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ORIGINAL INVESTIGATION

Effects of psychosocial stress on episodic memory updating

Bhaktee Dongaonkar · Almut Hupbach · Rebecca Gomez · Lynn Nadel

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Abstract
Rationale When a consolidated memory is reactivated, it becomes labile and modifiable. Recently, updating of reactivated episodic memory was demonstrated by Hupbach et al. (Learn Mem 14:47–53, 2007). Memory updating involves two vital processes—reactivation followed by reconsolidation. Here, we explored effects of psychosocial stress on episodic memory updating. Based on prior research, we hypothesized that stress before reactivation or stress before reconsolidation would impair memory updating.
Methods Participants learned a set of objects (list 1) on day 1. On day 2, some participants were reminded of list 1 before learning a second set of objects (list 2). Memory for list 1 was tested on day 3. Stress was administered either before reactivation of list 1 on day 2 (exp 1) or before reconsolidation of list 1, i.e., after reactivation and learning list 2 on day 2 (exp 2).
Results Memory updating involves the incorporation of list 2 items into list 1 memory, contingent upon the reactivation of list 1 memory. In exp 1, the reminder groups had higher intrusions than the no-reminder groups, but contrary to our predictions, stress did not reduce this reminder effect. Stress effects were, however, found in exp 2: the reminder group that was stressed *after* reactivation and new learning showed fewer intrusions than the control reminder group.
Conclusion The findings suggest that stress before reactivation does not impair memory updating but stress at the onset of reconsolidation can. Timing may determine the effects of stress on memory processing.

Keywords Episodic · Memory updating · Reactivation · Reconsolidation · Stress · Cortisol · Timing of stress

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Introduction

When a consolidated memory is reactivated, it can be modified before subsequent reconsolidation. Reactivation can be considered the reverse of consolidation in that it involves a protein degradation process (Alberini et al. 2006; Kaang and Choi 2011; Lee 2008) that renders the reactivated memory labile and allows changes to be made to the existing representation. Following reactivation, another round of protein synthesis is required to reconsolidate the reactivated memory representation (Miyashita et al. 2008). Depending on the circumstances, reactivation can act to either strengthen the original memory (Lee 2008; Marin et al. 2010; Sara 2000a, b; Zhao et al. 2009), weaken it (Nader et al. 2000; Tollenaar et al. 2008a, 2009a; Zhao et al. 2009), or even update and hence change it (Hupbach et al. 2007, 2008).

Various brain regions, including the prefrontal cortex and hippocampus, are engaged during reactivation of an episodic memory representation and maintenance of this active state (Bechtereva et al. 2004; Bledowski et al. 2009; Blumenfeld and Ranganath 2007; Cabeza and Nyberg 2000a, b; Chan 2009; Cohen et al. 1997; Dietrich 2004; Nyberg et al. 1999, 2000; O'Reilly and Rudy 2001). Activity in these regions is sensitive to neuromodulators, especially glucocorticoids and catecholamines such as dopamine and norepinephrine. Typically, neuromodulatory effects in prefrontal cortex and hippocampus follow an inverted U-shaped function, such that moderate levels of glucocorticoids and catecholamines facilitate their activity

while levels that are either too low or too high impair their function (Arnsten 2000, 2009; Aston-Jones and Cohen 2005; Birnbaum et al. 2000; Joëls and de Kloet 1990, 1991; Kim and Diamond 2002; Lisman et al. 2011; Roozendaal et al. 2004; Yuen et al. 2009).

Stress-induced increases in glucocorticoid and catecholamine levels can suppress the function of memory-related brain regions and lead to impairments at memory retrieval (Buchanan and Tranel 2008; Buchanan et al. 2006; de Quervain et al. 2007; Domes et al. 2004; Kuhlmann et al. 2005; Merz et al. 2010; Tollenaar et al. 2008a, b, 2009a, b). However, little is known about the delayed consequences of reactivation under stress or the effects of stress on memory reconsolidation. In the few extant studies looking at stress effects on reactivation and reconsolidation, participants explicitly recalled the original memory in conjunction with a stress manipulation.

Tollenaar and colleagues (2008a) trained participants on 20 neutral and 20 negative paired associates. Participants returned 1 day or 5 weeks later, and were stressed or not (control), after which they performed cued recall. Six months after this second visit, all participants were tested for recall during a telephone session. The control and stress participants who returned a day later for their second session did not differ significantly in memory recall at 6 months. However, participants who were stressed 5 weeks later in their second session had impaired recall memory for neutral and negative paired associates compared to their control group. These results suggest that stress before recall (and the accompanying reactivation of a memory engendered by recall) can impair memory but that impairment is only seen if there is a fairly long interval between learning and reactivation. It is notable that this stress effect on reactivation persisted up to 6 months after reactivation. In a similar study conducted by Tollenaar and colleagues (2009a), participants learned 15 emotional and 15 neutral words in an initial session. Upon returning a week later for a second session, participants were administered 30 mg of hydrocortisone or placebo. After a delay of 75 min, which allows for synthetic cortisol to reach peak circulating concentration, participants were given a free recall test and then a cued recall test inducing reactivation. Participants returned for a third session a week later (2 weeks after the first learning session) and were tested for recall and then recognition. Participants from the hydrocortisone group had impaired recall memory for emotional and neutral words in the second session with the impairment persisting into the third session. The placebo group was unimpaired. These results suggest that reactivation of memories when cortisol levels are high leads to attenuation of those memories, regardless of emotionality. Together, these two studies show that stress prior to reactivation impairs memory and that this impairment persists over several days to months.

Hupbach and Fieman (2012), on the other hand, found enhancing effects of stress on memory retrieval. Their study involved a 3-day paradigm. On day 1, participants memorized a scientific passage. On day 2, participants were subjected to cold pressor stress (CPS) or a warm water control, and immediately afterwards were asked to recall the passage. Recall was tested again 24 h later. Memory was enhanced in the stress condition on days 2 and 3. Importantly, this effect was only observed in male participants, who reacted to CPS with a significant increase in cortisol. There was no increase in cortisol in the female sample, and no memory effects of CPS were observed. This suggests that the stress-induced memory enhancements were mediated by cortisol changes. One fact that might explain these discrepant results is that Tollenaar et al. observed higher baseline cortisol levels than Hupbach and Fieman (2012). Thus, as seen in consolidation studies, memory reconsolidation under stress might also reflect an inverted U-shaped function such that high and low levels of stress hormones impair memory while moderate levels of stress enhance it.

In a few recent studies, stress was applied only after memory reactivation, which occurred under non-stressful conditions, thereby allowing the analysis of reconsolidation directly. Schwabe and Wolf (2010) asked participants to recall recent neutral, positive, and negative autobiographical memories. After recall, participants performed a control or stress task. A week later, they recalled the same autobiographical memories that had been recalled earlier. The stressed group showed reduced recall for neutral memories, while positive and negative memories were unaffected. Memory recall in the control group remained constant in all conditions. These results suggest a specific stress-induced impairment of reconsolidation of neutral autobiographical memories. Marin and colleagues (2010) studied post-reactivation stress effects on immediate versus delayed memories for emotional and neutral information. Participants saw a video with emotional and neutral information on day 1. Two days later (day 2) they returned and were instructed to reactivate the story from day 1 followed by a stress manipulation or control task and then an immediate recall test. A delayed recall test was conducted 5 days later. The stress group showed an increase in immediate recall for emotional, but not neutral, information when compared to the control group. This enhancement persisted through delayed recall 5 days later. These results suggest that stress after reactivation enhanced memory for emotional, but not neutral, information in a lasting way. Zhao and colleagues (2009) tested effects of post-retrieval stress in abstinent heroin addicts. On day 1, participants learned a list of 10 heroin-positive, 10 heroin-negative, and 10 heroin-neutral words over two learning trials. A day later, they recalled as many words as possible and then underwent

stress induction or completed a control task. They returned 24 h later for another recall test. Four weeks later, all participants performed the experiment again but this time stress participants were assigned to the control group and vice versa. Post-reactivation stress increased recall for heroin-positive words and decreased recall for heroin-negative words, while recall of heroin-neutral words remained unchanged.

One needs to be cautious in interpreting these studies on stress and reconsolidation. The paradigms, materials, and characteristics of the participants differed widely, and stress affected emotional memories in some cases and neutral memories in others. What has been referred to as memory reconsolidation necessarily involves two processes: reactivation of a memory and its subsequent re-stabilization. While prior research suggests that stress can impair the first process—reactivation of the memory, more research is needed on the second process—the re-stabilization of a memory. Further, as noted above, the process of reconsolidation can result in a strengthened, weakened, or updated memory. If new, related, information is acquired after a memory has been reactivated, this new information can update that memory (Hupbach et al. 2007), pending successful re-stabilization. Effects of stress on this aspect of memory reconsolidation, critical to the phenomenon of updating, are not known.

The present study explored the effects of stress during these two phases—reactivation and re-stabilization—on memory updating. We hypothesized that stress at either of these times might impair the memory updating normally observed in a well-established paradigm (Hupbach et al. 2007, 2008). This updating paradigm involves learning a list of objects on test day 1. Two days later (test day 2), participants return to the lab and are reminded of their test day 1 experience in order to reactivate that memory representation, followed by learning another list of objects. Two days after test day 2, participants are tested for their memory of the objects learned on test day 1. In addition to correctly recalling objects from the first list, participants unknowingly recall objects from the second list. This updated memory results from being reminded of the test day 1 experience before the second round of learning. There is typically no updating effect in control participants who are not reminded of test day 1 before learning the second list.

To explore the effects of stress on memory updating, we carried out two related experiments: one stressing participants before memory reactivation (stress before reactivation), and the other stressing subjects after memory reactivation and the learning of list 2, just as the re-stabilization phase of the reconsolidation process was getting started (stress before re-stabilization). For stress before reactivation, we stressed participants on test day 2, before they were reminded of list 1. To investigate the effects of stress exclusively on re-stabilization, we stressed participants after reactivation of list 1 and list 2 learning on test day 2. Our initial hypothesis was that stress would impair reactivation possibly through its impact on the prefrontal cortex and hippocampus. Poorer reactivation should result in a failure to update properly or in a weakly updated list 1 memory with fewer items incorporated from list 2 than in a control, non-stressed group. Stress before re-stabilization, on the other hand, could impair a number of distinct processes: (1) the re-stabilization of list 1, (2) the updating of list 1 with list 2 items, and (3) the consolidation of list 2 itself.

Experiment 1: Stress before reactivation in episodic memory updating

Materials and methods

Participants

Forty-eight undergraduate students (29 females and 19 males; age between 18 and 23 years) from the University of Arizona consented to participate for course credits. The experimental procedures were approved by the Institutional Review Board at the University of Arizona. Participants were randomly assigned to the study groups.

Materials

List 1 and list 2 objects were identical to the objects from Hupbach et al. (2007). List 1 had 20 objects (balloon, bow, calculator, toy car, crayon, cup, dice, feather, flashlight, flower, glue, key, sock, sponge, spoon, sunglasses, teabag, toothbrush, tennis ball, and whistle). List 2 had 20 different objects (apple, Band-Aid, battery, book, cassette tape, cell phone, comb, dollar bill, toy frog, envelope, paper clip, toy pot, puzzle piece, rock, straw, thread, tissue, watch, shovel, and zipper).

Procedure

Figure 1 outlines the serial order of the tasks, and the administration of stress in exps 1 and 2.

List learning

The procedure was exactly the same as in Hupbach et al. (2007). Participants attended three sessions with a 48-h/2-day interval between each session (M-W-F or T-R-S). On their first visit (test day 1), the list 1 objects were removed from a basket, placed on the table one by one and then put back in a different container, a bucket. Participants were instructed to name the objects aloud when they were placed

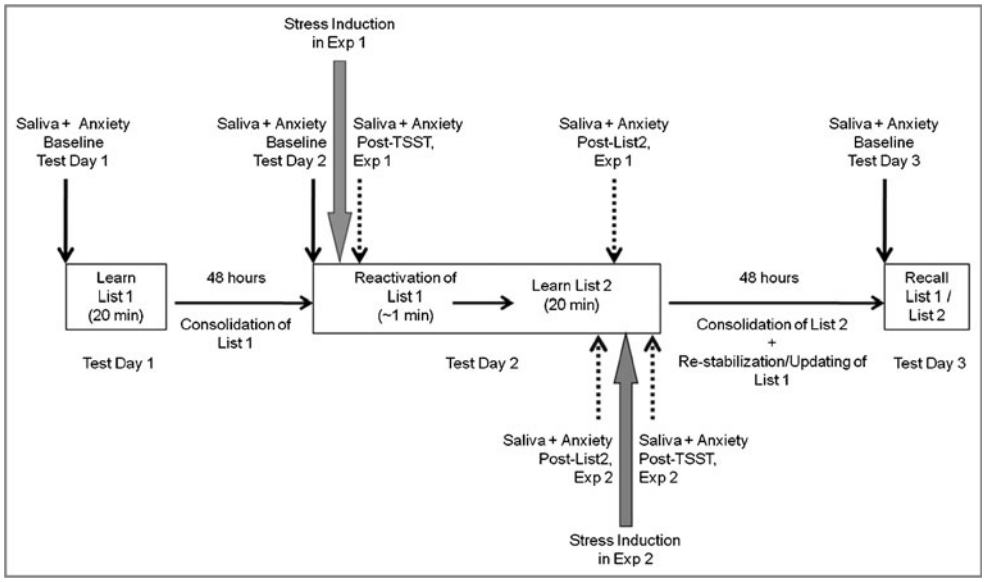

Fig. 1 Sequence of tasks in exps 1 and 2. The *gray arrows* indicate the time points of stress induction in experiments 1 and 2. The *vertical solid black arrows* indicate the time points of baseline saliva collection and baseline subjective anxiety ratings. The *vertical dotted black arrows pointing downwards* indicate time points of saliva and subjective anxiety measurements in exp 1, in addition to baseline measures. The *vertical dotted black arrows pointing upwards* indicate time points of saliva and subjective anxiety measurements in exp 2, in addition to baseline measures. Participants were stressed before reactivation in exp 1 and after list 2 learning, but before re-stabilization, in exp 2

on the table and to observe them carefully. The objects were then taken away and placed out of view following which the experimenter engaged the participant in a neutral conversation for 30 s that prevented rehearsing. Next, the participants had to recall as many of those 20 objects as possible. This cycle continued until participants reached a learning criterion of 17/20 objects or at the most four trials, whichever occurred first. The number of trials required to reach criterion on list 1 was denoted as the score for each participant. Participants who recalled less than 17 objects on their fourth trial received a score of 5. On their second visit (test day 2), participants learned another list of objects (list 2), different from those in the first session. In the second session, the learning procedure was modified to distinguish it from the procedure used in the first session. All objects were placed on the table in front of the participants, who had to name each object without touching it. They were given an additional 30 s to look at the objects carefully. The objects were then taken away and placed out of view followed by 30 s of neutral conversation. The participants then had to recall as many of the 20 objects as possible. The cycle continued until participants recalled 17/20 objects or had at most four learning trials. The number of trials to reach criterion were scored as for list 1. On their third visit (test day 3), participants were instructed to recall as many objects as possible from their session on test day 1. We conducted four recall trials, and between trials, the experimenter conversed with the participants for 30 s to temporarily disengage them from the task.

Reminder manipulation

Before list 2 learning began on test day 2, participants were randomly assigned to the reminder or no-reminder group. The reminder group had the same experimenter and went to the same room as on test day 1. In addition, they were shown the basket from test day 1 and were asked a reminder question, "Do you remember this basket and what we did with it?" The no-reminder group had a different experimenter and went to a different room, and participants were not asked the reminder question. Following these manipulations, list 2 learning ensued. On test day 3, all participants had the same experimenter and went to the same room as on test day 1.

Stress manipulation

On arrival on test day 2, participants were randomly assigned to the stress induction or control group. To induce stress, participants performed the Trier Social Stress Test (TSST; Kirschbaum et al. 1993). They were given 5 min to prepare a speech, which they then had to deliver for 5 min in front of two judges. The room had a video camera and microphone to give the impression that their speech was being recorded. They were informed that their verbal and non-verbal performance would be evaluated using a movement and voice inflection analyzer. At the end of 5 min, participants were instructed to perform a math task involving the backwards subtraction of a two-digit number from a four-digit number (e.g., 17 from 1,873) for 5 min. The control group wrote a job

essay for the first 5 min, then read and edited the essay for the next 5 min, and finally solved simple two-digit subtraction problems for 5 min.

Salivary cortisol and subjective anxiety measures

The experiments were conducted in the afternoon between 1 and 6 pm to control for circadian variations in cortisol. Saliva samples and subjective anxiety measures were collected at regular intervals on all three test days (see Fig. 1). Spielberger's State Anxiety Inventory was administered to assess subjective anxiety. The inventory includes 20 statements that the participants had to rate on a scale of 1 to 4. The score ranges from 20 (low anxiety, not stressed at all) to 80 (high anxiety, extremely stressed). The time points of assessing subjective anxiety were baseline upon arrival on test day 1, baseline on test day 2, immediately after TSST or the control task on test day 2 (20 min after test day 2 baseline), end of list 2 learning on test day 2 (35–40 min after test day 2 baseline), and baseline on test day 3. Additional subjective anxiety measures were taken after each list learning, after the TSST/control manipulation, and after recall to indicate how the participants felt while performing those tasks for a total of 11 subjective anxiety measures across three test days.

Saliva samples were collected using oral polymer swabs (product no. 5001.02), stored in tubes, and frozen at −20 °C (product no. 5001.05), until they were analyzed in our laboratory at the University of Arizona using high sensitivity salivary cortisol immunoassay kits (product no. 1-3002; stored at 6 °C) purchased from Salimetrics, LLC, USA (www.salimetrics.com).

Results

Subjective anxiety scores

Subjective anxiety was assessed using Spielberger's State Anxiety Inventory. As noted above, the scores on this test can range from a minimum of 20 to a maximum of 80. Subjective anxiety ratings collected across five time points on test day 2 are shown in Fig. 2. A mixed factorial ANOVA with stress and reminder conditions as between-group factors and subjective anxiety time points as within-subject factors revealed that subjective anxiety changed across some of the 11 different time points ($F_{5.66,\ 243.41}=43.11$, $p<0.001$, $\varepsilon=0.566$, Greenhouse–Geisser correction for sphericity violation). There was a significant interaction of the stress manipulation on subjective anxiety ($F_{5.66,\ 243.41}=10.11$, $p<0.001$, $\varepsilon=0.566$, Greenhouse–Geisser correction for sphericity violation), but no interaction involving reminder ($F<1$). A simple effect analysis showed that stressed participants reported being more stressed than control participants during TSST ($F_{1,\ 45}=20.45$, $p<0.001$) and immediately after TSST ($F_{1,\ 45}=22.44$, $p<0.001$) on test day 2. There were no significant differences in subjective anxiety between control and stress groups at any of the other time points over the three test days.

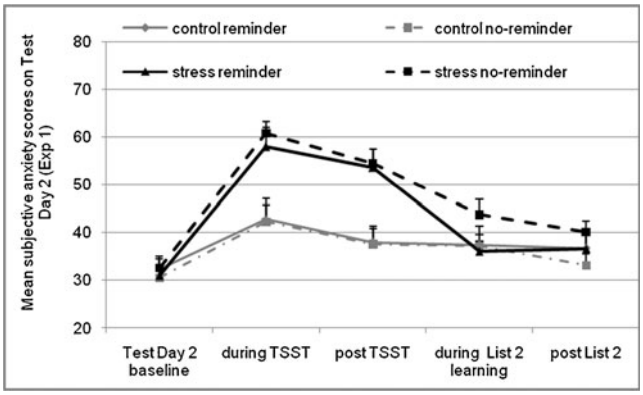

Fig. 2 Subjective anxiety scores on test day 2 in exp 1

Salivary cortisol levels

Changes in salivary cortisol levels across five time points and as a function of the reminder are shown in Fig. 3. A mixed factorial ANOVA with stress manipulation and reminder as between-group factors and the five salivary cortisol measures (or time) as within-group factors revealed a main effect of salivary cortisol change across the measured time points ($F_{2.37,\ 104.44}=12.26$, $p<0.001$; $\varepsilon=0.593$, Greenhouse–Geisser correction for sphericity violation). This analysis also revealed a main effect of stress ($F_{1,\ 44}=8.73$, $p=0.005$) and a significant interaction of stress and salivary cortisol concentration ($F_{2.37,\ 104.44}=13.09$, $p<0.001$; $\varepsilon=0.593$, Greenhouse–Geisser correction for sphericity violation) but no effect of reminder ($F_{2.37,\ 104.44}=1.53$, $p=0.22$; $\varepsilon=0.593$, Greenhouse–Geisser correction for sphericity violation). Simple effect analysis showed that cortisol levels were higher in the stress groups than in the control

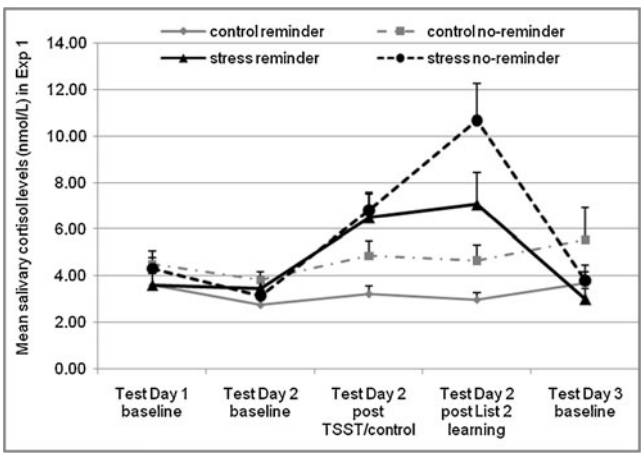

Fig. 3 Salivary cortisol (nanomoles per liter) across five different time points over three test days in exp 1

groups immediately post-TSST ($F_{1, 46}$=12.5, p=0.001) and remained high in the stress groups until the end of list 2 learning ($F_{1, 46}$=17.87, p<0.001) on test day 2. Paired sample t tests showed that cortisol levels in stressed participants increased from baseline to post-TSST (t_{24}=5.98, p< 0.001) and these increased cortisol levels persisted until the end of list 2 learning (t_{24}=4.71, p<0.001) on test day 2. There were no significant differences in salivary cortisol measures at other time points.

List 1 learning performance on test day 1

The score/number of trials to reach criterion on list 1 were averaged for each group; since the various groups on test day 1 followed the same procedures, it is not surprising that there were no significant differences in the number of trials to reach criterion between groups on this day as measured by a 2 (stress, control)×2 (reminder, no-reminder) ANOVA (F<1): stress reminder (M=2.92 trials, SEM=0.33), control reminder (M=3.33 trials, SEM=0.33), stress no-reminder (M=3.42 trials, SEM= 0.26), and control no-reminder (M=2.91 trials, SEM=0.32).

List 2 learning performance on test day 2

There were no main effects of reminder ($F_{1, 44}$=2.03, p=0.16) or stress ($F_{1, 44}$=3.76, p=0.069) on learning of list 2 on test day 2. Nor was there an interaction (F<1): stress reminder (M=2.77 trials, SEM=0.26), control reminder (M=2.58 trials, SEM=0.26), stress no-reminder (M=3.58 trials, SEM=0.39), and control no-reminder (M=2.64 trials, SEM=0.28).

Recall performance on test day 3

List 1 recall was scored for correctly recalled objects from list 1 and falsely recalled objects from list 2 (intrusions) for every trial. Figure 4 shows the mean percentage of recalled list 1 objects (averaged over four trials) and the mean percentage of list 2 intrusions into list 1 (averaged over four trials) in exp 1.

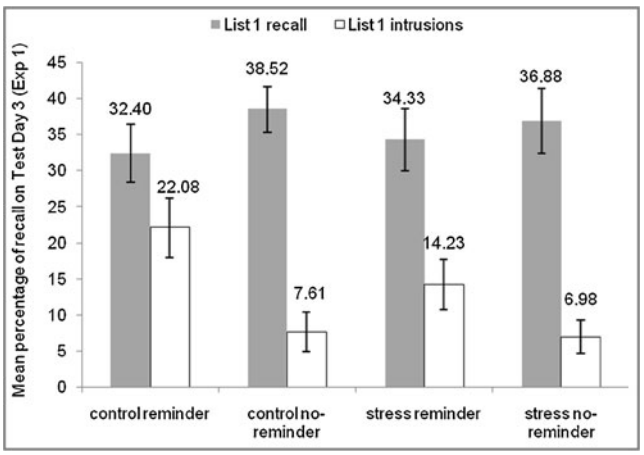

Fig. 4 Mean percentage recall of objects from list 1 and intrusions in list 1 (falsely recalled list 2 objects) on test day 3 in exp 1. *Error bars* indicate standard error of the mean

Recall of list 1

A 2 (stress, control)×2 (reminder, no-reminder)×4 (recall trials) mixed factorial ANOVA revealed a main effect of recall trial ($F_{1.97, 86.44}$=9.70, p<0.001; ε=0.655, Greenhouse–Geisser correction for sphericity violation). Within-subjects polynomial contrasts confirmed a linear trend in recall across trials ($F_{1, 44}$=14.51, p=0.001) such that the number of list 1 objects recalled increased as the trials progressed. There were no effects of stress manipulation (F<1) or reminder condition ($F_{1, 44}$=1.16, p=0.29) on recall of list 1 objects.

Intrusions

A 2 (stress, control)×2 (reminder, no-reminder)×4 (recall trials) mixed factorial ANOVA revealed a main effect of reminder ($F_{1, 44}$=10.09, p=0.003, r=0.43) with more intrusions in the reminder than in the no-reminder condition. Stress had no main effect on the number of intrusions ($F_{1, 44}$=1.54, p=0.22, r=0.18). Nor was there interaction of stress and reminder ($F_{1, 44}$=1.11, p=0.3, r=0.16). While the stress reminder group had fewer intrusions than the control reminder group, this decrease was not significant (t_{23}=1.42, p=0.17), counter to our hypothesis.

Discussion

Experiment 1 explored the effects of stress on memory updating when participants were stressed before receiving a reminder reactivating their test day 1 memory. The objective was to observe whether stress before reactivation would impair the typical updating of list 1 with list 2 items. There were no differences in the recall of list 1 due to stress or reminder manipulations. Although the reminder groups had significantly higher intrusions than the no-reminder groups and although there was an 8 % reduction in intrusions in the stress reminder group compared to the no stress reminder group, this effect failed to reach significance.

We conducted two follow-up experiments to assess some of the effects of stress: (1) on list 2 learning and (2) on explicit reactivation, instead of subtle reactivation, of a well-learned list 1. In exp 1, stress effects on subtle reactivation could only be tested indirectly on test day 3, in terms of the number of intrusions. Explicit recall on the other hand allows us to directly confirm whether stress has any effect on retrieval of a well-learned memory representation before its modification. If we find that stress has no effect on explicit retrieval of a well-learned list 1 on test day 2, then we can strengthen the conclusion that stress does not

significantly interfere with reactivation of list 1 memory. Given space constraints, we report only the main findings. In the first follow-up study, using only the reminder condition, stress (n=12) and control (n=12) participants recalled list 2 on test day 3. All other procedures were the same as for the reminder conditions in exp 1. We found no difference between the stress reminder and control reminder for recall of list 2 ($\%M_{stress}$=43.65, SEM=4.22; $\%M_{control}$=44.17, SEM=3.86) or for intrusions ($\%M_{stress}$=1.98, SEM=0.68; $\%M_{control}$=2.19, SEM=0.86) from list 1 into list 2, suggesting that there was no effect of stress on the learning of list 2 on test day 2, F<1. In the second follow-up study, participants learned list 1 objects on test day 1 (as in exp 1) and were simply asked on test day 2 to recall list 1 objects under stress (n=7) or control (n=8) conditions. We found no difference between the two groups in recall of list 1 items, suggesting that stress did not impair reactivation of criterion-based learning ($\%M_{stress}$=66.07, SEM=4.22; $\%M_{control}$=61.25, SEM=4.19), F<1. Given these results that stress prior to reactivating a memory has little impact, the second experiment focused on the effect of stress after list 2 learning, at a time when it can affect only the re-stabilization phase of reconsolidation.

Experiment 2: stress before re-stabilization in episodic memory updating

Materials and method

Participants

Thirty-seven undergraduate students of the University of Arizona consented to participate and were given course credit for participation (13 females and 24 males; age between 18 and 27 years). Participants were randomly assigned to the two groups in the experiment (stress vs. control). The experimental procedures were approved by the Institutional Review Board at the University of Arizona.

Materials

The same lists 1 and 2 objects used in exp 1 were also used in exp 2.

Procedure

The procedure on test days 1, 2, and 3 was the same as in exp 1 except in the timing of the stress induction on test day 2 and recall on test day 3. Stress was induced after list 2 learning, which followed the list 1 reminder manipulation on test day 2 (see Fig. 1). Collection and analysis of cortisol and subjective anxiety measures was also identical with the exception that the collection timing of "post-TSST" and "post-list 2 learning" saliva sample and subjective anxiety measure was switched on test day 2 as illustrated in Fig. 1. In addition, recall of list 1 (four trials) was followed by recall of list 2 (four trials) on test day 3. We omitted the no-reminder groups as we were primarily interested in the effects of stress in the reminder condition.

Results

Subjective anxiety scores

Spielberger's State Anxiety Inventory was used to record subjective anxiety over 11 time points. The means for five time points on test day 2 are shown in Fig. 5. A repeated measures ANOVA with stress as the between-group factor and the 11 time points as within-group factors revealed that subjective anxiety varied at some of the 11 time points in the two groups ($F_{3.57,\ 60.74}$=22.15, p<0.001; ε=0.357, Greenhouse–Geisser correction for sphericity violation). On test day 2, TSST induced significantly higher levels of stress in the stress reminder participants ($F_{3.57,\ 60.74}$=4.99, p=0.002; ε=0.357, Greenhouse–Geisser correction for sphericity violation). Independent samples t tests showed that stress reminder participants reported higher anxiety during TSST (t_{35}=7.05, p<0.001) and post-TSST (t_{35}=6.52, p<0.001) than control reminder participants on test day 2. There were no differences in subjective anxiety between control and stress groups at the remaining time points.

Salivary cortisol levels

Figure 6 shows mean changes in cortisol levels in the stress reminder and control reminder groups over five time points across test days 1–3. A repeated measure ANOVA with stress as a between-subjects variable and time of assessing cortisol as a within-subject variable revealed a main effect of stress on salivary cortisol levels ($F_{1,\ 33}$=3.94, p=0.05) on test day 2 as well as a significant interaction of stress and time of assessment ($F_{3.41,\ 112.64}$=3.52, p=0.014; ε=0.853, Hyunh–Feldt

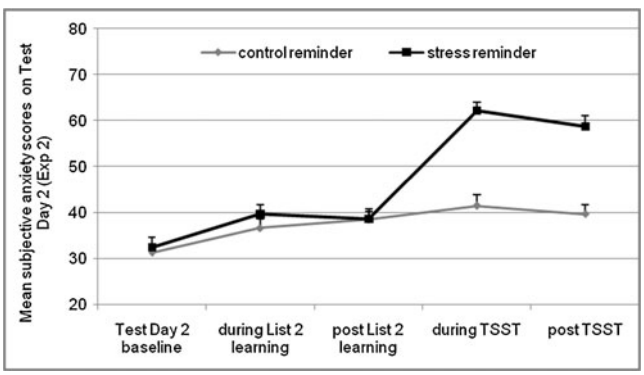

Fig. 5 Subjective anxiety scores on test day 2 in exp 2

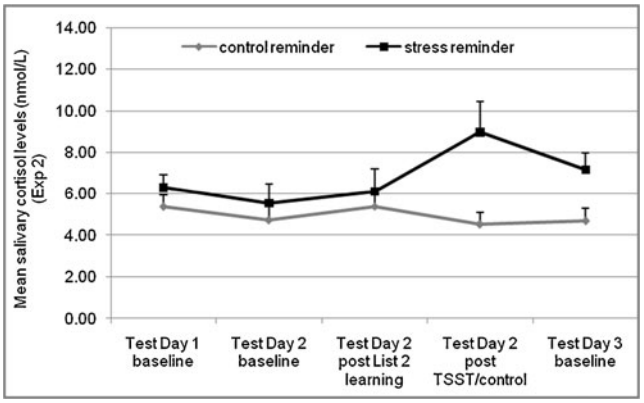

Fig. 6 Mean salivary cortisol in stress reminder and control reminder groups in exp 2

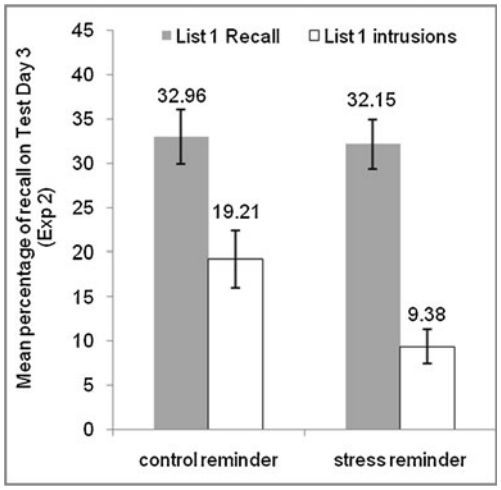

Fig. 7 Mean percentage recall of objects from list 1 and intrusions in list 1 (falsely recalled list 2 objects) on test day 3 in exp 2. *Error bars* indicate standard error of the mean

correction for sphericity violation). Independent samples *t* tests revealed higher cortisol levels after the TSST in for the stress reminder group compared to the control reminder group (t_{35}=3.17, p=0.003, r=0.95) on test day 2 as well as significant increases in salivary cortisol levels from post-list 2 learning to post-TSST in the stress reminder participants (t_{17}=2.79, p=0.01). Although stress reminder participants had higher baseline cortisol levels on test day 3 than control reminder participants (t_{33}=2.03, p=0.05, r=0.33), there was no significant difference between groups in their corresponding baseline subjective anxiety on test day 3 (t_{35}=1.73, p=0.09, r=0.28), and a paired sample *t* test comparing baseline levels of salivary cortisol on test days 2 and 3 in the stress reminder participants showed that cortisol was within baseline levels on test day 3 (t_{16}=1.97, p=0.06). There were no significant differences in salivary cortisol measures at the remaining time points.

List 1 learning performance on test day 1

An independent samples *t* test showed that there was no difference in the number of trials taken to reach criterion between the stress reminder and control reminder groups (t<1): stress reminder (M=3.22 trials, SEM=0.24), control reminder (M=2.95 trials, SEM=0.26).

List 2 learning performance on test day 2

An independent samples *t* test revealed no difference in the number of trials taken to reach criterion between the stress reminder and control reminder groups (t<1): stress reminder (M=2.33 trials, SEM=0.21), control reminder (M=2.47 trials, SEM=0.28).

Recall performance on test day 3

Recall on test day 3 was scored in the same manner as for exp 1. Figure 7 illustrates the mean percentage recall of list 1 objects (averaged over four trials) and mean percentage intrusions into list 1 (averaged over four trials) in exp 2.

Recall of list 1

A 2 (stress, control)×4 (recall trials) mixed factorial ANOVA revealed a within-subjects main effect of recall trial ($F_{3,\ 105}$=13.88, p<0.001). Within subjects polynomial contrasts confirmed a linear trend of recall across trials ($F_{1,\ 35}$=28.24, p<0.001). The number of objects recalled from list 1 increased as the trials progressed: trial 1 (M=28.11 %), trial 2 (M=31.35 %), trial 3 (M=34.05 %), and trial 4 (M=36.77 %). There was no main effect of stress (F<1; stress reminder M=32.15 %, SEM=2.8; control reminder M=32.96 %, SEM=3.1) and no interaction of stress and recall trials (F<1).

Intrusions into list 1 (from list 2)

A 2 (stress, control)×4 (recall trials) mixed factorial ANOVA revealed a main effect of stress on the number of intrusions ($F_{1,\ 35}$=6.72, p=0.014, r=0.4) such that the stress reminder group (M=9.37 %, SEM=1.94) had significantly fewer intrusions than the control reminder group (M=19.21 %, SEM=3.2). There was no effect of trial (F<1) or interaction of stress and recall trial ($F_{2.18,\ 76.48}$=2.55, p=0.08; ε=0.728, Greenhouse–Geisser correction for sphericity violation).

Recall of list 2

A 2 (stress, control)×4 (recall trials) mixed factorial ANOVA revealed a within-subject main effect of trial ($F_{2.58,\ 90.14}$=4.89, p=0.005; ε=0.859, Hyunh–Feldt

correction for sphericity violation) with a linear trend of recall with progressive trials ($F_{1, 35}$=10.77, p=0.002): trial 1 (M=35.43 %), trial 2 (M=38.0 %), trial 3 (M=38.0 %), and trial 4 (M=39.14 %). There was neither an effect of stress on recall of list 2 (F<1; stress reminder M=40.21 %, SEM=2.31; control reminder M=36.18 %, SEM=4.12) nor an interaction of stress and recall trial (F<1).

Intrusions in list 2 (from list 1)

A 2 (stress, control)×4 (recall trials) mixed factorial ANOVA revealed no effect of stress on the number of intrusions in list 2 ($F_{1, 35}$=1.74, p=0.2; stress reminder M =2.01 %, SEM=0.64; control reminder M=3.42 %, SEM= 0.84), no effect of trial ($F_{1, 35}$=3.57, p=0.07), and no stress by recall trial interaction (F values<1).

Discussion

In exp 2 we explored the effects of stress on the re-stabilization phase of memory updating, by stressing participants on test day 2 after learning list 2. Stress impaired memory updating at this time point such that stress reminder participants showed significantly fewer intrusions than control reminder participants. There were no effects of stress on recall of list 1.

The few studies in humans that have investigated the effects of stress on re-stabilization phase of reconsolidation have reported impairment of the original memory in some cases and enhancement in others (Marin et al. 2010; Schwabe and Wolf 2010; Zhao et al. 2009) suggesting that the original memory was altered under the influence of stress. In these studies, participants explicitly recall the original memory, in contrast to the more subtle form of reactivation used in our study. Note that stress did not affect list 1 recall, suggesting that whatever the nature of the memory reactivation initiated by our reminder manipulation, re-stabilization of that memory is not itself affected by stress. However, our subtle reactivation did trigger an update of the original memory with new objects from list 2, which is reflected in the high intrusions observed in the control reminder group. Stress after learning list 2 impaired, but did not entirely abolish, this updating. We conclude that stress impaired the updating of original memory (list 1) with new items from list 2, but it did not impair the ability to recall list 1.

General discussion

Our experiments were aimed at exploring the effects of stress on memory updating when stress was induced either before reactivation or before re-stabilization. Stress prior to reactivation does not appear to impair memory updating (exp 1). Stress after list 1 reactivation and list 2 learning, however, does seem to impair updating (exp 2).

Stress has frequently been reported to impair memory retrieval (Buchanan and Tranel 2008; Buchanan et al. 2006; de Quervain et al. 2007; Domes et al. 2004; Kuhlmann et al. 2005; Merz et al. 2010; Tollenaar et al. 2008a, b, 2009a, b; but see Hupbach and Fieman 2012). Most of these studies involved a single exposure to the stimulus to be remembered, while in our experiments, participants learned items to criterion. Such learning may render memory less susceptible to stress before reactivation (as demonstrated by the second follow-up study showing equal list 1 memory retrieval in stress and control conditions, described in the discussion of exp 1).

To date, three animal studies have reported impairing effects of stress on reconsolidation. Maroun and Akirav (2008) found that stress enhanced consolidation but impaired reconsolidation (re-stabilizing phase) of object recognition memory in rats. Similarly, conditioned place preference for morphine was disrupted when the memory was reactivated by re-exposing rats to the conditioning chamber followed by cold water stress. Here, stress disrupted the reconsolidation of morphine place preference (Wang et al. 2008). Finally, Cai et al. (2006) showed that glucocorticoids administered immediately after reactivation of a contextual fear memory led to reduced expression of fear in mice. These studies corroborate our finding from exp 2, which now extends the effects reported in the animal literature to human memory reconsolidation. However, our results differ from the rodent studies in one important respect: we do not see reduced retrieval of the original memory (list 1). Since only memory updating is reduced, it is possible that stress specifically affects a process critical to updating, such as linking list 2 items to the test context, or to the items on list 1.

An alternate explanation for the reduction in updating resulting from stress at reconsolidation is related to the saliency of the events on test day 2. Participants might be utilizing the stressful state to help distinguish objects learned on test day 2 from those learned on test day 1. Were this the case, however, it should be observed in both exps 1 and 2, since stress was induced on test day 2 in both. Although we see a small reduction in intrusions in exp 1, the reduction in memory updating is significant only in exp 2, where stress was induced post-reactivation. Stress prior to reactivation in exp 1 did not result in statistically reliable impairment, suggesting that the saliency of the events occurring on test day 2 is not the cause of the reduction in memory updating.

A limitation to our study is that although both experiments included control reminder and stress reminder groups, it was difficult to compare these groups across experiments

with statistical tests due to methodological differences in the two experiments. Although additional research is needed, the findings in exp 2 show a definite reduction in memory updating under stress, and the findings across experiments are important for suggesting that the timing of stress may affect updating.

In short, the effects of stress on memory updating appear to depend on when the stress occurs—the closer the stressful experience is to the onset of reconsolidation, the greater the impact. When an event itself is stressful (e.g., a fear inducing situation) or when stress is experienced within minutes of exposure to emotional information (e.g., negative pictures or words), the co-release of noradrenaline and glucocorticoids results in enhanced memory (Joëls et al. 2006, 2011; Kim and Diamond 2002; Roozendaal et al. 2004; Wiegert et al. 2008). However, the occurrence of stress from 40 min up to an hour before training can suppress new learning, as the slow genomic mechanisms triggered by glucocorticoids take effect (Joëls et al. 2006; Wiegert et al. 2006). In our experiments, learning occurred within 20 min of stress induction and therefore do not neatly fit the "timing" criteria for these effects. At this point, we conclude from our results that stress and the associated release of cortisol can alter the neural dynamics that normally lead to an updated episodic memory representation. Further work is needed to determine exactly how stress exerts these effects.

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