Research Article

Stress enhances reconsolidation of declarative memory

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Abstract

Retrieval of negative emotional memories is often accompanied by the experience of stress. Upon retrieval, a memory trace can temporarily return into a labile state, where it is vulnerable to change. An unresolved question is whether post-retrieval stress may affect the strength of declarative memory in humans by modulating the reconsolidation process. Here, we tested in two experiments whether post-reactivation stress may affect the strength of declarative memory in humans. In both experiments, participants were instructed to learn neutral, positive and negative words. Approximately 24 h later, participants received a reminder of the word list followed by exposure to the social evaluative cold pressor task (reactivation/stress group, n_{exp1} = 20; n_{exp2} = 18) or control task (reactivation/no-stress group, n_{exp1} = 23; n_{exp2} = 18). An additional control group was solely exposed to the stress task, without memory reactivation (no-reactivation/stress group, n_{exp1} = 23; n_{exp2} = 21). The next day, memory performance was tested using a free recall and a recognition task. In the first experiment we showed that participants in the reactivation/stress group recalled more words than participants in the reactivation/no-stress and no-reactivation/stress group, irrespective of valence of the word stimuli. Furthermore, participants in the reactivation/stress group made more false recognition errors. In the second experiment we replicated our observations on the free recall task for a new set of word stimuli, but we did not find any differences in false recognition. The current findings indicate that post-reactivation stress can improve declarative memory performance by modulating the process of reconsolidation. This finding contributes to our understanding why some memories are more persistent than others.

Key words: Reconsolidation, declarative memory, stress, SECPT

Introduction

The malleable nature of human memory is crucial for adequate adaptation to an ever-changing environment. Given that a stimulus or context may not predict danger or reward forever, it is essential that our memories remain open to modification. One process that provides an opportunity for such modification is memory reconsolidation. Upon retrieval, a memory trace may return into a labile, protein-synthesis dependent state where it is susceptible to modifications (Nader, 2003). Mere retrieval is however not sufficient to induce memory reconsolidation (Pedreira et al., 2004; Forcato et al., 2009; Lee, 2009; Sevenster et al., 2013). The experience of a prediction error – i.e., the mismatch between the actual and expected experience based on prior learning – appeared to be a prerequisite to destabilize the previously formed memory trace (Pedreira et al., 2004; Sevenster et al., 2013). This destabilization enables the memory trace to be updated either by simply changing the strength of the original memory trace (e.g., Nader et al., 2000; Frenkel et al., 2005; Kindt et al., 2009; Soeter and Kindt, 2010, 2011, 2012) or by integrating new information into the memory trace (e.g., Forcato et al., 2007; Hupbach et al., 2007).

Memory reconsolidation is typically demonstrated through the amnestic effects of pharmacological agents administered after memory reactivation that target protein synthesis directly (e.g., Nader et al., 2000) or indirectly by targeting the release of neurotransmitters (e.g., norepinephrine)(e.g., Dębiec and LeDoux, 2004; Kindt et al., 2009). Those pharmacological studies have added greatly to our knowledge on the neurobiological mechanisms of memory reconsolidation. However, they do not provide us with information on whether and how daily life experiences can change the content and/or strength of previously formed memories. One potential candidate for such a naturalistic experience that may affect memory reconsolidation is stress exposure. Indeed, a real-life stressor (i.e., water deprivation) following memory reactivation enhanced contextual memory in the crab *chasmagnathus*, indicating that a naturalistic event may strengthen memory reconsolidation (e.g., Frenkel et al., 2005). Likewise, in humans it has been demonstrated that a stressful event can enhance reconsolidation of declarative memory (Coccoz et

al., 2011; Coccoz et al., 2013; but see, Schwabe and Wolf, 2010). Confrontation with a stressful experience activates the autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis, which eventually leads to the release of catecholamines ((nor)adrenaline) and glucocorticoids. The hippocampus is critically involved in declarative memory processes and is highly sensitive to neuromodulators **triggered during the stress response** (Eichenbaum, 2004; Joëls and Baram, 2009). Thus, the finding that stress exposure affects memory reconsolidation may be explained by the effect of stress hormones on the neurocircuitry of reconsolidation

Previous studies on the enhancing effect of stress exposure during the reconsolidation-window have focused on declarative memory for neutral information, whereas research on learning and memory (*consolidation*) demonstrate that stress exposure and stress hormones typically affect memory performance for emotional stimuli (e.g., Cahill et al., 2003; McGaugh, 2004). The sensitivity of emotional memory to stress effects can be explained by the observed interaction between emotion-induced arousal elicited by the emotional stimuli and the enhanced levels of stress hormones (Roozendaal et al., 2009). Whether stress also differentially affects *reconsolidation* of emotional and neutral memories is yet unknown. A previous study in humans suggests that post-reactivation stress may specifically enhance memory of emotional information (Marin et al., 2010). However, these results could not be ascribed to enhanced reconsolidation given that post-reactivation stress exposure improved recall performance at an immediate retention test, whereas the required protein synthesis for reconsolidation takes at least several hours (Walker et al., 2003; Duvarci and Nader, 2004). More insight in the interaction between post-retrieval stress exposure and memory performance may advance our understanding of why emotional memories are so persistent. Indeed, retrieval of traumatic memories is often accompanied by feelings of distress. This post-reactivation stress may strengthen the process of memory reconsolidation thereby facilitating the persistence of those memories.

Here, we tested in two experiments the effects of post-reactivation stress exposure on reconsolidation of emotional and neutral memories. Participants learned a list of neutral, positive and negative words. The next day, they received a reminder of the word list and were subsequently exposed to a stress task (i.e., social-evaluative cold pressor test, SECPT) (reactivation/stress group) or a control task (reactivation/no-stress group). To control for non-specific stress effects, an additional control group was solely exposed to the stress task on day 2, without memory reactivation (no-reactivation/stress group). On day 3, memory performance was assessed by means of a free recall task and a recognition task. Based on previous research of Coccoz et al. (2011), we expected that post-reactivation stress would improve memory performance in the reactivation/stress group compared to both control groups (reactivation/no-stress group and no-reactivation/stress group). Moreover, we expected that the enhancing effects of post-reactivation stress would be more pronounced for the emotional words (Marin et al., 2010).

Experiment 1

Methods

Participants

Seventy-three healthy participants (32 men and 41 women), ranging in age between 18 and 29 years, participated in study I. Self-reported medical and psychiatric problems or the use of medication known to influence the HPA-axis (except for oral contraceptives; n=35) served as exclusion criteria. An additional exclusion criterion was a score above 18 on the Beck Depression Inventory (BDI)(Beck et al., 1996). Participants received either course credits or a small amount of money for their participation. The study was approved by the ethical committee of the University of Amsterdam and informed consent was obtained from all participants.

Design and general procedure

Fifty participants were randomly assigned to the reactivation/stress (n=25) or reactivation/no-stress (n=25) group. An additional group of participants (n=23) were non-randomly assigned to a no-reactivation/stress group. Participants were individually tested on three consecutive days. To reduce the impact of diurnal variation in cortisol level, all testing sessions took place between 12 pm and 7 pm.

Day 1. The first session took place in a laboratory setting in a closed cubicle (4.9 x 8.2 ft) containing a computer screen. Participants were informed about the nature and general procedure of the experiment. Participants were told that they were participating in a larger project consisting of several unrelated tasks (i.e., word task, cold pressor challenge and questionnaires) divided over consecutive days to minimalize the possibility that performance on the different tasks would interfere with each other. This cover-story was used to ensure that participants would not study the words outside the experimental context. Thereafter, participants signed the informed consent. Eligibility of the participant was screened using a self-report questionnaire. To assess participants' working memory capacity, the digit span subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981) was administered. Next, participants filled out the Anxiety Sensitivity Index (ASI) (Peterson and Reiss, 1992) and BDI to ensure that groups did not differ in processing of the emotional stimuli. To assess the current mood state of the participants, the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) was administered. Thereafter, participants performed the declarative memory task (see below).

Day 2. To allow for controlled saliva collection, participants were asked to refrain from caffeine, alcohol and excessive exercise from twelve hours before the start of the experiment, and to refrain from food, drinks (except for water), chewing gum, cigarettes and teeth brushing two hours prior to testing. Participants in the reactivation groups (stress/no-stress) were tested in the same experimental setting as day 1 (same cubicle and same experimenter). After a 10-min resting period, participants provided a first saliva sample and filled out the PANAS; baseline measurements of blood pressure (BP) and heart rate (HR) were assessed. Next, participants received the reactivation trial

and immediately afterwards the experimenter entered the cubicle and participants were subjected to the social evaluative cold pressor test (SECPT) or control task. To assess to what extent participants experienced the SECPT as stressful, painful and unpleasant, a likert scale from 0 ('not all all') to 9 ('extremely') was taken. BP and HR were measured at five time points: before, during, directly after, 1 min and 20 min after the SECPT or control task. Saliva samples were taken before memory reactivation, directly after and 20 min after SECPT or the control task.

Participants in the no-reactivation/stress group were tested in a different context, i.e., an office setting at a different location and with a different experimenter. This change in spatial context was incorporated to prevent the original learning context from reactivating the declarative memory trace (Hupbach et al., 2008). Apart from the reactivation trial, participants underwent the same procedure as both reactivation groups.

Day 3. The third session took place at the same location and with the same experimenter as on day 1. Participants filled out the PANAS and completed the final memory test consisting of a free recall task followed by a recognition task. At the end of the experiment, participants were asked about their expectations and motivation during the task and were debriefed about the stress procedure and the surprise memory test.

Declarative memory task

Encoding. On the first day of the experiment, participants performed the declarative memory task (adapted from Smeets et al., 2006). To create emotional and neutral memories, participants were shown 20 neutral, 20 positive and 20 negative words, intermixed. The words were presented visually and aurally. Each word was shown for 2 sec on the computer screen followed by a 2 sec inter-trial interval. Participants were instructed to carefully listen to and read the words presented to them. Subsequently, participants were confronted with a free recall task, in which they were asked to retrieve as many words they could remember within 4 min. After a 1 min resting period, participants were exposed to the words for a second time and were explicitly instructed to learn the words. Next,

the free recall task was administered. Word order was random for the first presentation and remained the same at the second presentation to stimulate the creation of a memory trace of the word list. The words were chosen from a validated dataset (Hermans and De Houwer, 1994). Based on the data of Hermans and De Houwer (1994), the word categories differed in terms of valence, but not in terms of familiarity or word length (see supplementary results).

Memory reactivation. On day 2, participants in the reactivation groups were instructed to recall the words that they learned on the first day and to perform a free recall task within 4 min. The instructions for the free recall task were similar to the instructions that they received the previous day. However, as soon as participants started to type in their response, the free recall task ended abruptly. Thus, participants were not allowed to type in the words they remembered. This procedure was used to induce a prediction error (Pedreira et al., 2004; Forcato et al., 2007).

Memory test. Memory performance was assessed with a free recall task and a recognition task. The free recall task was similar to the task on day 1. Participants were instructed to recall the words learned on the first day within a 4-min interval. The recognition task contained the 60 words of day 1 randomly intermixed with 60 new words (Hermans and De Houwer, 1994) that were not studied before. Participants were asked to indicate whether they recognized the words as 'old' or 'new' and to give a confidence rating for their answer on a scale from 1 ("not at all certain") to 9 ("certain"). The task was self-paced and reaction times were recorded.

Stress manipulation

Psychosocial stress was induced with the SECPT (Schwabe et al., 2008). Participants were asked to immerse their hand to the wrist into ice water (0-4 °C) as long as possible, but with a maximum of 3 min. Given that this procedure can be very uncomfortable, participants were informed that they could remove their hand at any time if the procedure became unbearable. Participants that kept their hand for 3 min were instructed at that point to remove their hand. On average, participants immersed their hand for 2 min and 44 sec in cold water (range: 38 sec – 3 min). In addition to the

regular CPT, a social-evaluative element is incorporated in the SECPT. During hand immersion, participants were videotaped and monitored by the (female) experimenter. Abundant evidence indicates that activity of the HPA-axis is associated with uncontrollability and social evaluative threat of the stress task (for a meta-analysis, see Dickerson and Kemeny, 2004). Accordingly, the SECPT was shown to be more effective in eliciting an increase in cortisol level and autonomic activity than the regular CPT (Schwabe et al., 2008). Two participants experienced adverse effects of the SECPT: one participant passed out and one participant felt dizzy during the SECPT. The control task consisted of hand immersion in warm water (35-39 °C) without being monitored or videotaped by the experimenter.

Saliva sampling and cortisol analysis

Saliva samples were obtained using cotton salivette collection devices (Sarstedt, Nümbrecht, Germany). The saliva samples were stored at -30 °C until biochemical analysis performed by the Technische Universität Dresden. Free cortisol concentrations were measured using a commercially available chemiluminescence immune assay (CLIA) with high sensitivity of 0.16 ng/ml (IBL, Hamburg, Germany). Cortisol levels were determined to test whether the stress manipulation succeeded. Therefore, we only analyzed the samples taken at baseline and at 20 min after SECPT (i.e., peak level).

Data analysis

Sample characteristics were analyzed using one-way analyses of variance (ANOVAs) with Group (reactivation/stress, reactivation/no-stress and no-reactivation/stress) as between-subject factor. To assess the effects of the stress manipulation, mixed ANOVAs were conducted with Time as within-subject factor and Group as between-subject factor. For BP, HR and the negative affect scale of the PANAS, three time points were entered in the analyses (before, during/directly after, and 20 min after the SECPT or control task). For the analyses of the cortisol levels, two time points were used

(baseline and peak level (i.e., 20 min after SECPT or control task). To increase normality of the distributions, PANAS ratings were log-tranformed and cortisol levels were square-root transformed. The subjective level of stress was analyzed by means of one-way ANOVAs.

Recall performance was defined as percentage correct recall on day 3 relative to recall performance on day 1 (second free recall task). Percentage correct recall was calculated for each valence category separately (positive, negative and neutral words). For recognition memory we used Hit rate ('old' words that were correctly recognized as old) and false alarm (FA) rate ('new' words that were erroneously recognized as old). Memory performance (i.e., mean recall performance, FA rate and Hit rate) was screened for possible outliers (-2<Z>2). Outliers were only discarded for analyses of the specific task (recall performance [n=3], Hit rate [n=1] and FA rate [n=2]). Encoding on day 1, memory recall and recognition on day 3 were analyzed with mixed ANOVA with Valence (positive, negative and neutral) as within-subject factor and Group as between-subject factor.
For the one-way ANOVAs, the Welch statistics are reported when the assumption of homogeneity of variance was violated. For the mixed ANOVAs, Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. In case of significant results, partial eta squared (η_p^2) is reported as a measure of effect size. Follow-up analyses were conducted with ANOVAs. An alpha-level of .05 was used for all statistical tests.

Results

Participant characteristics

Seven participants (five from the reactivation stress group and two from the reactivation/no-stress group) were excluded prior to analysis; four participants did not adhere to instructions, one participant passed out during the stress task, at which occasion the experiment was stopped, one participant did not sleep before the memory reactivation session (<1h) and one participant indicated disproportional alcohol use before the test session. The final sample consisted of 66 participants with a mean age of 21.30 years (SD=2.57), 20 in the reactivation/stress group (11 male), 23 in the

reactivation/no-stress group (10 male) and 23 in the no-reactivation/stress group (10 male). The groups did not differ on age, digit span performance, ASI or BDI scores (all *Fs*<2.46, *ps*>.1). In the exit interview, participants indicated to be motivated to complete the experimental tasks (*Fs*<2.26, *ps*>.1).

Stress responses to the SECPT

Blood Pressure. Table 1 displays the stress responses to the SECPT and control task. As expected, the analyses for systolic and diastolic BP revealed significant interactions between Time and Group ($F(3.31,102.73)=19.33$, $p<.001$, $\eta_p^2=.38$ and $F(3.38, 104.72)=16.89$, $p<.001$, $\eta_p^2=.35$, respectively). In addition, significant main effects for Time and Group emerged (*Fs*>6.45, *ps*<.003, $\eta s_p^2>.17$). Follow-up analyses showed that groups differed on systolic and diastolic BP during the SECPT (*Fs*>19.20, *ps*<.001), but not at baseline or 20 min after the SECPT (*Fs*<1.20). Planned contrasts confirmed that both stress groups (reactivation/no-reactivation) showed significantly higher systolic and diastolic BP than the reactivation/no-stress group during the SECPT (*ps*<.001). The two stress groups did not differ from each other in BP (*ps*>0.1). Furthermore, pair-wise comparisons confirmed an increase in systolic and diastolic BP from pre-SECPT to SECPT assessment in both stress groups (*ts*>4.43, *ps*<.001). The reactivation/no-stress group showed a decrease in systolic BP ($t(21)=3.62$, $p=.002$), but no significant change in diastolic BP (*t*<1.31).

Heart Rate. The HR analysis yielded a significant interaction between Time and Group ($F(3.63,112.38)=4.04$, $p=.006$, $\eta_p^2=.12$) and a significant main effect of Time ($F(1.81,112.38)=28.61$, $p<.001$, $\eta_p^2=.32$), in the absence of a main effect of Group (*F*<1.0). Follow-up analyses did not reveal a difference in HR between the reactivation/no-stress group and both stress groups (reactivation/no-reactivation) during the SECPT or at pre or post assessment (*Fs*<1.60, *ps*>.10). Yet, pair-wise comparisons revealed that both stress groups showed the expected increase in HR from baseline (*ts*>3.11, *ps*<.006), which was absent in the reactivation/no-stress group ($t(21)=1.79$, $p=.09$).

Salivary Cortisol. The analysis showed that the groups differed in their salivary cortisol response to the manipulation task (Time x Group: $F(2,63)=11.33$, $p<.001$, $\eta_p^2=.27$; Time: $F(1,63)=14.98$, $p<.001$, $\eta_p^2=.19$; Group: $F(2,63)=3.42$, $p=.039$, $\eta_p^2=.10$). Follow-up analyses showed that groups differed at peak level ($F(2,63)=7.45$, $p=.001$, $\eta_p^2=.19$), but not at baseline ($F<1.13$). Planned contrasts revealed that only the reactivation/stress group showed a stronger cortisol responses at peak level compared to the reactivation/no-stress group ($p<.001$) and also compared to the no-reactivation/stress group ($p=.012$). Importantly, both stress groups showed a significant increase in cortisol response from baseline to peak level ($ts>3.11$, $ps<.006$), whereas the reactivation/no-stress group showed a decrease in cortisol response ($t(22)=2.13$, $p=.045$).

It is suggested that an increase in cortisol response equal to or larger than 2.5 nmol/L reflects a cortisol secretory episode (Van Cauter and Refetoff, 1985). In the current study, 51% of the participants in the stress groups (N=22/43) showed this increase in cortisol level in response to the SECPT.

Subjective Ratings. There was a significant difference between groups on the subjective ratings of pleasantness, painfulness and stressfulness of the task ($Fs>18.36$, $ps<.001$, $\eta s_p^2>.36$). As expected, participants in the reactivation/stress and no-reactivation/stress group rated the SECPT as more unpleasant, painful and stressful than participants rated the control task in the reactivation/no-stress group ($ps<.001$). The reactivation/stress group experienced the task also as more unpleasant than the no-reactivation/stress group ($p=.009$), but not as more stressful or painful ($ps>.1$).

Negative affect. The analysis for the log-transformed PANAS ratings showed a significant main effect of Time ($F(1.76,109.32)=12.44$, $p<.001$, $\eta_p^2=.17$) and Group ($F(2,62)=3.84$, $p=.027$, $\eta_p^2=.11$), as well as an interaction between Time and Group ($F(3.53,109.32)=4.06$, $p=.006$, $\eta_p^2=.12$). Follow-up analyses showed no differences between groups at baseline ($F<1.0$) or at post-assessment ($F(2,62)=2.71$, $p=.08$). As expected, groups differed directly after the SECPT in negative affect ($F(2,62)=6.68$, $p=.002$,

η_p^2=.18). Planned contrasts indicated that only the reactivation/stress group showed higher negative affect ratings than the reactivation/no-stress group (*p*=.001). Furthermore, the reactivation/stress group showed also somewhat higher negative affect ratings than the no-reactivation/stress group (*p*=.051). Furthermore, as can be seen in Table 1 only the reactivation/stress group showed the expected increase in negative affect from pre to stress measurement (Time: *t*(19)=2.54, *p*=.02).

Taken together, the stress manipulation was generally successful. The SECPT caused an increase in cortisol response and autonomic activity. On a subjective level, the SECPT resulted in stronger negative affect ratings and was experienced as stressful, painful and unpleasant. Note however, that the stress groups differed in their subjective stress response and cortisol peak level.

Memory performance

Encoding (Day 1). On day 1, recall performance increased significantly from the first to the second retention test (Time: *F*(1,60)=563.09, *p*<.001, η_p^2=.90). On average, participants recalled 27 words out of 60 (range: 15-41) on the second retention test. In line with previous studies, participants recalled more emotional than neutral words (Valence: *F*(2,120)=44.41, *p*<.001, η_p^2=.43) (Kensinger and Corkin, 2003) (see supplementary Table 1). There were no differences between groups on recall performance on day 1 (*Fs*<1.0), suggesting that the groups did not differ in encoding of the words.

Recall Performance (Day 3). Percentage recall performance differed between groups (Group: *F*(2,60)=3.78, *p*=.029, η_p^2=.11, see Figure 1a). There was neither a main effect of Valence nor an interaction between Valence and Group (*Fs*<1.34; see Figure 1b). Planned contrasts demonstrated that participants in the reactivation/stress group showed better recall performance than participants in either the no-reactivation/stress group (*p*=.025) or the reactivation/no-stress group (*p*=.015). Moreover, re-analyzing the latter planned contrast between the reactivation/stress group and the reactivation/no-stress group with cortisol peak level, unpleasantness and negative affect ratings as

covariates did not alter the results ($F(1,34)=3.96$, $p=.055$, $\eta_p^2=.10$). Thus, stress exposure after memory retrieval (i.e., during the reconsolidation window) resulted in improved memory recall without a specific effect of valence.

Recognition Performance (Day 3). Table 3 shows the mean Hit rate and FA rate for the recognition task for the three groups (day 3). There was no difference between groups on Hit rate, as revealed by the mixed ANOVA (Group: $F<1$; Valence x Group: $F<1$). There was also no general effect of Valence ($F(1.74,108.03)=1.24$, $p=.29$). Analysis on the FA rates showed a main effect of Valence ($F(2,122)=33.43$, $p<.001$, $\eta_p^2=.35$) and a marginally significant main effect of Group ($F(2,61)=2.88$, $p=.064$, $\eta_p^2=0.09$). Overall, participants made more errors for positive and negative words than for neutral words ($ts>6.23$, $ps<.001$). Interestingly, planned comparisons showed that participants in the reactivation/stress group recognized more 'new' words as 'old' than participants in the reactivation/no-stress group ($p=.023$) irrespective of valence, but not compared to the no-reactivation/stress group ($p=.096$). Re-analyzing FA rates between the reactivation/stress group and the no-reactivation/stress group with cortisol peak level, unpleasantness and negative affect ratings as covariates did not change this result ($F(1,37)=2.88$, $p=.098$). Note that the differences in FA rates were not related to the participants' confidence of memory performance (see supplementary results).

To summarize, the current results demonstrate that post-reactivation stress can enhance the reconsolidation of declarative memory resulting in improved recall performance. Stress exposure after memory reactivation may additionally enhance false recognition. A replication experiment was conducted to further test the hypothesis that stress during the reconsolidation window would enhance false recognition. To optimize the possibility that stress could affect recognition memory, we doubled the number of study items.

Experiment 2

Method

The materials and procedure of experiment 2 were identical to experiment 1, with the exception that participants now learned 120 words (40 neutral, 40 positive and 40 negative) instead of 60 words. For a full description of the methods, see supplementary material.

Results

Participant characteristics

Three participants (one from the reactivation/stress group and two from the reactivation/no-stress group) were excluded prior to our analyses: 1 participant indicated disproportional alcohol use and not a proper night of sleep before the test session (<5 h) and 2 participants did not adhere to the instructions. The final sample comprised 57 participants with a mean age of 21.51 years (SD=2.75), with 18 participants in the reactivation/stress group (10 male), 18 participants in the reactivation/no-stress group (8 male) and 21 participants in the no-reactivation/stress group (11 male). Groups did not differ in terms of age, ASI, BDI, PANAS or DUDIT score (all *Fs*<1.60). There was a marginal difference between groups on the digit span task ($F(2,53)=2.87$, $p=.066$, $\eta_p^2=.10$). Groups were equally motivated to complete the experimental tasks (*Fs*<1.0). Importantly, none of the participants indicated having studied the words outside the experimental context. Participants immersed their hands on average for 2 min and 53 sec in cold water (range: 40 sec to 3 min).

Stress responses to the SECPT

Blood Pressure. Table 2 displays the stress responses to the manipulation (i.e., SECPT and control task) for the three groups. As expected, there was a significant difference between groups on systolic and diastolic BP (Group x Time: *Fs*>20.68, *ps*<.001, ηs_p^2>.43; Group (*Fs*>4.74, *ps*<.014, ηs_p^2>.14). Furthermore, significant main effects of Time (*Fs*>56.45, *ps*<.001, ηs_p^2>.51) occurred for

systolic and diastolic BP. Follow-up analyses showed that groups did not differ in BP at pre or post assessment (*F*<1.44), but only during the SECPT (*Fs*>16.88, *ps*<.001, ηs_p^2>.38). Planned contrasts confirmed that both stress groups showed higher systolic and diastolic BP during the stress task than participants in the reactivation/no-stress group (*ps*<.001). There were no significant differences between the stress groups (*ps*>.1). Furthermore, pair-wise comparisons confirmed an increase in BP from baseline to the SECPT in both stress groups (*ts*>5.49, *ps*<.001). For the reactivation/no-stress group, pairwise comparisons indicated a significant decrease in systolic BP from baseline to the control task (*t*(19)=4.56, *p*<.001) and no effect on diastolic BP (*t*<1.0).

Heart Rate. There were no differences between groups on HR (*Fs*<1.0).

Salivary Cortisol[1]*.* There were no significant differences between the three groups on salivary cortisol response (Fs<1.17). In contrast to experiment 1 and previous studies (e.g., Schwabe et al., 2008), the percentage of participants that could be classified as cortisol responder was rather low (26%; N=10/39).

Negative Affect. There were no differences between groups on negative affect ratings (*Fs*<1.1).

Subjective Ratings. As expected, the groups differed on the subjective ratings of the SECPT and control task (*Fs*>9.07, *ps*<.005, ηs_p^2>.17).The SECPT was evaluated as being unpleasant, painful and stressful compared to the control task (*ps*<0.05). There were no differences between the reactivation/stress group and no-reactivation/stress group (*ps*>.079).

[1] Excluding participants that showed a deviant cortisol response (i.e., increase in cortisol response in the reactivation/no-stress group and decrease in cortisol response in both stress groups; *n*=19) did not alter the results on memory performance. Therefore, the reported analyses are performed over the entire group (see supplementary results for re-analyses without this subgroup).

To summarize, the SECPT caused an increase in autonomic activity and subjective experience of stress. Note that although the stress manipulation was successful in some respect, it was clearly less successful in inducing stress than in our first experiment.

Memory Performance

Encoding (Day 1). As expected, recall performance on day 1 increased from the first to the second retention test (Time: $F(1,53)=345.26$, $p<.001$, $\eta_p^2=.87$). On the second retention test participants remembered on average 32 out of 120 words (range: 14-54). There was a significant effect of Valence ($F(2,106)=22.52$, $p<.001$, $\eta_p^2=.30$), showing that participants recalled more emotional than neutral words (see supplementary table 2). There were no differences between groups in recall performance on day 1 ($Fs<1.34$).

Recall Performance (Day 3). As can be seen in Figure 2a, there was a significant main effect of Group ($F(2,53)=4.07$, $p=.023$, $\eta_p^2=.13$). Planned contrasts demonstrated that on day 3 participants in the reactivation/stress group performed significantly better on the free recall task than participants in the no-reactivation/stress group ($p=.006$) and marginally better than the reactivation/no-stress group ($p=.068$). There was no effect of Valence (Valence: $F(2,106)<1$; Valence x Group: $F(4,106)<1$, see Figure 2b). So, the current results replicate the results of our first experiment and demonstrate that stress exposure after memory reactivation enhances memory performance irrespective of valence.

Recognition Performance (Day 3). Analyses on Hit rates revealed a main effect of Valence ($F(1.76, 93.15)=19.54$, $p<.001$, $\eta_p^2=.27$), but no differences between groups ($Fs<1.0$). In contrast to the findings of experiment 1, we also found no group differences in FA rates ($Fs<1.0$) (see Table 3). For FA rates there was also a main effect of Valence ($F(2,102)=80.34$, $p<.001$, $\eta_p^2=.61$). A possible explanation for the absence of an effect of Group may be that participants in the second experiment

lost their motivation during the recognition task, since the task contained twice as many words as in the first experiment. However, additional analyses over the first part of the task (i.e., the first 20 neutral 'old' words, 20 neutral 'new' words, etcetera) did not reveal any group differences either (*Fs* < 1.41).

Explorative Analyses

A question that arises from both experiments is whether the relation between group and recall performance could be ascribed to stress responsiveness. Given that the stress response typically consists of a physiological and psychological component, we explored whether the different components of stress *mediated* the effect on recall performance between the two reactivation groups. In order to increase statistical power, we combined the data from the reactivation groups of experiment 1 and experiment 2 (*n*=79). We performed a multiple mediation analysis with increase in cortisol (i.e., index of glucocorticoid activity), increase in heart rate (i.e., index of sympathetic arousal) and the subjective experience of stress (i.e., indicator of psychological stress) as potential mediators of the effect between the independent variable group (reactivation/stress = 1; reactivation/no-stress = -1) and overall recall performance as the dependent variable. For HR we calculated the increase in response by subtracting the pre-stress response from the response during the task. Increase in cortisol was calculated by subtracting baseline level of cortisol from cortisol peak level (square root transformed). For the subjective experience of the SECPT, we calculated a sum score of the likert-scale ratings (i.e., unpleasantness, stressfulness, painfulness). All mediators were centered around the grand mean.

Preacher and Hayes (2008) method was followed for assessing multiple mediation, with 5000 bootstrap iterations and 95% bias corrected confidence intervals, using Mplus version 6.1 (Muthén and Muthén, 2007). As shown in Figure 3, the direct effect between Group and Recall performance disappeared when cortisol, HR and subjective stress was entered as mediators (path c). The mediators fully explained the relation between Group and Recall performance (path c'). Examination

of the contribution of individual mediators showed that only subjective stress (b=5.07, 95% CI: 1.68 – 8.71) was a significant mediator[2]. Thus, an increase in subjective stress resulted in an increase in recall performance. Note that if an increase in negative affect was entered as an index of psychological stress in the mediation model, no significant mediation effect was observed.

See supplementary results for explorative analyses on gender differences.

General discussion

In two independent experiments, we demonstrated that stress exposure after memory reactivation improved recall performance 24 h later. This finding implies that mild stress can enhance reconsolidation and thus strengthen declarative memory performance. A crucial finding is that memory enhancement cannot be attributed to non-specific stress effects, given that stress exposure without memory reactivation did not alter recall performance. Remarkably, post-reactivation stress enhanced recall performance, irrespective of the valence of the word stimuli. The finding that a stressful event can enhance memory reconsolidation is consistent with the idea that reconsolidation serves an adaptive function. The possibility to boost memories by enhancing reconsolidation may serve the function to specifically maintain those memories that are relevant (Lee, 2009; Hardt et al., 2010).

The current results are in line with previous findings in humans and animals showing that stressful events can enhance memory reconsolidation (e.g., Frenkel et al., 2005; Coccoz et al., 2011; Coccoz et al., 2013). In contrast to our expectations, post-reactivation stress did not differentially affect memories of emotional versus neutral content. This result seems to be at odd with the study of Marin et al., (2010), which showed that post-reactivation stress only affected memories of

[2] Excluding participants of experiment 2 that showed a deviant cortisol response (*n*=10) altered the results of the mediation analysis to some extent. Subjective stress as well as increase in HR significantly contributed to the relation between stress exposure and memory performance (see supplementary results).

emotional content. A possible explanation for this apparent discrepancy might be the success of encoding. In contrast to Marin et al., (2010), we controlled for initial encoding effects, because we indexed recall performance by the percentage of recalled words on day 3 relative to the recalled words on day 1 for each valence category separately. By doing so, we controlled for potential differences in encoding due to the emotional content of the to be remembered material. As expected, initial learning was better for emotional words than neutral words (Kensinger and Corkin, 2003). However, post-reactivation stress did not further enlarge this difference, but affected memory performance for all words. It is suggested that the superiority of memories for emotional content is the result of the interaction between arousal – evoked by the emotional material – and stress (Kensinger and Corkin, 2004). Given that participants in the current study were only generally reminded of the study words without explicit recall, it is likely that this procedure did not elicit stimulus specific arousal, which may explain the absence of a post-reactivation stress effect on emotional content. Note that we did not incorporate a post-reactivation short-term memory test. Since reconsolidation is a time-dependent process, memory performance should not be affected at a post-reactivation short-term memory test. Hence, the current design does not allow being conclusive on whether the stress manipulation actually affected the process of reconsolidation.

The explorative multiple mediation model revealed that an increase in stress response fully explained the between-group (reactivation/stress and reactivation/no-stress group) effect on recall performance. Participants that experienced the SECPT as more stressful showed stronger improvements in recall performance. Remarkably, an increase in cortisol response did not independently contribute to the mediating effect of stress. The observed changes in cortisol levels in response to the SECPT were rather low. Another potential shortcoming of cortisol assessments is that both the baseline levels as well as the response to the SECPT are known to show large inter-individual variability (Kudielka et al., 2009). The relatively small stress response combined with the large inter-individual differences may have suppressed the (potential) role of cortisol on memory performance in the mediation model. The advantage of using the SECPT over pharmacological

manipulations is that it mimics a real-life stress experience and elicits a wide range of physiological and psychological reactivity. Mediation analysis provides the opportunity to clarify the specific contribution of each stress response system. Nevertheless, the response systems cannot be completely disentangled given that activation of those systems act in concert. Given that the cortisol response did not contribute to the mediating effect of stress, it could be that enhanced recall performance was simply due to arousal elicited by the experience of an aversive event. A previous study in humans indeed showed that post-retrieval arousal elicited by aversive pictures enhanced recall performance (Finn and Roediger, 2011). It bears mentioning that the study of Finn and Roediger (2011) was performed on one day and is therefore not informative on reconsolidation effects.

Earlier work in animals indicated that post-reactivation stress may disrupt memory reconsolidation and impair rather than enhance memory performance (Akirav and Maroun, 2012). The impairing effects of stress in animals can be induced by post-reactivation stress exposure (Maroun and Akirav, 2008; Wang et al., 2008) or infusion of corticosterone (Cai et al., 2006; Abrari et al., 2008), but also by infusion of glucocorticoid receptor (GR) antagonists (Jin et al., 2007; Tronel and Alberini, 2007; Taubenfeld et al., 2009). The paradoxical observation that post-reactivation infusion of both GR-agonists and GR-antagonists can disrupt memory reconsolidation underlines the complexity of the interaction between stress experience, stress hormones and memory processes (Akirav and Maroun, 2012). Possibly, effects of stress on memory *reconsolidation* follow an inverted U-shape curve (Marin et al., 2011), similar to the observed effects of stress on memory *consolidation* (Abercrombie et al., 2003). In addition, two human studies showed that stress exposure after memory retrieval impaired memory recall of neutral autobiographical memories in healthy participants (Schwabe and Wolf, 2010) and drug-related words in abstinent heroin addicts (Zhao et al., 2009). The opposing results may be explained by experimental differences between those studies and ours, such as stimulus material (i.e., word stimuli, heroin-related words or

autobiographical memories), differences in delay-interval between reactivation and memory test (i.e., delay interval of one day compared to one week) and the strength of the stress response.

Stress during the reconsolidation window only improved memory performance on the free recall task and not on the recognition task. Both experiments showed no differences between groups in correct recognition rates. This discrepancy may be explained by differences between recognition and recall memory. Remarkably, in the first experiment we showed that participants in the reactivation/stress group erroneously recognized more 'new' words as 'old' (i.e., FA rate) than participants in the reactivation/no-stress group, but not compared to the no-reactivation/stress group. This enhancement in false recognition in the absence of enhanced correct recognition may suggest that stress during the reconsolidation process facilitates generalization of declarative memory (Payne et al., 2002; Adolphs et al., 2005). Nevertheless, given that the difference in false recognition rate was not observed between the reactivation/stress group and no-reactivation/stress group, we cannot rule out that stress exposure enhanced false recognition in a non-specific way rather than by affecting reconsolidation. To clarify this finding, we doubled the number of study items in the second experiment to optimize the possibility that stress could affect recognition memory (Hit rate and FA rate). In the second experiment we found no effect of post-reactivation stress on false recognition. However, by doubling the amount of study words, the encoding context was also changed. An alternative way to further explore whether or not stress during the reconsolidation window can affect false recognition is to extend the time between memory reactivation and test.

To conclude, the current findings demonstrate that exposure to a stressful event after memory reactivation can enhance memory reconsolidation and strengthen declarative memories, irrespective of the emotional content of those memories. Our findings may have implications for the understanding of the persistence of declarative memories in humans. Following a traumatic event, memory retrieval is often accompanied by the experience of stress. This post-reactivation stress may amplify the traumatic memory trace by enhancing the reconsolidation process thereby contributing

to the persistence of traumatic memories. Taken together, this study emphasizes the adaptive value of memory reconsolidation by showing that stress exposure after memory retrieval can improve memory performance.

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	Reactivation/ stress group n =20	Reactivation/ no-stress group n=23	No-reactivation/ stress group n=23
Systolic BP			
Pre	115.75 ± 3.79	111.82 ± 2.84	117.70 ± 2.86
Stress	130.75 ± 4.90**	107.55 ± 2.68	137.78 ± 3.29**
Post	111.15 ± 2.98	106.45 ± 2.41	111.65 ± 2.77
Diastolic BP			
Pre	72.95 ± 2.03	71.55 ± 1.56	71.04 ± 1.61
Stress	87.35 ± 3.16**	70.09 ± 1.60	90.22 ± 2.37**
Post	69.50 ± 1.91	69.77 ± 1.91	72.87 ± 1.79
HR			
Pre	66.40 ± 2.84	65.05 ± 2.30	64.74 ± 1.80
Stress	74.05 ± 3.92	66.82 ± 2.34	71.57 ± 2.35
Post	62.85 ± 2.03	63.50 ± 2.25	66.04 ± 2.08
Cortisol			
Pre	8.67 ± 1.05	8.92 ± 0.91	6.99 ± 0.45
Post	16.77 ± 2.50**	7.53 ± 0.67	9.58 ± 0.86
Negative Affect			
Pre	1.21 ± 0.07	1.12 ± 0.03	1.20 ± 0.05
Stress	1.40 ± 0.10**	1.07 ± 0.03	1.20 ± 0.06
Post	1.16 ± 0.06	1.05 ± 0.02	1.07 ± 0.02
Subjective Ratings			
Unpleasant	7.15 ± 0.32**	1.30 ± 0.18	6.00 ± 0.37**
Painful	5.85 ± 0.49**	0.17 ± 0.10	4.96 ± 0.45**
Stressful	4.10 ± 0.57**	0.30 ± 0.16	3.43 ± 0.59**

Table 1 Experiment 1 – Systolic and diastolic blood pressure (BP; in mmHg), heart rate (HR, in beat per minute), cortisol (Nmol/l) and negative affect ratings after 10 minute resting period (pre), during (stress) and after (post) the stress manipulation for the three experimental groups. Subjective experience of stress was rated directly after the stress manipulation. Means ± S.E.M.

** $p<.01$ compared to the reactivation/no-stress group.

	Reactivation/ stress group n=18	Reactivation/ no-stress group n=18	No-reactivation/ stress group n=21
Systolic BP			
Pre	118.00 ± 3.08	113.78 ± 2.81	113.86 ± 1.71
Stress	131.28 ± 3.34**	107.39 ± 2.68	130.47 ± 3.73**
Post	112.17 ± 2.07	109.17 ± 2032	107.43 ± 2.17
Diastolic BP			
Pre	73.89 ± 1.74	70.89 ± 1.45	72.29 ± 1.53
Stress	86.17 ± 1.86**	71.22 ± 1.38	90.14 ± 2.37**
Post	72.50 ± 1.60	70.28 ± 1.07	69.19 ± 1.47
HR			
Pre	70.11 ± 2.94	66.94 ± 4.08	64.57 ± 3.19
Stress	74.94 ± 4.00	69.00 ± 3.37	71.09 ± 3.12
Post	66.39 ± 2.78	65.00 ± 2.34	63.24 ± 2.70
Cortisol			
Pre	7.96 ± 1.06	7.57 ± 1.00	8.33 ± 1.03
Post	9.38 ± 1.30	7.21 ± 0.74	9.65 ± 0.88
Negative Affect			
Pre	1.09 ± 0.05	1.23 ± 0.09	1.13 ± 0.03
Stress	1.26 ± 0.09	1.19 ± 0.09	1.15 ± 0.04
Post	1.19 ± 0.09	1.13 ± 0.07	1.10 ± 0.04
Subjective Ratings			
Unpleasant	6.56 ± 0.48**	1.83 ± 0.36	6.38 ± 0.33**
Painful	4.00 ± 0.59**	0.06 ± 0.06	5.14 ± 0.50**
Stressful	2.92 ± 0.64**	0.56 ± 0.26	2.33 ± 0.49*

Table 2 Experiment 2 - Systolic and diastolic blood pressure (BP; in mmHg), heart rate (HR, in beat per minute), cortisol (Nmol/l) and negative affect ratings after 10 minute resting period (pre), during (stress) and after (post) the stress manipulation for the three experimental groups. Subjective experience of stress was rated directly after the stress manipulation. Means ± S.E.M.

* $p<.05$, ** $p<.01$ compared to the reactivation/no-stress group.

	Experiment I			Experiment II		
	Reactivation/ stress group	Reactivation/ no-stress group	No-reactivation/ stress group	Reactivation/ stress group	Reactivation/ no-stress group	No-reactivation/ stress group
Hit Rate						
Neutral	.86 ± .02	.85 ± .03	.82 ± .03	.69 ± .04	.68 ± .04	.68 ± .04
Negative	.86 ± .02	.84 ± .03	.87 ± .02	.77 ± .02	.81 ± .03	.82 ± .02
Positive	.87 ± .02	.86 ± .02	.86 ± .02	.75 ± .03	.77 ± .03	.75 ± .03
Overall	.86 ± .01	.85 ± .02	.85 ± .02	.73 ± .03	.75 ± .03	.75 ± .02
FA Rate						
Neutral	.18 ± .03	.13 ± .02	.14 ± .02	.15 ± .02	.18 ± .03	.20 ± .02
Negative	.30 ± .04	.22 ± .03	.25 ± .03	.30 ± .03	.37 ± .05	.39 ± .04
Positive	.36 ± .04	.23 ± .03	.26 ± .03	.30 ± .03	.35 ± .05	.39 ± .03
Overall	.28 ± .03	.19 ± .02*	.22 ± .02	.25 ± .02	.30 ± .04	.33 ± .03

Table 3 Recognition performance (day 3)

Mean (S.E.M) values of Hit rate and False Alarm (FA) rate as a function of valence for the experimental groups. * $p<.05$ compared to the reactivation stress group.

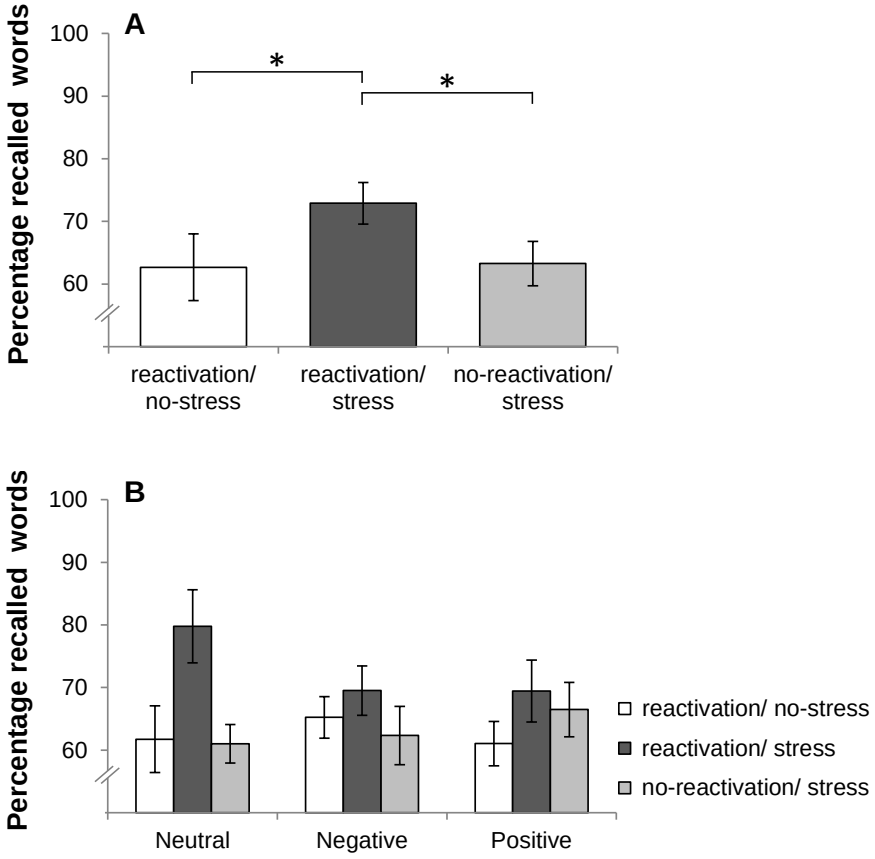

Figure 1 Experiment 1 – Percentage Recall performance (day 3)

Recall performance indexed by percentage recalled words at test (day 3) relative to performance on day 1. Panel A shows that overall recall performance was better in the reactivation/stress group compared to the reactivation/no-stress group and no-reactivation/stress group. Panel B depicts recall performance as a function of Valence and Group.

* p<.05; Error bars represent S.E.M.

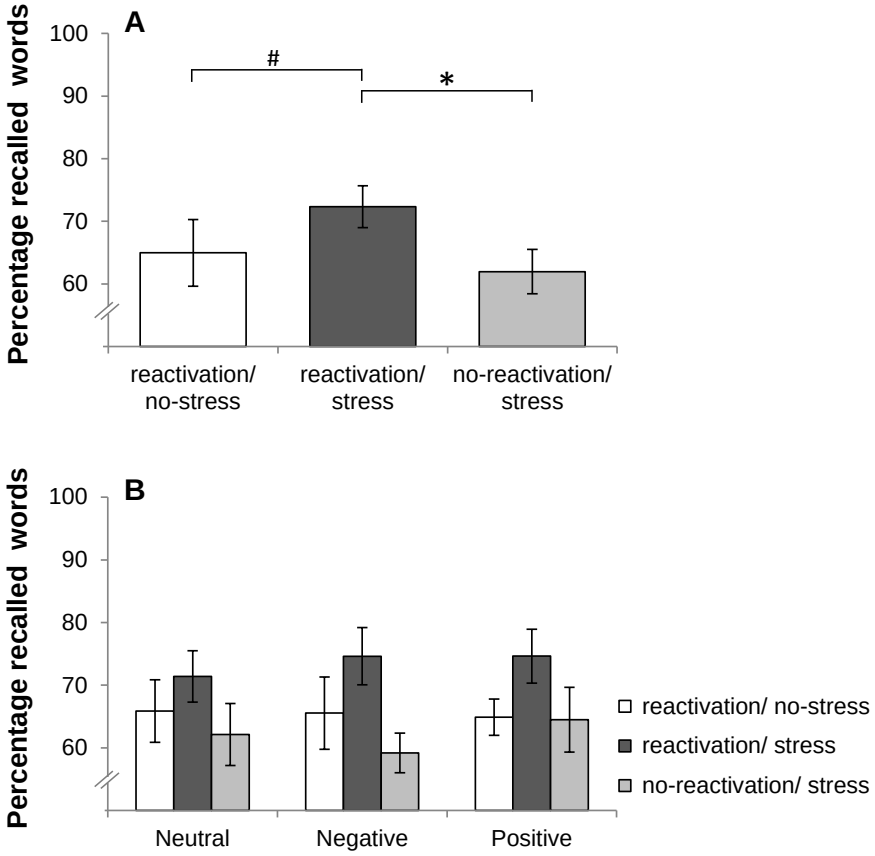

Figure 2 Experiment 2 – Percentage Recall performance (day 3)

Recall performance indexed by percentage recalled words at test (day 3) relative to performance on day 1. Panel A shows that overall recall performance was better in the reactivation/stress group compared to the reactivation/no-stress group and no-reactivation/stress group. Panel B depicts recall performance as a function of Valence and Group.

[#] $p < .07$; * $p<.05$; Error bars represent S.E.M.

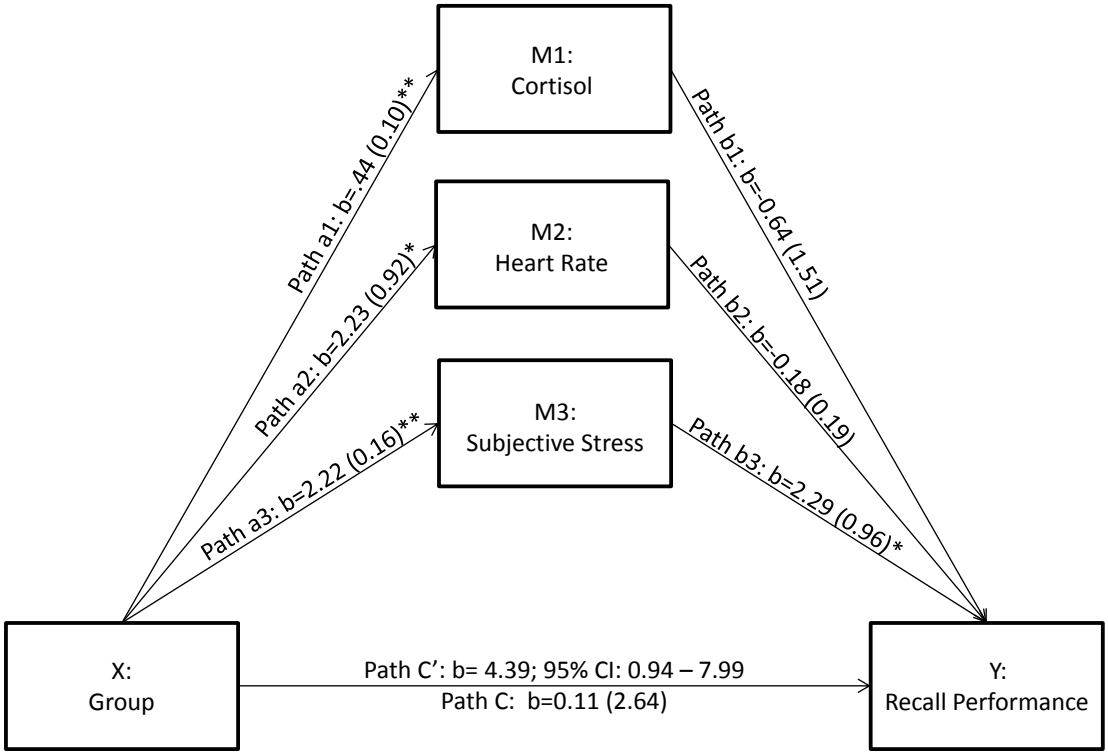

Figure 3 Multiple mediation model of the relation between group (reactivation/stress and reactivation/no-stress) and recall performance with cortisol response, heart rate and subjective stress as mediators. The point estimates (S.E.M) are presented in the figure.

* p<.05; ** p<.01

Supplementary Material for

Stress enhances reconsolidation of declarative memory

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Supplementary Results – Experiment 1

Declarative memory task

The words for the declarative memory task were selected from a validated dataset of 740 Dutch words (Hermans and de Houwer, 1994), which were rated by 352 first year students on subjective familiarity and valence. Based on these data, the three word categories (i.e., neutral, negative and positive) differed significantly in terms of Valence ($F(2,32.52)=1438.10$, $p<.001$, $\eta_p^2=.99$), but not on Familiarity or Word length ($Fs<1.36$, $ps>0.1$). Planned comparisons confirmed that all word categories differed from each other ($ps<.001$).

Recognition performance

Confidence Ratings. To assess whether the differences in FA rates were related to a subjective sense of memory performance, we divided the FA rates into three categories of memory confidence based on the self-report ratings from *certain*, *slightly certain* to *uncertain* (Qin et al., 2011). Mixed analysis of variance (ANOVA) showed that the experimental groups did not differ in their confidence ratings of the falsely recognized words (Group x Confidence Rating: $F<1.30$; Group x Valence x Confidence Rating: $F<1$).

Supplementary Method – Experiment 2

Participants

Sixty healthy participants (31 men and 29 women) between 18 and 31 years participated in experiment 2. Self-reported medical and psychiatric problems and use of medication know to influence the HPA-axis served as exclusion criteria. Additional exclusion criteria were a BDI score above 18 and use of psychoactive drugs on a regular basis, screened with the drug use disorder identification test (Berman et al., 2004). All female participants used oral contraceptives. Participants received either course credits or a small amount of money for their participation. The study was approved by the ethical committee of the University of Amsterdam and informed consent was obtained from all participants.

Design and general procedure

The general procedure of the experiment was identical to experiment 1, with the exception that participants now learned 120 words (40 neutral, 40 positive and 40 negative) instead of 60 words. The words were selected from a recently validated dataset (Moors et al., 2012). From the 120 words used in experiment 1 (i.e., free recall and recognition task), 113 words were used again in experiment 2. Seven words were replaced by new words because the words were not included in the recently validated data set of Moors et al., 2012 or for matching purposes. Based on the data of Moors et al., 2012, the three word categories differed in terms of valence, but not in terms of familiarity or word length. Moreover, emotional words differed on arousal ratings from the neutral words (see supplemental results). Participants were randomly assigned to the reactivation/stress group, reactivation/no-stress group and no-reactivation/stress group.

Data analysis

The procedure of data analysis was similar to experiment 1. Memory performance was screened on outliers and outliers were subsequently discarded (recall performance [n=1], Hit rate [n=1]; FA rate [n=1]).

Supplementary Results - Experiment 2

Declarative memory task

The words of the declarative memory task were selected from a recently validated data set of 4300 Dutch words (Moors et al., 2012), which were rated by 224 students on several dimensions, including valence and arousal. Based on these data, the three word categories (i.e., neutral, negative and positive) differed significantly in terms of Valence ($F(2,119)=1751.16$, $p<.001$, $\eta_p^2=.99$) and Arousal ($F(2,119)=29.90$, $p<.001$, $\eta_p^2=.34$). Planned contrasts showed that all categories differed in Valence ratings (*ps*<.001). On arousal ratings, there was only a significant difference between the emotional and the neutral category (*ps*<.001), but not between the negative and positive category ($p=.093$). Furthermore, there were no differences in Familiarity and Word length (*Fs*<2.24, *ps*>0.1).

Salivary Cortisol. Given that there were no significant differences between the three groups on salivary cortisol response, we re-analyzed the data excluding participants that showed a deviant cortisol response; cortisol increase in the reactivation/no-stress group (i.e., cortisol response > 2.5 nmol/L; n=3) and a cortisol decrease in both stress groups (reactivation/stress group: n = 7; no-reactivation/ stress group: n = 9). In the supplementary results below, we will present the results with these participants excluded from the analyses, leaving 15 participants in the reactivation/no-stress group, 11 participants in the reactivation/stress group and 11 participants in the no-reactivation/stress group.

Re-analysis of the cortisol data revealed a significant main effect of Time ($F(1,34)=14.11$, $p=.001$, $\eta_p^2=.29$) and a Time x Group interaction ($F(2,34)=11.82$, $p<.001$, $\eta_p^2=.41$). Follow-up analysis

showed that groups did not differ in cortisol level at baseline (F<1.40) and only trended towards a significance at peak level (F(2,34)=2.67, p=.084). Nevertheless, pair-wise comparisons revealed that both stress groups showed the expected increase in cortisol level from baseline (ts>3.06, ps<.012), whereas the reactivation/no-stress group showed a decrease in cortisol level (t(14)=2.56, p=.023). In addition, 45 percent of the participants in the stress group (n=10/22) could be classified as cortisol-responders.

Recall Performance (Day 3). The results of the subgroup are in line with the results presented in the article. A mixed ANOVA showed a main effect of Group (F(2,33)=3.34, p=.048, η_p^2=.17), in the absence of an effect of Valence (F<1.0) or an interaction between Valence and Group (F<1.0). Planned contrasts demonstrated that participants in the reactivation/stress group performed significantly better on the free recall task than participants in the no-reactivation/stress group (p=.02) and marginally better than the reactivation/no-stress group (p=.054).

Recognition Performance (Day 3). Analyses on Hit rates and FA rates only revealed a main effect of Valence (Fs>12.97, ps<.001, ηs_p^2>.28), but no effect of Group (Fs<1.0) nor an interaction effect between Group and Valence (Fs<1.0).

Supplementary explorative analyses – mediation analyses

We re-analyzed the multiple mediation model excluding the participants that showed a deviant cortisol response in experiment 2 (n=10; seven participants of the reactivation/stress group and three participants of the reactivation/no-stress group). The mediators (subjective stress, increase in HR and cortisol) fully explained the relation between Group and Recall performance (path c': b=5.02, 95% CI: 0.31 – 8.97). Examination of the unique contribution of each mediator showed that subjective stress (b=6.38, 95% CI: 2.31 – 10.68) as well as increase in HR (b=-0.95, 95% CI: -2.43 to -0.14) significantly mediated the effect of stress on recall performance. Remarkably, an increase in subjective stress resulted in an increase in recall performance, whereas an increase in HR was related to a decrease in recall performance. Thus, there is a competing mediation effect between subjective experience of stress and change in HR. It is unclear how to fathom these opposing roles of subjective and physiological stress. However, as we observed an improved recall performance in the reactivation/stress group, the subjective experience of stress seems to have more weight in affecting reconsolidation of words than the physiological stress response.

Supplementary explorative analyses - gender differences

Explorative analyses were performed to examine 1) gender differences in stress response and 2) gender differences in the effect of stress on reconsolidation of declarative memory. In experimental settings, men typically show higher cortisol responses than women (Kudielka and Kirschbaum, 2005). Stress responses in women seem to depend on their hormonal cycle; women in the luteal phase and women using oral contraceptives show diminished cortisol responses (Kirschbaum et al., 1999). For reasons of convenience, many studies test only male participants to examine the effects of stress on learning and memory thereby discarding potential gender differences in stress-related learning and memory performance. The few studies that have addressed gender differences indicate that the effects of stress and stress hormones may differentially affect memory processes for men and women (e.g., Wolf et al., 2001, Andreano and Cahill, 2006; Cornelisse et al., 2011).

By combining the data from experiment 1 and experiment 2, our sample of male and female participants is large enough to detect possible gender differences[3]. First, we examined whether men and women responded differently to the SECPT. Mixed ANOVAs with Time as within-subject factor and Gender as between-subject factor revealed that, men showed a higher systolic BP than women (main effect of Gender: $F(1,80)=38.62$, $p<0.001$, $\eta_p^2=.33$) and a lower HR ($F(1,80)=3.63$, $p=.06$, $\eta_p^2=.04$) , but no significant interactions were found between Time and Gender (BP, HR and Cortisol) ($Fs<2.01$, $ps>0.1$). Thus, on a physiological level men and women did not differ in their response to the SECPT. Furthermore, no gender differences were observed for the response on the negative affect scale of the PANAS ($F<1.0$). Interestingly, men and women differed in how they subjectively experienced the SECPT. Women rated the SECPT as being more stressful (women $M=3.88 \pm .40$, men $M=2.60 \pm .40$), painful (women $M=5.85 \pm .29$, men $M=4.21 \pm .38$) and unpleasant (women $M=7.03 \pm .23$, men $M=6.00 \pm .28$) ($Fs>5.36$, $ps<.05$, $\eta s_p^2=.06$) than men.

Second, we examined whether the observed differences in subjective stress experience resulted in gender differences in recall performance. A repeated-measure ANOVA with Valence as within-subject factor and experimental Group and Gender as between-subject factors showed no interactions between Gender and Group or Gender, Group and Valence ($Fs<1.58$, $ps>0.1$). The analysis did yield two main effects of gender. Male participants in general performed slightly worse on the free recall task ($F(1,113)=3.69$, $p=.057$, $\eta_p^2=.03$) and they erroneously recognized more 'new' words as 'old' in the recognition task ($F(1,113)=11.28$, $p<.001$, $\eta_p^2=.08$).

In sum, our explorative analyses showed no differences between men and women in cortisol response or in the effect of stress after memory reactivation on recall performance. Although, many experimental stress manipulations induce higher cortisol responses in men than in women (Kudielka and Kirschbaum, 2005), the results on the CPT are equivocal (al'Absi et al., 2002; Andreano and Cahill, 2006). Note, however, that the current analyses were explorative in nature. To examine the differences between men and women in stress responsiveness and the effect of post-reactivation

[3] Excluding participants that showed a deviant cortisol response in the second experiment did not alter the explorative analyses on gender differences.

stress on memory performance future research is needed that take hormonal parameters, like natural menstrual cycle, into account.

Supplementary tables

	Reactivation/ stress group (n=19)[a]	Reactivation/ no-stress group (n=23)	No-reactivation/ stress group (n=21)
Free Recall task 1			
Negative	5.74 ± 0.61	5.83 ± 0.38	5.71 ± 0.32
Positive	4.32 ± 0.47	4.95 ± 0.42	4.67 ± 0.35
Neutral	3.58 ± 0.51	4.00 ± 0.39	3.48 ± 0.34
Overall	13.63 ± 1.23	14.78 ± 0.92	13.86 ± 0.74
Free Recall task 2			
Negative	9.95 ± 0.64	10.87 ± 0.46	9.95 ± 0.60
Positive	8.32 ± 0.76	9.04 ± 0.43	9.10 ± 0.46
Neutral	7.53 ± 0.61	8.30 ± 0.50	7.81 ± 0.54
Overall	25.84 ± 1.70	28.22 ± 0.96	26.81 ± 1.34

Supplementary Table 1 Recall performance (day 1) – experiment 1

The table presents recall performance on the first and second free recall task for each valence category (mean ± S.E.M). The data are presented for the three experimental groups separately.

[a] Note that one participant of the reactivation/stress group and two participants of the no-reactivation stress group were identified as outliers and removed from the analyses for the free recall task.

	Reactivation/ stress group (n=18)	Reactivation/ no-stress group (n=17)[a]	No-reactivation/ stress group (n=21)
Free Recall task 1			
Negative	5.77 ± 0.41	6.76 ± 0.87	6.24 ± 0.62
Positive	5.06 ± 0.49	6.06 ± 0.64	6.24 ± 0.55
Neutral	3.39 ± 0.51	4.59 ± 0.78	3.48 ± 0.40
Overall	14.22 ± 1.06	17.41 ± 1.87	15.95 ± 1.16
Free Recall task 2			
Negative	11.44 ± 0.81	11.53 ± .1.14	12.62 ± 0.75
Positive	11.11 ± 1.06	11.65 ± 0.88	10.90 ± 0.68
Neutral	9.39 ± 1.14	9.24 ± 1.24	9.57 ± 0.73
Overall	31.94 ± 2.26	32.41 ± 2.26	33.10 ± 1.73

Supplementary Table 2 Recall performance (day 1) – experiment 2

The table presents recall performance on the first and second free recall task for each valence category (mean ± S.E.M). The data are presented for the three experimental groups separately.

[a] Note that one participant of the reactivation/no-stress group was identified as outliers and removed from the analyses for the free recall task.

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