurements during the WM task, due to the fact that reaction times tasks require speedy hand movements, which interfere with blood pressure assessments, and produce movement artefacts. Salivary cortisol concentration was peaking at the start of the WM task, and HR had returned to baseline. However, although blood pressure was significantly lower at that time than during stress, it was still significantly elevated indicating some sympathetic activation was present during WM testing. Moreover, it can be argued that the task itself could have induced acute increases in sympathetic activation, particularly at high comparison loads that are very demanding and perhaps frustrating. If this was the case, then sympathetic activation would also have been increased during performance at low loads, since trials at high and low comparison loads were delivered randomly. This would imply that high sympathetic activation and high cortisol levels do not impair WM performance at low comparison loads, in contrast to high load performance. Taken together, the present data cannot definitely answer the question whether stress-induced WM impairments require concurrent (very) high sympathetic activation. Clearly, more WM studies are warranted to investigate the differential effects of sympathetic activations and cortisol at different workloads.

In line with a recent study (Buchanan et al., 2006), high salivary cortisol levels in the present study were associated with less recall of *moderately* emotional, but not of *highly* emotional paragraphs. Buchanan and colleagues (2006) found retrieval impairments associated with cortisol elevations in responders to the cold pressor test. Moderately arousing words learned 1h before elevation of cortisol levels, were recalled less well than highly arousing or neutral words. One possible explanation for this finding is that the memory trace of emotionally highly arousing material is more stable and thus less vulnerable to the modulatory effects of cortisol than moderately arousing material. However, our results should be interpreted with caution. First, mean recall of highly emotional paragraphs was clearly reduced after stress, but individual differences in recall of the highly emotional paragraphs were large in both stress and control group. Second, it was not assessed to what extend encoding was affected by the arousal properties of the material. Third, we could not compare these findings with recall of neutral, non arousing stimuli. Human data on the interaction of stress or GCs and

arousing stimuli (or material with different valence) are sparse and far from consistent. For instance, Domes and colleagues (2004) found that stress impaired the retrieval of positive words, but not of neutral or negative words. Kuhlmann and others (2005a, 2005b) found (a trend towards) retrieval impairment for positive and negative words after cortisol or stress treatment. Buss, Wolf, Witt, and Hellhammer (2004) found significant impairment in retrieval of neutral autobiographical episodes in young men, and only a trend for impaired retrieval of positive or negative episodes. Differences in timing, tasks and gender of the participants may be the reason for the divergence in direction of cortisol effects on memory retrieval of material with different valence and arousal properties (Maheu et al., 2005a; Wolf et al., 2004).

According to our expectations, impaired WM performance at high loads was associated with low retrieval performance. Since we did not assess intelligence, it is possible that the association between WM and retrieval impairment reflects an underlying variance in intelligence levels between the groups. Nonetheless, the sample came from an university population and the allocation to groups was random, which may have reduced the chance of large differences in IQ variance. Moreover, performance on both measures was also associated with cortisol. Cortisol may have parallel effects on the structures on which WM and memory retrieval are known to rely, the PFC and MTL, and this way independently affect WM and memory retrieval. However, there is evidence from imaging studies that show common activity of the MTL and the PFC during retrieval and WM (Buckner & Wheeler, 2001; Cabeza et al., 2002; Karlsgodt et al., 2005; Nyberg et al., 2003; Ranganath & D'Esposito, 2001; Simons & Spiers, 2003) and cortisol-induced decreases in those areas (Oei et al., 2007). Therefore, it could be speculated that apart from direct effects on specific areas cortisol impairs memory indirectly through general effects on a frontotemporal network. Low loads from the Sternberg paradigm have been associated with activations in the left ventrolateral PFC, and high loads with right dorsolateral PFC (Bunge, Ochsner, Desmond, Glover, & Gabrieli, 2001; Manoach et al., 1997). The latter area is linked to episodic memory retrieval (Cabeza et al., 2002). This suggests that of the 2 subprocesses of WM, 'manipulation' might be more sensitive to the effects of cortisol and stress, as opposed to 'maintenance' processes. These domains await further research using imaging techniques.

Many brain activations attributed to specific cognitive processes probably reflect general processes (Cabeza et al., 2003). Cabeza and colleagues (2003) found a common network for episodic memory retrieval and attention. They suggest that 'post-retrieval monitoring' as interpretation for these PFC activations

should be rephrased in terms of attentional processes. Selective attention was not assessed in our study. However, there is evidence that cortisol impedes selective attention, leading to lower sensory acuity (Fehm-Wolfsdorf et al., 1993), and stress-induced high cortisol levels have been associated with decreased inhibition of non-relevant information on a negative priming task, a standard measure of inhibitory attentional processes (Skosnik, Chatterton, Jr., Swisher, & Park, 2000). However, it still remains to be determined whether stress impairs memory retrieval through its effects on general attentional processes.

Taken together, these findings further substantiate the effects of stress and cortisol on memory functioning. Specifically, we found that stress impairs WM at high loads, but not low loads. Our sample was small and therefore conclusions should be made with caution. However, our results on the WM task converge with the findings of Lupien et al. (1999), which increases the validity of our findings. Future studies should use sensitive measures of WM and attention, when investigating effects of stress or cortisol on memory retrieval. In addition, stimuli with different arousal properties and their valence should be carefully employed when investigating the effects of stress or cortisol on memory.