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Memories in context: On the role of cortisol

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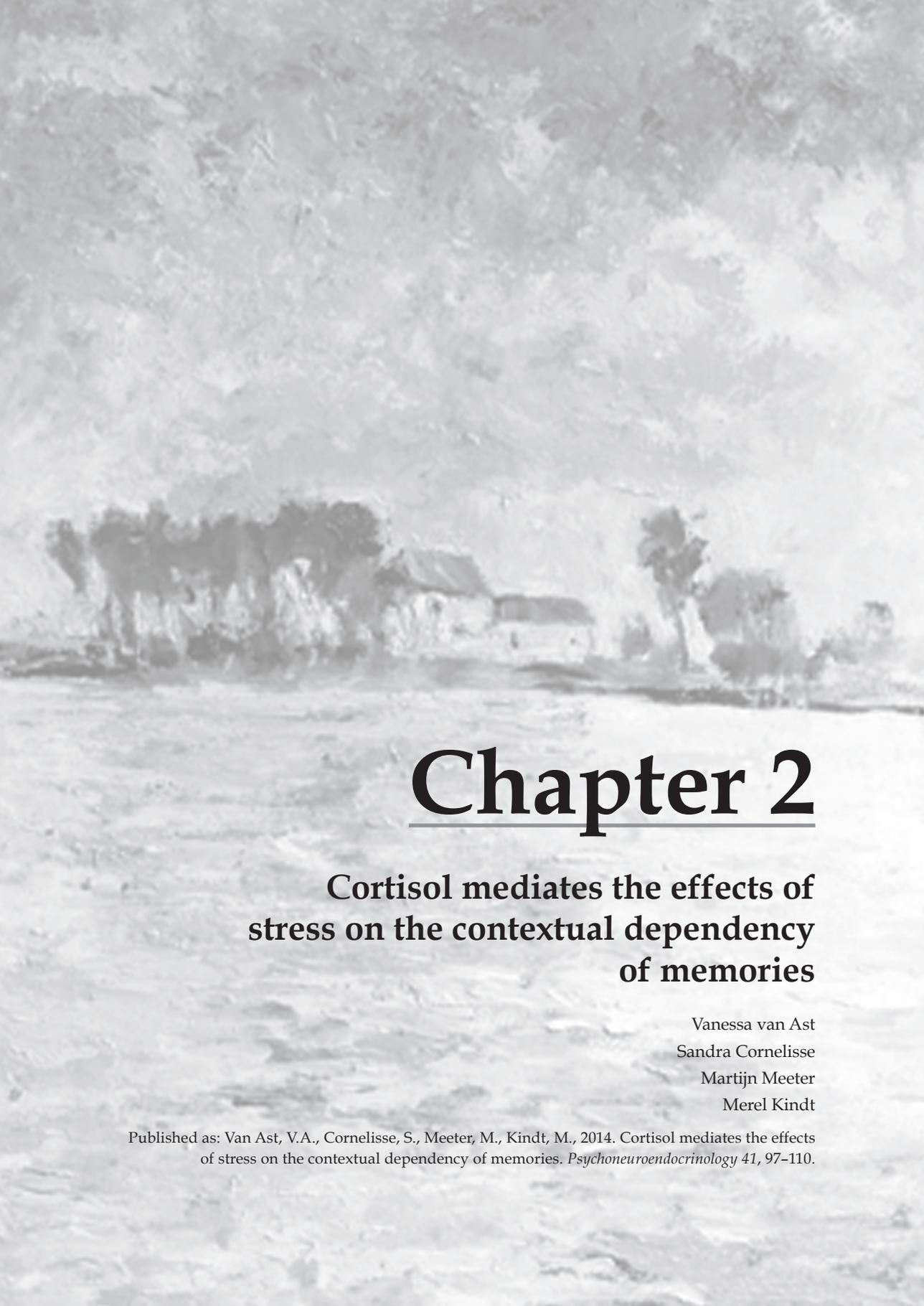
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Chapter 2

Cortisol mediates the effects of stress on the contextual dependency of memories

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SUMMARY

Stress is known to exert considerable impact on learning and memory processes. Typically, human studies have investigated memory for single items (e.g., pictures, words), but it remains unresolved how exactly stress may alter the storage of memories into their original encoding context (i.e., memory *contextualization*). Since neurocircuitry underlying memory contextualization processes is sensitive to the well-known stress hormone cortisol, we here investigated whether cortisol *mediates* stress effects on memory contextualization. Forty healthy young men were randomly assigned to a psychosocial stress or control group. Ten minutes after stress manipulation offset, participants were instructed to learn and remember neutral and negative words, each of which was depicted against a unique background picture. Approximately 24 hours later, memory was tested by means of cued retrieval and recognition tasks. To assess memory contextualization half of the words were tested in intact item-contexts pairs, and half in rearranged item-context combinations. Recognition data showed that cortisol, but no other indices of stress such as heart rate or subjective stress, *mediated* the effects of stress on contextualization of neutral and negative memories. The mediation analysis further showed that stress resulted in increases in cortisol and that cortisol was positively related to memory contextualization, but unrelated to other measures of memory. Thus, there seems to be a specific role for cortisol in the integration of a central memory into its surrounding context.

1. INTRODUCTION

Memory retrieval is generally enhanced when the encoding context and retrieval context are similar (Godden and Baddeley, 1975). As such, the ability to store declarative memories into their original encoding context (i.e., memory *contextualization*) is highly adaptive since it aids in subsequently retrieving memories that are likely to be appropriate in a specific context. The hippocampus has been suggested to underlie context effects on memory (e.g., O'Reilly and Rudy, 2000), by binding together multiple elements of an experience into a novel conjunctive representation (Eichenbaum, 2004; O'Reilly and Rudy, 2000). Also, the hippocampus is known to be sensitive to corticosteroids (de Kloet et al., 2005) that are released from the adrenal cortex in response to stress. Thus, by means of stress hormone effects on memory neurocircuitry, stress may affect the contextualization of memories. However, as of yet, little research with humans has examined how stress influences memory contextualization processes. Instead, studies focused on single items to be memorized, such as words or pictures. Because memories are often interrelated in complex associative networks rather than stored in isolation, investigating the effects of stress on memory contextualization might be a more ecological valid approach to investigating stress effects on memory.

Animal research has shown that glucocorticoid receptor (GR) activation by cortisol seems to be a prerequisite for the storage of information (de Kloet et al., 2005). In humans, stress typically enhances consolidation (Buchanan and Lovallo, 2001), and can enhance encoding (Cornelisse et al., 2011b; de Quervain et al., 2009; but see Elzinga et al., 2005; Van Ast et al., 2013). Such alterations in memory have indeed been shown to positively relate to stress-induced cortisol levels (e.g., Cornelisse et al., 2011b; Smeets et al., 2008). Thus, one might predict that stress enhances memory contextualization. However, enhanced *item* memory (e.g., pictures, words) by stress does not necessarily mean that binding the item into its original encoding context will be enhanced as well. In agreement, the 'arousal-impairs-binding' theory (Payne et al., 2003), poses that stress (likely through corticosteroid effects) enhances memory for item information from an arousing event at the cost of contextual binding, since these two types of memory formation depend upon different brain regions that on their turn differ in their sensitivity to corticosteroids (see also Mather, 2007).

Context has been broadly defined as the internal (cognitive and hormonal) and external (environmental and social) background against which psychological processes operate (Spear, 1973). Consequently, previous studies investigating the effects of stress on contextual binding have operationalized context in various ways. One study demonstrated that social stress enhanced memory for words related to person-

ality, but not a category unrelated to personality, which was interpreted as enhanced context congruent memory by stress (Smeets et al., 2007). Another study found that stress enhanced memory for the stress manipulation itself (Quas and Lench, 2007), but here context was not explicitly manipulated, neither was an appropriate control group included, precluding causal conclusions on the role of stress in the contextualization of memory. Notably, stress effects in both studies were positively related to cortisol. Another study manipulated thematic arousal independently of the to-be remembered material, and showed that social stress specifically enhanced high arousing themes. In addition, this memory-enhancing effect was most pronounced for elements central to the to-be-remembered event (Echterhoff and Wolf, 2012), which was again positively associated with cortisol. Confirming a crucial role for cortisol in memory contextualization, we have shown that cortisol may enhance or impair memory contextualization, depending on the timing of cortisol elevations relative to memory encoding (Van Ast et al., 2013). Other studies did not report possible relationships of cortisol with stress effects on contextual dependency of memory. One study manipulated context by the physical environment in which encoding and retrieval took place (i.e., change of room and odor) and found that stress impaired the typical memory enhancement by context congruency (Schwabe et al., 2009a). A second study showed that social stress enhanced memory for objects that were central to the stressor, but not for unrelated items (Wiemers et al., 2013).

Summarizing the above findings, cortisol likely plays an important role in the relationship between stress and memory contextualization. Therefore we hypothesized that cortisol may *mediate* the effects of stress on memory contextualization. This effect may be most pronouncedly observed in negative (i.e., of negative valence) memories as compared to neutral memories, by means of glucocorticoid modulation of emotion-induced noradrenergic activation (Roosendaal et al., 2006c). Since the direction of cortisol effects on contextualization is still debatable, and previous studies have reported mixed results (Echterhoff and Wolf, 2012; Schwabe et al., 2009a; Smeets et al., 2007; Van Ast et al., 2013; Wiemers et al., 2013), we left the direction of cortisol effects on contextualization open. We used mediation analysis (Baron and Kenny, 1986; Shrout and Bolger, 2002 see also **Figure 1A**) since it is a powerful method to assess whether cortisol explains a substantial amount of the covariance between stress and the extent to which a stimulus is remembered in a context-dependent manner. In addition, given that profound individual differences exist in memory performance as well as in (psycho)physiological responses to stress, mediation analysis is preferable over methods that merely test for group differences (Kosslyn et al., 2002). To critically test a unique role for cortisol in mediating stress effects on memory contextualization, we also tested whether other indices of stress

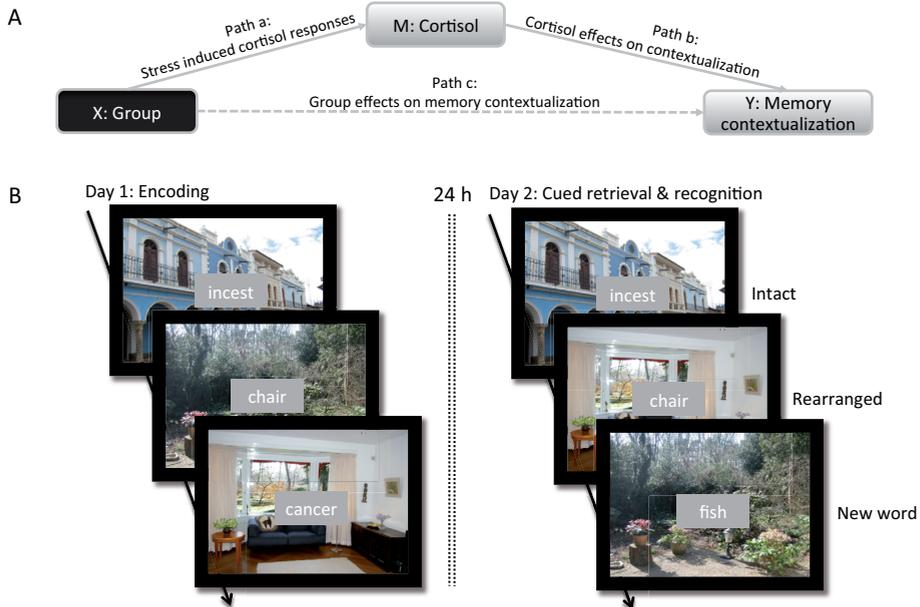


Figure 1. (A) Mediation diagram. For cortisol to *mediate* effects of stress on memory contextualization, three relationships should be observed: 1) Group (TSST = 1; control = -1) should result in elevated cortisol responses (path *a*), 2) Cortisol should relate to memory contextualization while controlling for the effects of stress (path *b*), and 3) The relationship between stress and contextualization should be significantly reduced when controlling for the effects of cortisol, which is being referred to as the mediation test or the *axb* test. The relationship between Group and memory contextualization is depicted by path *c*. **(B)** Experimental paradigm. To induce neutral versus emotional declarative memories during encoding, participants were presented with 30 negative and 30 neutral words on a small gray rectangle (image not to scale), each in combination with unique color pictures of natural scenes or city landscapes. During retrieval testing the following day participants were presented with the word stems (first two letters), which were shown in a grey rectangle depicted against a color picture. Word stems were unique, and participants were asked to use the stem as a memory cue and to complete the word by typing in the remaining letters. During recognition (here depicted), participants were presented with the 60 old words (30 negative and 30 neutral) that were presented during encoding on day 1, intermixed with 60 foil words (30 negative and 30 neutral) that were not presented before. Crucially, to assess context dependency of memories, half of the old words were presented in the same word-context combination (intact) as at the first session while the other half of the word-context pairs were rearranged (rearranged) to form new pairs. The foil items were presented against backgrounds viewed during the first session. Participants indicated whether they recognized the word as having seen during the imagination task by responding “old” or “new”. After indicating the word was old, the remember/know choice appeared.

such as heart rate, heart rate variability (HRV; an index of adaptive regulation of peripheral control: Thayer et al., 2012), alpha amylase (i.e., a marker of noradrenergic activity) or subjective mood functioned as mediators. Memory performance was assessed by cued retrieval and recognition tasks. Recognition performance is thought to originate from two independent memory processes; it may either be based on a detailed vivid feeling of reexperience (recollection), or on a sense that the item has been previously encountered (a sense of familiarity; Yonelinas, 2002). Therefore, we also tested whether cortisol mediated either of these processes specifically.

2. METHODS

2.1. Participants

Forty male participants participated in the experiment with a mean age of 22 years ($SD=3.76$, 18-39) and mean BMI of 21.75 ($SD=3.27$, 15-27) gave written informed consent and completed the study. Inclusion criteria as assessed by self-report were: no past or present psychiatric or neurological condition, no diagnosis of dyslexia, and age between 18 and 40 years. Men having any somatic or endocrine disease (e.g., acute asthma), or taking any medication known to influence central nervous system or endocrine systems were excluded from participation. Further, participants were asked to refrain from taking any drugs three days prior to participation, and to get a night of proper sleep, refrain from heavy exercise, alcohol and caffeine intake 12 hours prior to participation, and not to eat, drink, smoke or brush teeth two hours before participation. Participants were rewarded for their participation with course credits or paid € 24,-. The local ethical committee of the University of Amsterdam approved the study, which was performed in accordance with the Helsinki declaration.

2.2. Stress and control manipulations

Social stress was manipulated by means of the TSST (Kirschbaum et al., 1993) and consisted of a 3 min preparation period, a free 5 min speech simulating a job interview, as well as a 3 min mental arithmetic task. The speech and mental arithmetic took place while standing in front of a nonresponsive audience and were video- and audio-taped.

In line with recommendations by Het and colleagues (2009), the control condition was designed in such a way that it closely paralleled important modulating variables such as physical (e.g., participants are standing in both conditions) or cognitive load of the TSST, but left out the uncontrollability and social-evaluative threat central to the TSST (Dickerson and Kemeny, 2004). During the control group tasks, the experimenter left the room.

2.3. Memory tasks

2.3.1. Encoding

During encoding participants were shown 30 neutral and 30 negative words on a small gray rectangle presented against color pictures depicting for example natural scenes or city landscapes (see **Figure 1B**). Words were concrete nouns drawn from a validated database (Hermans and de Houwer, 1994), consisted of 5 to 10 letters and contained no more than 3 syllables. Neutral and negative words did not differ in terms of familiarity ratings, length, and/or amount of syllables, but did significantly differ in terms of valence. Background pictures were selected from Talamini et al. (2010) and from personal collection. The images contained no distinguishing objects, thus distinctiveness of the contexts relied on their unique spatial configuration. Word-context combinations were presented twice for 4 seconds, in the same order, with in between each word a gray screen with a fixation cross presented for 1 second. Participants were instructed to learn the words, and that their memory performance would be tested the next day. Note that any context effect on memory is incidental, as no instructions were given regarding the pictures. The task began with three practice trials that at the same time functioned as a buffer for possible primacy effects on subsequent memory performance. All memory tasks were programmed in E-prime (version 2.0; Psychology Software Tools, Inc.; Pittsburgh, PA).

2.3.2. Cued retrieval

For the first memory test of the session participants were presented with the first two letters of all the words presented during the memory encoding task. The stems were shown in a grey rectangle presented against a color picture. Word stems were unique, and participants were asked to use the stem as a memory cue and to complete the word by typing in the remaining letters. In addition, they were told that the background picture could be the same as the previous day, but not necessarily so. Only those items that could be recalled were to be completed. In case the participants could not remember the word, they were asked to respond with 'xxx'. The task was self-paced. Reaction times were recorded. Crucially, half of the words (i.e., 15 emotional and 15 neutral) were presented in the same context as the previous day ('intact context' condition), while the other half was presented in a context that was previously seen, but not in this specific word-context combination ('rearranged context' condition) (Talamini et al., 2010). By doing so, we created one condition in which unique contextual information is available to aid memory for the word, and one in which contextual information was present (i.e., the context was previously seen), but not related to the word at hand.

2.3.3. Recognition

See **Figure 1B** for an example of some memory task (recognition) trials. During the recognition test, participants were presented with the 60 old words that were presented during memory encoding on day 1, intermixed with 60 foil words (30 negative and 30 neutral) that were not presented before. Again, half of the old words were presented in the same word-context combination as at the encoding session, while the other half of the word-context pairs (same pairs as at recall) were rearranged to form new pairs (Talamini et al., 2010; Tsivilis et al., 2001). All of the foil words were combined with old contexts, in such a way that contexts previously associated with a neutral or negative word were equally divided over the foil neutral and negative words. The test was self-paced. Participants were instructed to indicate whether they recognized the word as having seen during the encoding task, by responding “old” or “new.” After each old/new decision, participants underwent a typical ‘remember/know’ procedure (Tulving, 1985): When responding “old” (whether correctly or not), participants then indicated whether they “remembered” seeing the word, or merely “knew” that they had previously seen the word. Prior to the recognition test, the experimenter orally explained the distinction between remember and know responses, making use of validated instructions (Geraci et al., 2009; Rajaram, 1993). Shortly, participants were asked to give a “remember” response when the word brought back to mind a specific detail from the episode in which the word had been experienced, such as a sensory detail, a thought, or a feeling. They were asked to give a “know” response when they knew having encountered the word, but did not consciously recollect anything about its actual occurrence or what happened or what was experienced at the time of its occurrence. Reaction times of the responses were recorded for old/new recognition.

2.4. Physiological and subjective measurements

2.4.1. Salivary cortisol & alpha-amylase

Saliva samples were obtained using Salivette devices (Sarstedt, Nümbrecht, Germany) at 5 time points throughout the experiment (**Figure 2**). After testing, the salivettes were stored at -25 °C. Upon completion of the entire study, samples were sent out to the Technische Universität Dresden, Germany for biochemical analysis. Salivary free cortisol concentrations were assessed using a commercially available chemiluminescence immuno-assay (CLIA) with high sensitivity of 0.16 ng/ml (IBL, Hamburg, Germany). Alpha-Amylase was assessed by a quantitative enzyme kinetic method.

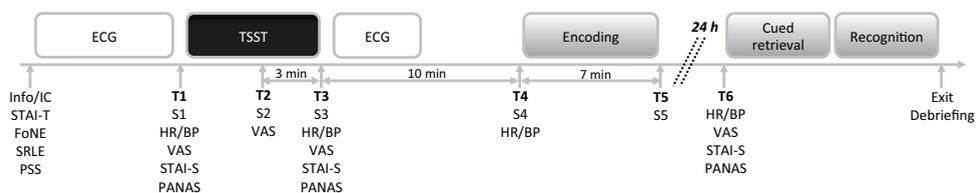


Figure 2. Timeline of the experiment. Upon arrival, participants filled out the informed consent (IC) and several personality questionnaires followed by a 10-minute baseline ECG recording. Blood pressure (BP) and heart rate (HR) were measured where after baseline mood questionnaires (PANAS, VAS and STAI-S) along with a baseline saliva sample (T1) were taken. Then, participants were subjected to the stress (TSST) task or a control procedure. In between the speech and arithmetic, another sample (T2) and VAS were taken. Directly after arithmetic offset blood pressure and heart rate were measured and another set of mood questionnaires was filled out along with a third saliva sample (T3). Again, ECG was measured for 5 minutes after which instructions for the encoding task were given, before and after a fourth (T4) and fifth (T5) saliva sample were taken, respectively. Memory testing took place approximately 24 hours after encoding. This session commenced with the mood questionnaires, BP and HR measurement (T6), followed by the cued retrieval and recognition tests.

2.4.2. Blood pressure

Blood pressure was measured with a cuff attached to the left upper arm, using an electronic sphygmomanometer (Omron, model HEM-780-D).

2.4.3. ECG

Two ECG disposable electrodes were placed left and right on the chest of the participant, and one reference electrode was placed on the abdomen. ECG was recorded with the VSRP98 software package (Versatile Stimulus Response Registration Program, 1998; developed by the Department of Psychology, University of Amsterdam).

2.4.4. Subjective measures

To probe possible differences between the experimental groups in personality variables that may affect responses to the stress manipulation participants filled out Dutch versions of the trait scale of the State-Trait Anxiety Inventory (STAI-T; Spielberger et al., 1970), the Brief Fear of Negative Evaluation revised scale (FoNE; Carleton et al., 2006), the Perceived Stress Scale (PSS; Cohen et al., 1983) and the Survey of Recent Life Events (SRLE; Kohn and Macdonald, 1992). To assess the effects of the stress versus the control manipulation on subjective mood, participants filled out the State-Anxiety Inventory (STAI-S; Spielberger et al., 1970), the Positive Affect and Negative Affect Schedule (PANAS; Watson et al., 1988) and a visual analogue scale (VAS; a 10 cm horizontal line that we measured in random units ranging from

0-100) that assessed to what extent the participant was feeling stressed ranging from 'not at all stressed' to 'very stressed'. Using VAS measures, a final post-experimental questionnaire assessed motivation and concentration to complete the speech and arithmetic tasks, the encoding tasks and memory tasks.

2.5. Procedure

A schematic outline of the experimental procedure is depicted in **Figure 2**. Participants were randomly assigned to either the stress or placebo procedure. Testing took place in between 1 pm and 7 pm, when endogenous cortisol levels are stable and relatively low (Pruessner et al., 1997). Upon arrival, participants read the information brochure, were screened by means of an interview to assess eligibility for participation and signed the informed consent followed by completion of the trait scale of the STAI-T, FoNE, PSS, and SRLE. Then, a 10-minute baseline ECG recording took place, during which participants watched a fragment of a relaxing movie. Thereafter, blood pressure and heart rate was measured using an electronic sphygmomanometer and baseline mood questionnaires (PANAS, VAS and STAI-S) along with a baseline saliva sample (T1) were taken. Then, the experimenter introduced the participant to either the stress or control instructions. In between the speech part and arithmetic part, another saliva sample (T2) and VAS were taken. Directly after arithmetic offset BP and HR were measured and another set of mood questionnaires was filled out along with a third saliva sample (T3). Again, ECG was measured for 5 minutes after which instructions for the encoding task were given, with a fourth saliva sample (T4). Time in between arithmetic offset and encoding onset was always exactly 10 minutes to ensure that peak stress induced cortisol levels (Kirschbaum et al., 1993) coincided with encoding and initial consolidation processes. After encoding a final sample (T5) was taken. To distinguish cortisol's effects on memory formation from its effects on retrieval, memory testing took place approximately 24 hours later. The second test session commenced with the mood questionnaires (PANAS, VAS and STAI-S; T6), BP and HR measurement followed by the cued retrieval and recognition tests. Finally, the exit questionnaire was filled out and the experimenter debriefed the participant.

2.6. Data reduction and analysis

The ECG signal was visually inspected and artifacts were corrected. Interbeat intervals were imported to Kubios HRV Package (Tarvainen et al., 2009). We calculated average heart rate and HRV (Thayer et al., 2012) during baseline and post stress. HRV was estimated by the root mean square of successive difference (RMSSD).

In order to obtain a single cortisol (and alpha-amylase) parameter for the mediation analysis an area under the curve index with respect to the increase (AUC_i; Pruessner et al., 2003) was calculated out of the four measurements for each participant. This measure was subsequently log transformed and centered around the mean.

Initial statistical analyses were performed using the SPSS statistical software package. To assess group differences in sample characteristics, univariate ANOVAs with the between-subject factor Group (Stress, Control) were employed. Physiological and subjective measures of stress were assessed by means of a mixed ANOVA with the within-subject factor Time (baseline, mid (if applicable), post) and between-subjects factor Group.

To assess participants' *retrieval* memory performance as a function of context and emotion, the proportion of correct answers was calculated per condition (i.e., neutral intact, neutral rearranged, negative intact, negative rearranged). Similarly, to assess *recognition* memory performance as a function of context and emotion, hit rate (i.e., correct classification of previously presented words as "old") was calculated for each condition. False alarm rates (i.e., misclassification of new words as "old") were calculated as a function of emotion. Using the hit and false alarm rates d' sensitivity index was calculated, according to signal detection theory. Hit rates >0.975 were truncated at 0.975, and false alarm rates <0.025 at 0.025 (Stanislaw and Todorov, 1999). To assess the *subjective* quality of memories for the different conditions, we calculated recollection ($\text{remember}_{\text{hit}} - \text{remember}_{\text{fa}} / (1 - \text{remember}_{\text{fa}})$) and familiarity using an adapted signal detection procedure ($d' = (\text{know}_{\text{hit}} / (1 - \text{remember}_{\text{hit}})) \text{ vs. } (\text{know}_{\text{fa}} / (1 - \text{remember}_{\text{fa}}))$) (Yonelinas et al., 1998). These scores take into account the fact that the probability of making a know response to a presented word was constrained by the number of remember responses made to presented words, because the participants were instructed to respond "know" to items that were familiar and not recollected (Yonelinas et al., 1998).

To assess contextual dependency of neutral and negative words and overall effects of stress on retrieval and recognition memory, these data were first analyzed by means of ANOVAs with the repeated-measures factors Emotion (neutral, negative) and Context (intact, rearranged) and Group as the between-subjects factor. A Greenhouse-Geisser procedure was used in case of violation of the sphericity assumption in ANOVAs.

To assess whether cortisol *mediated* effects of stress on memory contextualization, we first calculated *contextualization* indices by subtracting memory performance measures and subjective memory of the rearranged condition from the intact condition, for negative and neutral memories independently. Thus, a larger contextual-

ization index reflects greater contextual dependency of memories. For convincing evidence that cortisol *mediates* stress effects on contextualization, three conditions should hold. These conditions are outlined in the graphical model in **Figure 1A** and comprise the effects tested in a formal mediation analysis (Baron and Kenny, 1986; Zhao et al., 2010). First, group should predict cortisol responses (path *a*), second, cortisol should relate to memory contextualization while controlling for the overall effects of group (path *b*) and finally, cortisol should formally mediate the group-contextualization relationship (path *axb*) (Baron and Kenny, 1986; Zhao et al., 2010). This latter test assesses whether the mediator explains a significant amount of the effect of the manipulated variable on the measured outcome. We entered Group into the model as the predictor X (Stress = 1, Control = -1), memory contextualization as the dependent variable Y, and cortisol (i.e., the centered and log transformed AUCi measure) as the mediator, M.

Path *a*, path *b* and mediation effects can all be written in simple regression equations following the national convention of Kenny et al., (2003) and were tested within a single structural equation model, using a custom Matlab toolbox (Wager et al., 2009). To test the significance of *a*, *b*, and *axb* effects, bootstrap tests (1000 samples) were used, which provides a more sensitive test of mediation than the approximate test based on normality assumptions (Shrout and Bolger, 2002). Alpha was set at .05 for all statistical analyses.

3. RESULTS

3.1. Participant characteristics

The stress and control groups did not differ in terms of age, body mass index, trait anxiety, subjective experienced chronic stress, or fear of negative evaluation (all $F < 0.64$, all $p > 0.431$). The number of smokers in the groups did not differ (Stress: 6, Control: 4, $\chi^2_{(1)} = 0.53$, $p = 0.465$), neither did the average of cigarettes smoked differ per group ($F_{1,38} = 1.16$, $p = 0.288$). Participants from both groups had been awake for a comparable amount of time prior to testing ($F_{1,38} = 0.77$, $p = 0.385$), and were as motivated and concentrated to complete all experimental tasks (all $F < 0.43$, all $p > 0.514$). Participant characteristics can be found in **Supplementary Table 1**.

3.2. Physiological and subjective measurements of stress

3.2.1. Salivary cortisol levels

The stress induction resulted in altered cortisol responses for the two groups (Group×Time; $F_{4,152} = 20.37$, $p < 0.001$, $\eta_p^2 = 0.35$; **Figure 3A**). Planned comparisons

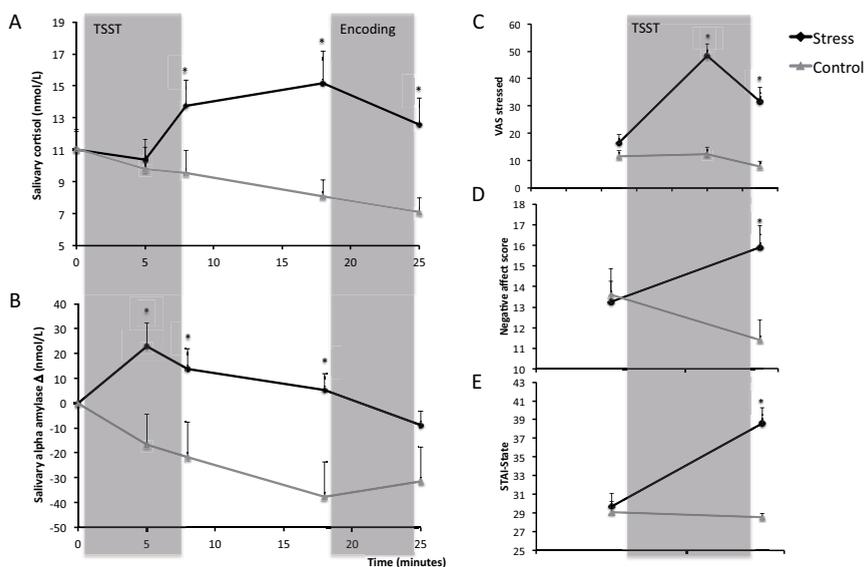


Figure 3. Physiological and subjective measurements of stress. **(A)** Mean cortisol responses. **(B)** Baseline-corrected alpha-amylase responses for the stress and placebo groups. **(C)** Subjective stress (VAS). **(D)** Negative affect. **(E)** State anxiety ratings. Error bars represent standard error of the mean (s.e.m.). Significant differences with placebo are depicted with * = $p < 0.001$.

confirmed that the stress group displayed significantly elevated cortisol levels after the TSST (T3; $F_{1,38} = 4.79$, $p = 0.035$, $\eta_p^2 = 0.11$), before memory encoding (T4; $F_{1,38} = 12.32$, $p < 0.001$, $\eta_p^2 = 0.25$) and after memory encoding (T5; $F_{1,38} = 10.91$, $p = 0.002$, $\eta_p^2 = 0.22$), but not at baseline or during the TSST (all $F < 0.23$, all $p > 0.636$). There was no significant interaction effect with Group and Smoking ($F_{1,36} = 2.79$, $p = 0.103$).

3.2.2. Salivary alpha-amylase levels

Analyses were performed on baseline corrected alpha-amylase levels because the control group displayed larger alpha-amylase levels at baseline. A significant Group \times Time interaction effect ($F_{4,152} = 4.20$, $p = 0.003$, $\eta_p^2 = 0.10$) indicated that the stress induction resulted in altered amylase responses in the two groups (**Figure 3B**). Planned comparisons confirmed that stress successfully elevated alpha-amylase levels of the stress group already during the TSST (T2; $F_{1,38} = 7.37$, $p = 0.010$, $\eta_p^2 = 0.16$), right after the TSST (T3; $F_{1,38} = 5.35$, $p = 0.026$, $\eta_p^2 = 0.12$) and right before memory encoding (T4; $F_{1,38} = 7.52$, $p = 0.009$, $\eta_p^2 = 0.17$), but not after memory encoding (T5; $F_{1,38} = 2.56$, $p = 0.118$).

3.2.3. Blood pressure (BP)

The stress induction was successful in altering systolic BP (Group×Time; $F_{1,38}=30.15$, $p<0.001$, $\eta_p^2=0.459$). Planned comparisons confirmed that systolic BP in the stress group was significantly elevated after the TSST as compared to the control group (T3; $F_{1,38}=23.00$, $p<0.001$, $\eta_p^2=0.377$), but also tended to differ at baseline (T1; $F_{1,38}=3.55$, $p=0.068$). A similar analysis did not reveal a significant Group×Time interaction effect ($F_{1,38}=1.25$, $p=0.272$) for the diastolic BP data. Nevertheless, planned comparisons showed that diastolic BP values of the two groups were significantly different after stress induction (T3; $F_{1,38}=5.87$, $p=0.020$, $\eta_p^2=0.13$), but not at baseline (T1; $F_{1,38}=1.95$, $p=0.171$). Systolic ($F_{1,38}=2.21$, $p=0.15$) and diastolic ($F_{1,38}=3.45$, $p=0.071$) BP did not differ at the second day of testing.

3.2.4. Heart rate

There was no significant Group×Time interaction ($F_{1,38}=3.31$, $p=0.077$) indicating that the stress induction resulted in altered HR (as measured by the sphygmomanometer) in the two groups, and planned comparisons did not confirm that HR was significantly different between the stress and control group after the TSST (T3; $F_{1,38}=2.31$, $p=0.137$). However, HR did significantly increase for the stress group from baseline ($F_{1,38}=7.31$, $p=0.010$, $\eta_p^2=0.16$) but not for the control group ($F_{1,38}=0.17$, $p=0.896$). HR did not differ between the two groups on day 2 (all $F<0.24$, all $p>0.88$). As measured by ECG, there were no significant Group×Time interactions for HR ($F_{1,38}=0.00$, $p=0.811$) or HRV ($F_{1,38}=0.03$, $p=0.613$).

3.2.5. Subjective measures

Subjective indices of stress are presented in **Figure 3C-F** and in **Supplementary Table 2**. Significant Group×Time interactions for VAS, negative affect and state anxiety indicated that the TSST was successful in inducing stress (all $F_{1,38}>14.13$, all $p<0.001$). Planned comparisons confirmed that the stress manipulation indeed led to increases on all of these measures (all $F_{1,38}>15.38$, $p<0.001$), while groups did not differ at baseline (all $F_{1,38}<2.99$, all $p>0.122$). Stress did not affect positive affect ($F=1.17$, $p=0.29$). None of these subjective measures differed between the two groups on day 2 (all $F<1.90$, all $p>0.17$).

3.3. Memory performance

3.3.1. Retrieval

Overall, stress did not seem to affect contextualization, since there were no significant interactions with Group (all $F_{1,38}<0.35$, all $p>0.353$) and no main effect of Group

($F_{1,38}=1.00, p=0.341$). Retrieval of negative vs. neutral words depended on context (Context \times Emotion interaction; $F_{1,38}=13.05, p<0.001, \eta_p^2=0.26$) with increased retrieval for negative relative to neutral memories in rearranged contexts ($F_{1,38}=29.00, p<0.001, \eta_p^2=0.43$), indicating that negative memories were relatively less context-dependent. Further planned comparisons indicated that both neutral ($F_{1,38}=40.85, p<0.001, \eta_p^2=0.52$) and negative words ($F_{1,38}=8.43, p=0.006, \eta_p^2=0.18$) were generally better remembered in intact contexts versus rearranged contexts. Within intact contexts, neutral and negative words were equally well remembered ($F_{1,38}<0.01, p>0.99$), but within rearranged contexts negative words were better remembered than neutral words ($F_{1,38}=29.00, p<0.001, \eta_p^2=0.43$). These effects are depicted in **Figure 4A**.

Mediation analyses¹ with neutral and negative memory contextualization indices as the dependent variables confirmed that stress did relate to cortisol (path $a=0.16, Z=3.85, p<0.001$), but cortisol did not mediate the contextualization of either neutral (path $a \times b=0.04, Z=0.98, p=0.327$) or negative (path $a \times b=0.04, Z=1.36, p=0.172$) memories, neither did cortisol directly relate to the contextualization of neutral (path $b=0.21, Z=0.96, p=0.335$) or negative memories (path $b=0.22, Z=1.33, p=0.182$). Together, no evidence was found that stress affected cued recall, contextualization of recall, or that stress effects were mediated by cortisol.

3.3.2. Recognition: d-prime

At first sight, analysis of d-prime did not seem to affect contextualization or other memory indices (e.g., overall memory performance), since there were no significant interactions with Group (all $F_{1,38}<1.22, p=0.276$) nor a main effect of Group ($F_{1,38}=0.24, p=0.626$). Unlike the retrieval data, there was no Context \times Emotion interaction ($F_{1,38}=1.04, p=0.749$), but context did exert a beneficial effect on recognition (Context; $F_{1,38}=34.63, p<0.001, \eta_p^2=0.48$). Memory performance for negative words was worse than for neutral words (Emotion; $F_{1,38}=6.41, p=0.016, \eta_p^2=0.14$). Note that the typical memory enhancement for negative words was found on hit rate data (see **Table 1**), but false alarm rates were even higher (see **Table 2**). This resulted in worse memory

1. As an explorative complement to the mediation analysis we classified participants belonging in the Stress group as TSST cortisol high and low responders based on a median split in this group (Domes et al., 2004; Nater et al., 2007). Participants from the Control group were classified as Control cortisol non-responder if their maximum cortisol increase was below 0 nmol/l, $n=18$). The retrieval data were then again analyzed by means of ANOVAs with the repeated-measures factors Emotion and Context, and Group (TSST cortisol high-responders versus Control cortisol non-responders) as the between-subjects factor. This analysis did not reveal any significant interactions with Group ($F<1.89, \text{all } p>0.184$) and no main effect of Group ($F=0.04, p=0.842$).

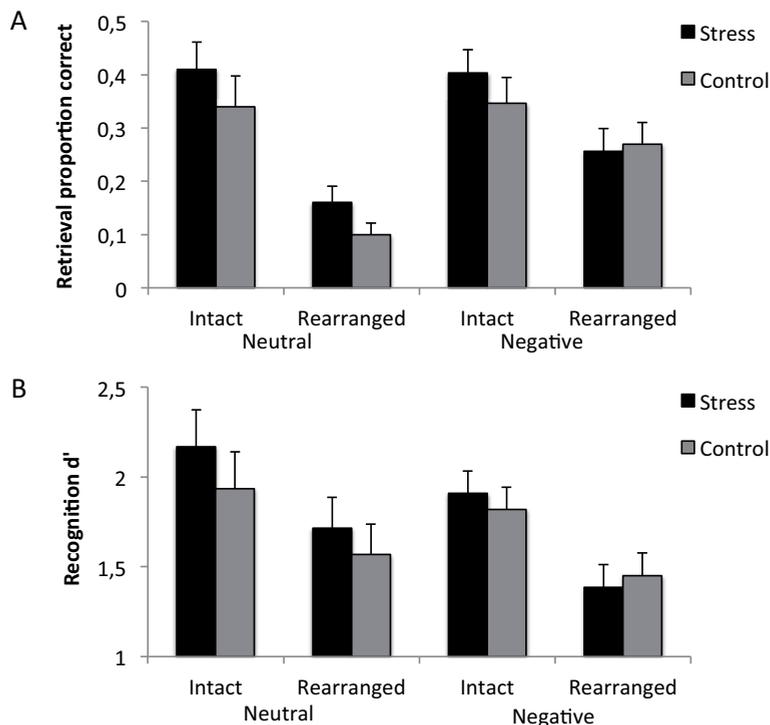


Figure 4. (A) Cued retrieval data. Mean proportion correct responses during the cued recall memory task at day 2, as a function of group (stress or control), context (intact or rearranged) and emotion (neutral or negative). (B) Recognition performance. Graphs depict memory performance indexed by D-prime as a function of group, context and emotion. Error bars represent standard error of the mean (s.e.m.).

Table 1. Raw memory hit rate and subjective quality of memory data (proportions) and reactions times (RT) as a function of group (stress, placebo), context (intact, rearranged) and emotion (negative, neutral). Standard errors (S.E) are depicted between parentheses.

Group	Condition	Hits	RT	Remember	Know
Stress	Intact neutral	0.81(0.15)	2354.30(891.13)	0.51(0.30)	0.30(0.22)
	Intact negative	0.87(0.11)	2317.77(793.64)	0.49(0.22)	0.38(0.22)
	Rearranged neutral	0.67(0.20)	2820.34(1149.88)	0.30(0.22)	0.36(0.16)
	Rearranged negative	0.74(0.16)	2858.00(923.71)	0.33(0.21)	0.40(0.20)
Control	Intact neutral	0.76(0.19)	2398.38(926.50)	0.46(0.27)	0.30(0.16)
	Intact negative	0.87(0.10)	2388.62(1040.48)	0.52(0.20)	0.35(0.17)
	Rearranged neutral	0.66(0.21)	2675.54(909.82)	0.35(0.17)	0.32(0.14)
	Rearranged negative	0.78(0.14)	2480.71(937.44)	0.39(0.19)	0.40(0.15)

Table 2. Raw memory false alarm rate and subjective quality of memory data (proportions) and reactions times (RT) as a function of group (stress, placebo), and emotion (negative, neutral). Standard errors (S.E) are depicted between parentheses.

Group	Condition	False alarms	RT	Remember	Know
Stress	Neutral	0.15(0.11)	3037.48(1013.02)	0.04(0.06)	0.10(0.10)
	Negative	0.27(0.13)	3132.01(920.89)	0.09(0.08)	0.17(0.12)
Control	Neutral	0.17(0.12)	2883.19(1112.64)	0.05(0.11)	0.13(0.11)
	Negative	0.28(0.13)	2914.52(923.50)	0.14(0.16)	0.18(0.10)

performance for the negative words as measured by d-prime. D-prime data are depicted in **Figure 4B**.

Following the same reasoning as for the retrieval data, we further tested whether cortisol played a mediating² role between Group (X) and contextualization of memories (Y). The mediation analyses confirmed that Group predicted cortisol responses in models for neutral (path $a=0.16$, $Z=3.83$, $p<0.001$) and negative memories (path $a=0.16$, $Z=3.73$, $p<0.001$). For the neutral and negative memories, cortisol positively predicted memory contextualization (path $b=0.92$, $Z=1.96$, $p=0.050$; path $b=1.10$, $Z=2.33$, $p=0.020$, respectively). That is, controlling for the effects of group, larger cortisol responses were positively related to contextual dependency of memories. More importantly, even though there was no significant relationship between stress and memory contextualization (path $c=0.04$, $Z=0.46$, $p=0.642$; path $c=0.08$, $Z=0.86$, $p=0.388$), cortisol did *mediate* the effects of stress on neutral (path $a \times b = 0.15$, $Z=2.07$, $p=0.034$) and negative (path $a \times b = 0.18$, $Z=2.44$, $p=0.015$) memory contextualization. Note that when the $X \rightarrow Y$ relationship is quite distal, as is presently the case with the dichotomous predictor, a significant $X \rightarrow Y$ relationship is not required for M to be a significant mediator, especially when there is strong theoretical reason to expect a mediation model (Shrout and Bolger, 2002; Zhao et al., 2010). Graphs of the significant mediation paths are depicted in **Figure 5**.

Importantly, these findings uniquely pertained to *contextualization*, as models using overall, neutral, negative, intact, or rearranged memory performance as the dependent variables did not reveal any significant relationships between cortisol and memory performance (path b), nor a significant mediating role (path $a \times b$) for cortisol. Also, when alpha-amylase was entered as mediator of group effects on contextualization neither path b nor the mediation effect was even approaching significance.

2. The analysis with the TSST cortisol high responders versus the Control cortisol non-responders did reveal a marginally significant Group \times Context interaction ($F_{1,23}=4.22$, $p=0.052$) pointing to a specific role for cortisol in the contextualization of both neutral and negative memories equally.

memories however, path b (path $b=0.95$, $Z=1.28$, $p=0.194$) and the mediation effect (path $a \times b = 0.17$, $Z=1.33$, $p=0.184$) were not significant anymore.

3.3.3. Recognition: Subjective quality of memories.

Analysis of recollection data (Figure 6) showed that context advanced recollection ($F_{1,38}=19.75$, $p<0.001$, $\eta_p^2=0.34$). Analysis of familiarity data revealed a significant Context \times Emotion interaction ($F_{1,38}=6.27$, $p=0.017$, $\eta_p^2=0.14$), that was due to more “know” responses to negative than to neutral words in intact contexts ($F_{1,38}=5.78$, $p=0.021$, $\eta_p^2=0.132$). No other effects reached significance for either recollection or familiarity (all $F_{1,38}<1.61$, all $P>0.212$).

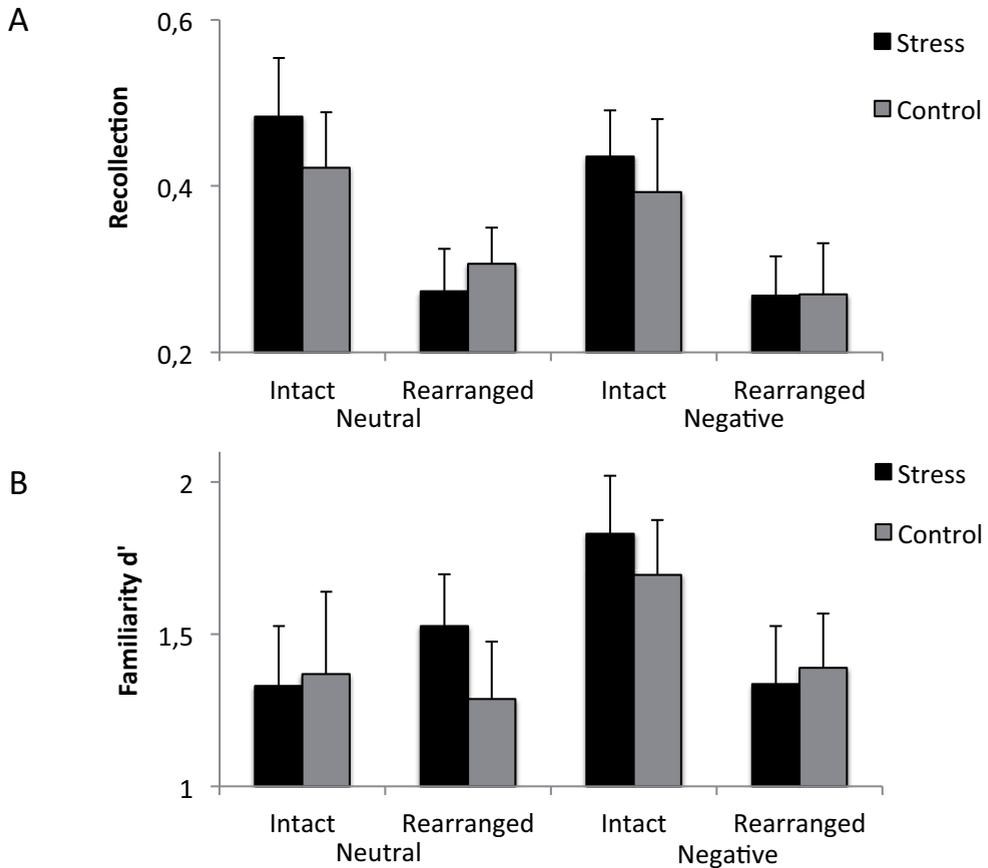


Figure 6. Subjective quality of memories. (A) Mean recollection and (B) familiarity scores as a function of group (stress of control), emotion (neutral or negative) and context (intact or rearranged). Error bars represent standard error of the mean (s.e.m.).

We further tested whether cortisol played a mediating³ role between Group (X) and contextualization of the subjective quality (recollection or familiarity) (Y) of negative or neutral memories. Cortisol did not mediate contextualization of recollection of negative (path $a \times b = 0.03$, $Z = 0.78$, $p = 0.386$) or neutral memories (path $a \times b = 0.01$, $Z = 0.28$, $p = 0.778$), or contextualization of familiarity of neutral words (path $a \times b = 0.03$, $Z = 0.10$, $p = 0.921$). However, in line with the d' data, cortisol did reveal to be a significant positive mediator of stress effects on contextualization of feelings of familiarity for negative words (path $a \times b = 0.32$, $Z = 3.13$, $p = 0.002$). Again, cortisol itself was positively related to contextualization (path $b = 1.91$, $Z = 3.21$, $p < 0.001$).

4. DISCUSSION

The aim of the present study was to assess whether cortisol mediates stress effects on the contextual dependency of negative and neutral memories. Recognition data showed that cortisol *mediated* the effects of psychosocial stress on memory contextualization. In other words, cortisol explained a substantial amount of the covariance between group and the extent to which a stimulus is remembered in a context-dependent manner. Cortisol uniquely mediated stress effects on memory; other important psychophysiological and psychological indices of stress did not appear to be significant mediators (path $a \times b$). The mediation analysis further showed that stress resulted in increases in cortisol (path a) and that cortisol was positively related to memory contextualization (path b), but unrelated to other measures of memory such as overall memory performance. Thus, there seems to be a specific role for cortisol in the integration of a central memory into its surrounding context. These findings have important implications for our understanding of how stress affects memory processes, which we will further discuss below.

The majority of human studies investigating stress effects on memory have investigated non-associative and distinct emotional stimuli (e.g., words, pictures, for review see; de Quervain et al., 2009). Though valuable, such an approach overlooks an essential feature of human episodic memory: the necessity to bind together disparate elements of an experience into an integrated representation. By inclusion of intact and rearranged context conditions we could concurrently assess memory performance when unique contextual information is available to aid memory, and memory performance when no additional contextual information is available to reactivate the memory. Since cortisol was indeed related to the difference between

3. The additional analysis (see footnote on retrieval data) based post-hoc on the cortisol data did not reveal any interactions with Group (all $F < 2.46$, all $p > 0.131$) nor a main effect of Group (all $F = 0.55$, $p = 0.817$).

intact and rearranged conditions (contextualization), one likely interpretation of the present findings is that cortisol aids in the process of making a conjunctive representation of a central memory in its context

We did not observe an overall effect of stress on memory contextualization (path c) but cortisol was a significant mediator of stress effects on contextualization, consistent with an 'indirect-only' mediation model (Zhao et al., 2010). Since typically about two third of individuals respond with a robust cortisol response to the TSST (e.g., Smeets et al., 2006), and the cortisol responses of the control and stress group were partly overlapping (see **Figure 5**), it is not surprising that the overall effect of stress was not present (though an overall stress effect was in fact present when TSST cortisol high responders versus Control cortisol non-responders were analyzed). Further, stress effects on object memory have often been related to cortisol (Cornelisse et al., 2011b; e.g., Smeets et al., 2008). Since these previous studies did not manipulate context, these findings are comparable with our 'intact context' condition. Perhaps then, stress does not only directly strengthen object memory but, through cortisol, additionally enhances the process of contextualization.

The results also showed that cortisol's effect on contextualization for the negative words operated via familiarity. The feeling of familiarity can be induced automatically at a reoccurrence of an event, and can emerge in the absence of any conscious recollection of the context in which the event originally occurred. It is possible that the cortisol effects revealed on recognition are at least partly driven by an enhanced feeling of familiarity, though the former was a more general effect, existing for both negative and neutral memories. Remarkably similar, another study also found that stress increased familiarity-based recognition without affecting either recollection or free recall (Yonelinas et al., 2011).

The present findings seem to be at odds with a study that investigated the effect of stress on context dependent memory of an object-location task (Schwabe et al., 2009a). It was found that stress disrupted contextual enhancement of performance on neutral objects. But since no relationships with cortisol were reported, it remains unclear what aspect of stress caused the effect. Other studies reported positive relationships between cortisol and contextual enhancement of memory, though context in these studies was defined more broadly than here (Echterhoff and Wolf, 2012; Smeets et al., 2007; Wiemers et al., 2013). To assess the specific role of cortisol in altered memory contextualization, we pharmacologically manipulated (by means of 10 mg hydrocortisone) cortisol in another study (Van Ast et al., 2013). We found that cortisol administered right before encoding *impaired* contextualization of negative memories. Though the paradigm differed in important aspects from the current paradigm (i.e., encoding instructions, cortisol timing versus encoding),

one particularly important factor underlying the discrepancy in findings may be variation in cortisol concentrations (Abercrombie et al., 2003): the hydrocortisone manipulation resulted in cortisol levels over 50 times as high (absolute peak cortisol levels *mean*: 447 with *SD*: 118) as here. Also, in the study by van Ast et al. (2013) the hydrocortisone manipulation uniquely impacted negative memories, which was explained by interacting cortisol and arousal-evoked central adrenergic release caused by the negative words (Kensinger, 2004; Roozendaal et al., 2006c). In the present study, cortisol affected both neutral and negative memories. Perhaps stress-induced adrenergic activation acting on neutral memories too has obscured possible differences in negative and neutral memories. Either way, the discussed studies all converge on the idea that cortisol can pronouncedly alter the way memories are integrated in their original encoding contexts.

It is interesting to speculate which aspects of memory formation may be most sensitive to stress and concomitant stress hormones, and what the underlying neural mechanisms may be. Considerable MR and GR co-expression is found in hippocampal pyramidal cell fields as well as the dentate gyrus, the amygdaloid and lateral septal nuclei, and some cortical areas (de Kloet et al., 2005). Recent studies have implicated adult-born hippocampal neurons -likely located in the dentate gyrus (Pereira et al., 2007)- in pattern separation, a process by which similar experiences or events are transformed into discrete, non-overlapping representations. Glucocorticoids and stressors have been shown to suppress neurogenesis in the dentate gyrus (Mcewen, 2001). It has been proposed that impaired pattern separation underlies the overgeneralization of fear networks often seen in anxiety disorders, specifically post-traumatic stress disorder, and therefore represents an endophenotype for these disorders (Kheirbek et al., 2012a). Thus, it is likely that especially these regions are susceptible to the effects of stress and cortisol (though we found an enhancing effect of cortisol, as opposed to the discussed impairing effects).

In contrast with the process of contextualization (that arguably may be subserved by pattern separation/completion), single item encoding, and the feeling of familiarity that single items can generate, have been proposed to be subserved by the perirhinal cortex (Brown and Aggleton, 2001), a region that has typically not been implicated to be sensitive to stress and concomitant hormones. Our results showed that stress enhanced contextualization of familiarity responses, which would link effects of cortisol to an area not sensitive to it. One way to reconcile these opposing findings is to assume that the hippocampus is in fact involved in familiarity. Perhaps cortisol in the present study may have primarily affected weak memories, strengthening their hippocampal components. This would be in line with suggestions that weak memories are most likely to fit a familiarity profile (Kirwan et al., 2010; e.g.,

Squire et al., 2007), and would also explain why cortisol emerged as a significant mediator of memory contextualization only on recognition and familiarity, but not on cued retrieval and recollection (though signs of the effects pointed into the same direction). All in all, based on the present findings we cannot claim which particular brain regions may have been most pronouncedly affected by our stress manipulation. Clearly, more in-depth investigations are worthwhile and needed, and neuroimaging may be the perfect tool to do so.

Theories on the origin of anxiety disorders such as PTSD emphasize impairment in the ability to bind fearful memories with their original encoding context (Brewin et al., 2010; Ehlers and Clark, 2000; Liberzon and Sripada, 2008). If cortisol indeed aids in integrating a central memory in its original encoding context, cortisol may serve a protective role against traumatic fear memory generalization. Accordingly, studies in humans have shown that *reduced* cortisol responses after trauma were associated with increased risk of developing subsequent PTSD (Yehuda et al., 1998). Furthermore, clinical trials showed that patients who were administered cortisol following trauma were less susceptible to develop full-blown PTSD symptoms (Schelling et al., 2004). Observations like these have prompted the idea that corticoids may in fact protect against the development of PTSD. In this respect, a robust cortisol response to impending threat is a highly adaptive response.

There are some important considerations and limitations of the present study that should be mentioned. First of all, we did not find any effects of stress on overall memory performance (either neutral or negative), on cued retrieval or recognition measures, which may be counterintuitive in comparison with many other studies (Cornelisse et al., 2011b; Smeets et al., 2007). However, not many studies have assessed recognition memory for *words*, and those that have remain equivocal. One study did not find any effect on recognition (Schwabe et al., 2008a), though this may have been due to a ceiling effect of memory performance. Concerning effects of stress on recall, enhancing (Smeets et al., 2009), impairing (Elzinga et al., 2005; Zoladz et al., 2011) or no effects (Schwabe et al., 2008a; Smeets et al., 2008) were found.

A related issue is that the majority of studies investigating context effects on (recognition) memory employed associative designs where participants had to indicate whether they recognized the cue, context, or the cue-context association (Schmidt et al., 2011; Uncapher et al., 2006). Recognition judgments in the present study were required only to the central word in the word-context pair. Thus, recognition of the item's context was incidental rather than integral to the memory task. Using a similar task one study showed that intact and rearranged context conditions elicited dissociable event-related potentials (Tsvivilis et al., 2001). Future studies will have to determine to what extent these types of memory tasks relate to each other, and how

they vary in their sensitivity to stress and stress hormones. Extensive animal research points to arousal as a possible moderator of cortisol effects on memory (Roozendaal et al., 2006c), which has been replicated in humans (e.g., Smeets et al., 2009) but here we did not find any evidence for such an effect. One possible explanation may be that the indexes of arousal (e.g., HR, BP, alpha-amylase) employed here perhaps do not represent pure arousal, and an approach with a pharmacological blocker of the adrenergic receptor may prove more fruitful.

Another consideration is that in etiological models of anxiety disorders sex specific sensitivity to stressful events has been repeatedly associated with the higher prevalence of mood and anxiety disorders in women (Cahill, 2006; Kessler et al., 1993). Here women were excluded from participation because they are known to display more variable HPA-axis reactivity than men (Kajantie and Phillips, 2006), and HPA reactivity (Kirschbaum et al., 1999) and memory (Andreano et al., 2008) depend on oral contraceptive use and menstrual cycle phase. Another moderating factor on HPA-axis reactivity is smoking (e.g., Childs and Wit, 2008). We therefore ran an explorative analysis excluding participants who were regular smokers, and though not significant, it seemed that overall cortisol responses of smokers in the Stress group were lower, while baseline cortisol was higher in the Control group. Since smoking has been shown to alter cortisol levels after traumatic events (Olf et al., 2006) future studies could further explore these relationships.

Finally, the current design does not allow distinguishing between stress effects on encoding and initial consolidation. Cognitive processes underlying encoding such as working memory (Blumenfeld and Ranganath, 2006) and attention (Miller and Cohen, 2001) have typically been shown to be impaired by stress (Elzinga and Roelofs, 2005; Henckens et al., 2011; Qin et al., 2009a; Schoofs et al., 2008), and are therefore unlikely to explain the present positive relationship with cortisol and long-term memory. Since many studies have reported that cortisol administration (e.g., Buchanan and Lovallo, 2001) or social stress (e.g., Smeets et al., 2009) during consolidation can enhance memory a consolidation interpretation here is likely. Nevertheless, for future studies it would be worthwhile to complement the study design with eye-tracking (for spatial attention) or a working memory task.

In conclusion, this study shows that stress-induced cortisol levels enhance the integration of declarative memories in their original encoding context. This finding advances our insights of the mechanisms by which stress, through cortisol, affects human memory processes. On a broader level, these results suggest that stress-induced cortisol responses serve a protective function against memory generalization.

SUPPLEMENTARY MATERIAL

Table S1. Participant characteristics for the Stress and Control groups

Variable	Control (n=20)	Stress (n=20)
Time day 1	14:34(1:46)	14:53(1:44)
Time day2	13:57(1:20)	14:37(1:43)
Age (years)	23.25(4.86)	20.75(1.97)
BMI	21.57(4.44)	21.93(1.79)
STAI-T	33.55(4.90)	34(7.53)
FoNE	11.85(5.18)	12.60(7.83)
SRLE	58.96(8.92)	61.40(10.43)
PSS	17.58(8.34)	17.80(7.55)
Number cigarettes day	2.5(1.22)	1.05(0.55)
Motivation TSST/Control (VAS)	50.80(25.32)	47.85(23.82)
Motivation encoding (VAS)	62.73(25.15)	57.90(21.07)
Concentration encoding (VAS)	62.09(27.16)	62.35(26.76)

Data are presented as mean (SD). Time of day (time of encoding at day 1 and time of retrieval at day 2) is written in hours:minutes from midnight. BMI, body mass index; STAI-T, Trait version of the Spielberger state trait anxiety inventory; FoNE, fear of negative evaluation; SRLE, survey of recent life events; PSS, perceived stress scale; VAS, visual analogue scale; Motivation TSST/Control motivation to complete the TSST and control procedure; encoding, the learning of the words; Concentration, to concentrate during learning.

Table S2. Responses to the TSST and control procedure, for the Stress and Control group, respectively.

Group	Variable	T1	T2	T3	T4	T5	T6
Control (n=20)	Cortisol (nmol/L)	11.04(5.02)	9.82(5.92)	9.57(6.14)	8.10(4.46)	7.09(3.94)	-
	sAA (nmol/L)	99.25(82.73)	82.46(59.78)	77.48(51.88)	61.56(40.02)	67.86(54.87)	-
	Systolic BP (mm/Hg)	114.90(10.62)	-	115.15(12.39)	111.95(13.30)	-	120.45(13.30)
	Diastolic BP (mm/Hg)	67.50(11.68)	-	73.05(9.02)	69.70(7.34)	-	68.60(5.70)
	HR (bpm)	58.60(8.62)	-	58.80(9.06)	59.65(8.56)	-	65.25(10.78)
	VAS ('stressed')	11.5(9.09)	12.3(11.94)	7.85(8.83)	-	-	14.15(11.33)
	STAI-S	29.10(5.68)	-	28.55(4.27)	-	-	30.50(6.23)
	PANAS positive	29.30(7.07)	-	30.80(5.60)	-	-	30.75(6.74)
	PANAS negative	13.60(5.17)	-	11.40(1.79)	-	-	12.55(2.87)
	ECG (bpm)	69.36(8.96)	-	65.81(9.25)	-	-	-
RMSSD	50.80(24.27)	-	60.12(37.69)	-	-	-	
Stress (n=20)	Cortisol (nmol/L)	11.43(5.82)	10.70(5.83)	14.23(7.26)	15.81(8.74)	13.09(7.12)	-
	sAA (nmol/L)	61.23(38.93)	86.23(58.51)	76.92(54.71)	66.29(48.88)	53.59(37.80)	-
	Systolic BP (mm/Hg)	121.25(10.69)	-	136.45(15.53)	125.25(12.54)	-	126.40(14.07)
	Diastolic BP (mm/Hg)	72(8.45)	-	81.05(11.70)	74.75(10.09)	-	73.10(8.73)
	HR (bpm)	59.85(8.80)	-	63.95(12.14)	62.35(10.11)	-	67.90(11.48)
	VAS ('stressed')	17.79(13.85)	48.45(19.53)	31.30(21.99)	-	-	13.15(15.60)
	STAI-S	29.45(6.01)	-	37.09(7.07)	-	-	32.4(7.26)
	PANAS (pos)	28.65(6.03)	-	28.56(5.15)	-	-	27.00(5.68)
	PANAS (neg)	12.95(4.26)	-	17.70(4.53)	-	-	12.45(2.84)
	ECG (bpm)	72.45(17.68)	-	68.41(16.23)	-	-	-
RMSSD	53.97(31.77)	-	62.29(41.04)	-	-	-	

Data are presented as mean (SD). sAA, salivary alpha-amylase; BP, blood pressure, HR, heart rate; VAS, visual analogue scale; STAI-S, state version of the Spielberger state trait anxiety inventory; PANAS, positive affect and negative affect schedule; ECG, electrocardiogram; RMSSD, root mean successive differences. T1-T6 indicate time points throughout the experiment (see Figure 2). T6 took place on the second day of testing.