Published in final edited form as:

Cogn Emot. 2014 ; 28(2): 361–374. doi:10.1080/02699931.2013.822346.

Does Stress Enhance or Impair Memory Consolidation?

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Abstract

Three experiments examined the hypothesis that stress-induced arousal enhances long term memory for experiences associated with an arousing events. Contrary to expectations, in each experiment exposure to a stressor (arm immersion in ice water) interfered with, rather than enhanced, long term memory for associated material. Despite varying the stimuli (words, pictures), their emotional value (positive, negative, neutral), the time between learning and stress inductions (0 to 1 minute), and opportunities for post-learning rehearsal, each experiment produced a significant reversal of the hypothesized effect. That is, in each experiment, exposure to a stressor interfered with, rather than enhanced, long term memory for associated material. We conclude that the relationship between stress and memory consolidation is more bounded than previously believed.

Keywords

AROUSAL; STRESS; LONG TERM MEMORY CONSOLIDATION; CORTISOL; COLD PRESSOR STIMULATION

“An impression may be so exciting emotionally as almost to leave a scar upon the cerebral tissues”

—William James, 1890

The idea that emotionally arousing experiences are especially memorable has been around a long time. Many memories do seem to involve emotional arousal—a heart-stopping car crash, our first public speech, or the excitement of a wedding. But can arousal, and in particular, stress, also impair memory? In the reported studies, we examine the reliability with which stress-induced arousal consolidates experience in long term memory.

Although various conceptualizations of emotion and arousal exist, emotion is often perceived as comprising two dimensions: valence and arousal. Valence is commonly seen as how “good” (positive) or “bad” (negative) something is, whereas arousal indicates how urgent or important it is. Arousal is often quantified by measures of the activation of autonomic and endocrine systems (e.g., increases in heart rate or stress hormones such as

cortisol). Two of the most popular continua for defining arousal are that arousal is the inverse probability of falling asleep (Corcoran, 1965), or that arousal reflects the intensity of emotion (Whissell, Fournier, Pelland, Weir, & Makarec, 1986). For the purposes of this research, and following Storbeck and Clore (2008), stress-induced arousal can be characterized as an emotional and physiological reaction to aversive stimuli involving activation of the sympathetic, autonomic, and/or the endocrine systems.

Measures of stress or arousal are often uncorrelated across systems (autonomic nervous system (ANS), electrocortical, endocrine, somatic, etc.). In fact, even within a system, such as the ANS, measures are commonly uncorrelated (Cacioppo & Petty, 1983). However, hormonal changes or patterns of responses across different measures are generally accepted as a reflection of changes in arousal (Cacioppo & Petty, 1983). Because the effects of cortisol and epinephrine are reasonably well understood, and because these hormones are typically released under stress, levels of these hormones have been used in many studies as an indicator of stress-induced arousal (see Nicolson, 2007, for a review on cortisol measurement and stress). Typically, at higher doses of stress hormones, effects on memory are more pronounced. For example, Cahill and Alkire (2003) found that at 80 ng/kg/min for 3 minutes, epinephrine improved memory consolidation, whereas 40 ng/kg/min of epinephrine and saline showed no such improvement. Cortisol in particular has been shown to correlate with observed differences in memory and performance in many studies (e.g., Cahill, Gorski, & Le, 2003), and is a widely used measure. For example, Abercrombie, Speck, and Monticelli (2006) found a positive relationship between cortisol increase and memory in those who experienced high negative affect related to the stressor. The degree of cortisol response is also important at retrieval; those who showed a larger increase in cortisol to stress show greater memory impairment than those with a lower cortisol response (Buchanan, Tranel, & Adolphs, 2006; Tollenaar, Elzinga, Spinhoven, & Everaerd, 2008). However, it is not simply that the higher the cortisol levels, the greater the influence on memory. Elzinga, Bakker, and Bremner (2005) found a surprising negative relationship between cortisol and memory consolidation, and Cahill et al. (2003) found no relationship between cortisol and memory. Thus, cortisol serves as a reliable measure of stress that often, but not always, correlates with memory performance.

One of the most studied factors in arousal and memory research is the relationship between the timing of arousal and recall. A number of studies have shown that both pre- and post-learning arousal enhances memory consolidation. For example, participants who were administered cortisol before viewing pictures recalled more high arousal pictures (but not more neutral pictures) than those who received a placebo (Buchanan and Lovallo, 2001; Kuhlmann & Wolf, 2006). Arousal induced after learning, such as through the use of epinephrine administration (Cahill & Alkire, 2003), negative physiological stress (cold pressor: arm immersion in ice water; Cahill et al., 2003), negatively arousing stimuli (Cahill & McGaugh, 1995), negatively stressful (oral surgery) and positively arousing (comedic) videos (Liu, Graham, and Zorawski, 2008), similarly resulted in enhanced recall, particularly for stimuli that were themselves arousing. However, arousal experienced at retrieval impairs memory. For example, Smeets, Otgaar, Candel, & Wolf (2008) found that stress induced through cold pressor stimulation impaired memory when it was introduced at retrieval. Similarly, Kuhlmann, Piel, & Wolf (2005) demonstrated impaired memory when

the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) occurred shortly before retrieval, (see Roozendaal, 2002 for a review of arousal and memory at retrieval). Different methods of inducing arousal (physiological stress, social stress, cortisol administration, epinephrine administration, pictorial stimuli, and video stimuli) result in similar effects. The key factor for arousal enhancement of memory appears to be that arousal occurs close in time to learning and dissipates before recall. However, while it also appears that affective items show an effect of arousal while neutral items do not (Cahill & Alkire, 2003: epinephrine administration; Cahill et al., 2003: cold pressor; Abercrombie et al., 2006: TSST; Liu et al., 2006: arousing videos), it should be noted that neutral items do sometimes show a memory benefit (Nielson, Yee, & Erickson, 2005: arousing videos; Anderson, Wais, & Gabrieli, 2006: arousing pictures; Preuß & Wolf, 2009: TSST).

Most research has concerned the beneficial effects of arousal on declarative memory consolidation for presented items. But in other types of memory, evidence has been more mixed. A review of eyewitness testimony for arousing events suggested that arousal does enhance memory, as victims of crime, for example, often recall more than bystanders (Christianson, 1992). However, arousal can also be detrimental to eyewitness memory. Victims of rape or assault and individuals sustaining injuries often provide less adequate descriptions of assailants than victims of less arousing crimes, such as robbery (Christianson, 1992). Thus, the literature shows that arousal sometimes impairs but more typically benefits memory consolidation, particularly for declarative memory. In this research, we explored the factors that might govern when stress-induced arousal enhances and when it impairs memory.

Experiment 1

Many studies of declarative memory use complex stimuli, such as pictures or videos, making it somewhat unclear which factors promote and which interfere with later recall. Although simple stimuli such as word lists have been used previously in studies of stress (Payne et al., 2002; Smeets et al., 2008), these studies typically concerned false memories emerging from presentation of lists of interrelated words, a process that relies on associative memory. In contrast, our first experiment focuses on memory consolidation for simple stimuli in the form of unrelated word lists. We expected that, as in prior research (e.g., Cahill et al., 2003), induced stress would enhance recall after two days.

Method

Participants—97 undergraduate participants (53 stress, 44 control; 59 women, 38 men, M age = 18.97, SD age = 1.15) volunteered to participate in exchange for partial course credit. Participants with Reynaud's syndrome, recent surgery, Cushing's disease, or other medical conditions where stress inductions would be inadvisable were excluded from participation.

Word Lists—Word lists were created from ANEW (Affective Norms for English Words; Bradley & Lang, 1999). Three lists (negative, neutral, positive) consisting of 10 words each were matched for word frequency. An ANOVA revealed that the lists did not differ in word frequency: $F(2, 28) = .587, p = .56$, word length: $F(2, 28) = .69, p = .51$, or semantic cohesion $F(2, 127) = .104, p = .902$. The negative ($M = 2.26, SD = .51$), neutral ($M = 5.01$,

$SD = .32$), and positive ($M = 8.13$, $SD = .51$) lists differed significantly in valence, $F(2, 29) = 417.25$, $p < .001$; post-hoc LSD tests revealed that all differed significantly from each other: all p 's $< .001$. Finally, the lists differed in arousal: $F(2, 29) = 190.41$, $p < .001$, such that negative ($M = 7.33$, $SD = .42$) and positive ($M = 7.48$, $SD = .88$) were equivalent to each other ($p = .55$), but both were significantly higher in arousal than the neutral list ($M = 3.10$, $SD = .15$), p 's $< .001$.

Cold Pressor Stimulation—Cold pressor stimulation has been used in experiments of stress and memory (e.g., Cahill et al., 2003; Smeets et al., 2008) and has been shown to reliably induce stress (Lofvallo, 1975), resulting in increased cortisol concentrations (al' Absi, Petersen, & Wittmers, 2002). Hence, participants were instructed to place their non-dominant arm up to the elbow in either a bucket of ice water (0-3° C: stress condition) or warm water (37-40° C: control condition) for one minute. Participants were encouraged to complete the task, but were told that they could remove their hand at any time without penalty if they did not wish to continue. Participants were observed during the cold pressor task to ensure proper arm immersion.

Stress Questionnaire—As in Cahill et al. (2003), participants rated the amount of pain or discomfort they experienced from the arm immersion on a scale of 0 (no pain or discomfort) to 8 (worst pain or discomfort imaginable).

Procedure—All materials and procedures were approved by the IRB of the University of Virginia, and informed consent was obtained from all participants. During session 1, participants sat in a small testing room that contained a chair and a desk with a computer. The computer presented the words in each list for 1 second each, and the order of lists and words within lists was randomized. There was a 45-second break between lists. After the final list, the computer instructed participants to inform the experimenter that they were ready to begin the next task. Participants then exited the small booth into a larger room and underwent either the cold pressor stress manipulation or the warm water control condition. Next, participants filled out personality measures and a self-report measure of stress. Participants were told to return in 48 hours, ostensibly to view more stimuli. Approximately 48 hours later, participants returned for session 2 and were surprised with a recall test. They were instructed to recall, in no particular order and with no time constraint, all of the words they could remember from the word lists two days prior.

Results

Self-Report Measure of Discomfort—An ANOVA was conducted to determine the effect of condition on self-reports of pain/discomfort. Those in the stress condition ($M = 3.91$, $SD = 1.75$) reported significantly more pain/discomfort than those in the control condition ($M = .23$, $SD = .56$), $F(1, 96) = 177.34$, $p < .001$, $\eta^2 = .65$. No main effects or interactions were found for gender.

Recall—Recall was analyzed with the factors of affect, gender, and stress in a normal identity generalized estimating equation. There was a main effect of affect such that both negative words ($M = 2.36$, $SE = .14$) and positive words ($M = 2.20$, $SE = .14$) were recalled

at a higher rate than neutral ($M = 1.61$, $SE = .14$, $p < .001$ and $p = .002$, respectively), Wald χ^2 (2, $N = 291$) = 20.43, $p < .001$, QICC = 568.25. There was also a main effect of gender, such that women ($M = 2.35$, $SE = .10$) recalled more words than men ($M = 1.76$, $SE = .13$), Wald χ^2 (1, 291) = 8.96, $p = .003$, QICC = 568.25, see Figure 1. A main effect of condition also revealed that those who were not stressed ($M = 2.24$, $SE = .13$) recalled more words than those who had been stressed ($M = 1.88$, $SE = .11$), Wald χ^2 (1, $N = 291$) = 3.43, $p = .06$, QICC = 568.25, see Figure 1. There were no interactions.

Discussion

The results showed three notable effects on memory after a two-day delay. First, arousing words were recalled at higher rates than neutral words—a finding that is consistent with the literature (see Buchanan & Adolphs, 2002 for a review). Second, women recalled more words than men, consistent with findings that women often perform better than men in tasks involving word list recall and verbal memory (e.g., Kail Jr. & Siegel 1978; Kimura and Seal, 2003, Mann, Sasanuma, Sakuma, & Masaki, 1990).

Surprisingly, however, delayed recall was better in the control condition than in the stress condition, a finding that is at odds with prior reports of stress-enhanced declarative memory. This is especially noteworthy given that our procedure was extremely similar to other studies in the literature (e.g., Cahill et al., 2003). However, most prior studies used pictorial stimuli, so it is possible that the stress impairment of recall found here was due to the use of non-related word lists. Although others have used word lists in prior studies (e.g., Payne et al., 2002; Smeets et al., 2008), they used lists designed for false memory studies, such that words in a given list were all related to one central concept, or critical lure. Thus, recall in those studies should have strongly reflected relational processing. It is possible that if we had used different stimuli, such as related word lists, pictures, or videos (as in Beckner et al., 2006), we might have found a stress enhancement.

A second possibility for these results is the use of a shorter time period—one minute—for the stress induction than the typical time (three minutes) used in other research (e.g., Cahill et al., 2003). It is possible that the shorter stress induction did not allow the stress to fully develop. While self-report data showed that the two conditions differed significantly in stress, self-report measures are not always ideal. Experiment 2, therefore, included a 3-minute stress induction as well as salivary cortisol measurements of stress. Additionally, it allowed us to determine the reliability of the finding that, rather than enhancing long term memory, stress can lead to significantly poorer long term recall.

Experiment 2

Experiment 1 provided evidence that stress is detrimental to memory consolidation when unrelated words lists are used and exposure to the stressor is brief. It is unclear, however, whether the cold pressor task was effective in inducing actual physiological changes in stress in addition to the observed changes in self-reports of stress. Thus, Experiment 2 involved the collection of cortisol at two time points—both before and 15 minutes after the stressor. Self-report of discomfort was not collected, because experiment 1 showed the ice water to be arousing, and it is possible that the self-report procedure caused participants to

associate stress exclusively to the stress task and not to viewing the experimental stimuli. The mood literature has shown that if participants attribute their mood to a mood manipulation procedure, typical experimental findings disappear or even reverse (Schwarz & Clore, 1983). If giving self-reports of stress causes participants to associate or attribute their stress to the ice water task, it is possible that an analogous effect would occur; in fact, this may explain why the findings from experiment 1 (that non-stressed participants recall more) differ from the typical findings. This possibility is discussed further in the general discussion.

In addition, to be more consistent with prior studies, which typically used pictorial stimuli, Experiment 2 was designed to be a close replication of Cahill et al. (2003), a well-known study of stress-induced arousal and memory consolidation.

Method

Participants—One hundred and thirty-one undergraduates (63 aroused, 68 control; 69 women, 62 men; M age = 18.47) volunteered to participate for partial course credit. As in Experiment 1, those with medical conditions where a stress induction could be harmful were not allowed to participate.

Stimuli—Stimuli consisted of 31 pictures (11 neutral, 10 positive, 10 negative) taken from IAPS (International Affective Picture System; Lang, Bradley & Cuthbert, 2005). The pictures were chosen so that negative, neutral, and positive pictures differed in valence ($M = 2.28, 4.94, 7.85$, respectively; $F(2, 28) = 707.29, p < .001$), with negative ($M = 4.78$) and positive pictures ($M = 4.75$) being equivalently high in arousal and more arousing than neutral pictures ($M = 2.25$; $F(2, 28) = 113.34, p < .001$). In addition, they were chosen to be similar in arousal and valence ratings to those used by Cahill et al. (2003).

Procedure—All materials and procedures were approved by the IRB of the University of Virginia, and informed consent was obtained from all participants. To reduce variability due to diurnal changes in cortisol, all participants were tested between the hours of 12 and 5 pm. Participants were contacted via email the day before the experiment and asked to refrain from drinking alcohol for 12 hours prior to the experiment and from eating a large meal or consuming products with high dairy, acidity, or caffeine 60 minutes prior to the experiment, as these factors have been shown to influence cortisol samples (King, Munisamy, de Wit, & Lin, 2006; Lemmens, Born, Martens, Martens, & Westterp-Plantenga, 2011; Lovallo et al., 2005; Nicolson, 2007; Witbracht, Van Loan, Adams, Keim, & Laugero, 2013). After signing the consent form, participants washed their hands and rinsed out their mouths with water. Participants then placed a synthetic salivette swab under their tongue for two minutes to saturate the swab with saliva. They then placed the swab in the salivette tube, which was stored at -20°C until it was assayed.

As menstrual cycle and oral contraceptive use has been shown to affect cortisol reactivity to stress (see Kudielka & Kirschbaum, 2005, for a review), women were asked additional questions (such as the date of their last menstruation, typical menstrual cycle length, and oral contraceptive use) to determine menstrual cycle stage.

Next, participants entered a small testing booth. A computer presented each picture in random order, except that one particular neutral picture (a lamp) always appeared first. Each picture was shown for 15 seconds with a 0 ms inter-stimulus interval. In order to ensure that participants were paying attention, 5 seconds into the presentation of each picture, the computer gave a prompt to generate and type a name or short phrase for that picture. Immediately after exposure to the lists, participants exited to the main room and underwent the cold pressor stimulation described in Experiment 1, but for a duration of three minutes instead of the one minute in Experiment 1.

Participants were told to return in 48 hours, ostensibly to view more stimuli. Approximately 48 hours later, they returned for session 2 and were given a surprise recall test. They were instructed to recall, in no particular order and with no time constraint, all of the pictures they could remember seeing two days prior by using the name or phrase they had generated, and also to record any details they could recall about each picture.

The saliva samples were sent to the General Clinical Research Center (GCRC) lab at the University of Virginia, where they were stored at -20 °C until analysis. After thawing, saliva samples were centrifuged at 1500 g for 15 minutes, which resulted in a clear supernatant of low viscosity. Cortisol levels were determined employing Enzyme-Linked Immunosorbent Assay (ELISA) methodology. 96-well-Maxisorb microtiterplates were coated with monoclonal mouse anti-cortisol antibodies. All reagents were brought to room temperature and mixed before use. Plates were brought to room temperature and prepared for use with NSB (non-specific binding) cells. Each tube was prepared with 24 mL of assay diluents, and 25 mL of standards, controls, and saliva samples were pipetted into the appropriate wells. The assay diluents were pipetted into zero and NSB wells. A final 1:1600 dilution of conjugate (15 mL into 24mL assay) was mixed and 200mL were added into each well. Each plate was mixed for 5 minutes at 500rpm, and was incubated for 55 minutes at room temperature. The plates were then washed 4 times with 1X wash buffer (100 mL of 10X wash buffer concentrate mixed with 900 mL of deionized H₂O), and blotted. 200mL of TMB substrate solution was added to each well, and plates were mixed for 5 minutes at 500rpm. They were then incubated in dark at room temperature for 25 minutes. 50mL of stop solution was then added to each well, and mixed for 3 minutes at 500rpm. With a computer-controlled program a standard curve was generated and the cortisol concentration of the samples was calculated. The intra-assay coefficient of variation was 8 and the corresponding inter-assay coefficient of variation was 2.7.

Results

Cortisol—The samples of three participants were not able to be assayed due to insufficient saliva. There was no difference between the ice water ($M = 5.56$ nmol/L, $SD = 4.02$) and warm water ($M = 5.87$, $SD = 4.78$) groups in baseline cortisol, but the change in cortisol 15 minutes after the CPS task was significantly higher in the ice water group ($M = +1.99$ nmol/L, $SD = 3.71$) compared to the warm water group ($M = +.02$, nmol/L, $SD = 2.56$), $t(129) = 3.52$, $p = .001$. Baseline and post-stress cortisol did not differ as a function of gender.

Picture Recall—Recall was analyzed with the factors of picture type, gender, and stress condition in a normal identity generalized estimating equation. As positive and negative pictures did not differ from each other in recall, they were collapsed into one “arousing” picture category.

Condition: Those who were not stressed ($M = 3.14$, $SE = .13$) recalled more pictures than those who had been stressed ($M = 2.64$, $SE = .13$), Wald $\chi^2(1, N = 254) = 5.25$, $p = .02$, QICC = 653.60, see Figure 2. As in prior studies (e.g., Cahill, et al, 2003), a Pearson product correlation revealed that memory performance was not correlated with cortisol (see Table 1), all $ps > .10$.

Gender: As in Experiment 1, women ($M = 3.24$, $SE = .13$) recalled more pictures than men ($M = 2.53$, $SE = .14$), Wald $\chi^2(1, N = 254) = 10.70$, $p < .01$, QICC = 653.6, see Figure 2. Women reported the date of their last period, and also how long their cycle typically lasts. Many women, however, either did not answer the question about cycle length, or responded with numbers (e.g., “4-5”) that indicated that they misinterpreted the question to be about length of menstruation rather than overall cycle length. For these women, menstrual cycle was computed using a chart for unknown cycle length (S.S. Dickerson, personal communication, Oct 30, 2009). Thus, in Experiment 2, of the women who reported cycle data, 18 women were in menses (6 stress, 11 control), 17 women were in the follicular stage (12 stress, 5 control), 12 were in the follicular/luteal phase (6 stress, 6 control), and 20 were in luteal phase (8 stress, 12 control). In addition, 24 (13 stress, 11 control) women were taking oral contraceptives, and 45 (20 stress, 25 control) women were not. Analyses of menstrual cycle, oral contraceptives, cortisol changes, and their interactions showed no significant influences on recall and so are not further discussed.

Affect: Arousing pictures ($M = 3.99$, $SE = .13$) were recalled more than neutral pictures ($M = 1.79$, $SE = .13$), Wald $\chi^2(1, N = 254) = 215.88$, $p < .001$, QICC = 653.6.

Discussion

Experiment 2 replicates and extends Experiment 1. Experiment 2 included cortisol measures and pictures rather than word lists as stimuli. Despite the use of the same kinds of complex stimuli as in previous studies and the longer duration of cold pressor stress, control participants again recalled more pictures than stressed participants. In addition, women again recalled more than men, and arousing stimuli were recalled better than neutral stimuli. Cortisol measures showed that the ice water did indeed stress participants.

Experiment 3

Experiments 1 and 2 provided evidence that stress can impair memory consolidation. It is possible, however, that experiencing the ice water immediately after seeing the pictures interfered with participants’ ability to review and rehearse what they had seen. They may have been able to continue to think about the stimuli in the warm water condition—the water was comfortable and hence not especially distracting, and holding one’s arm in it should not have been attention demanding. The ice water, however, was quite uncomfortable, may have been distracting, and certainly required effort to keep one’s arm immersed. Perhaps,

therefore, those in the warm water condition continued to think about the stimuli, whereas those in the ice water condition were unable to do so. Experiment 3 addressed this possibility by having all participants count the time aloud while their arm was in water, a task demanding enough to prevent either group from engaging in mental practice, but not so demanding as to dampen the arousing potential of the ice water. In addition, both groups were given one minute after seeing the pictures to rest and think about them before the stress task.

If interference with mental rehearsal in the ice water condition is not the reason for the superior performance of the control condition, then the results from Experiment 3 should replicate those from Experiments 1 and 2. If, however, holding one's hand in the ice water does interfere with mental rehearsal, then stress effects should disappear or be replaced by consolidation effects such that in comparison to the warm water group, the ice water group shows superior rather than inferior memory.

Method

Participants—One hundred and twenty-seven undergraduates (69 women, 58 men; 64 stress, 63 control, M age = 18.88) volunteered to participate for partial course credit. As in prior experiments, those with conditions where stress inductions would be inadvisable were not allowed to participate.

Stimuli—Stimuli were the same as in Experiment 2, except that no positive pictures were used as there was no difference in recall between negative and positive pictures in Experiment 2.

Procedure—All materials and procedures were approved by the IRB of the University of Virginia, and informed consent was obtained from all participants. The procedure is the same as in Experiment 2, except: (1) all participants rested for one minute before undergoing the cold pressor task; (2) participants were given a stopwatch and instructed to count the seconds out loud while their arm was in water, and (3) cortisol was additionally collected at a third time point, 25 minutes after the CPS task, as cortisol responses are typically higher between 20-30 minutes post-stress (Dickerson & Kemeny, 2004).

The saliva samples were sent to the lab of Dr. Andrea Gierens at Universiteit of Trier, Germany, a lab that is noted for its continued rigor and research in cortisol analysis (see Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005, for an example of assay work investigating the influences of oral stimulants on cortisol assays). Samples were stored at -20 °C until analysis. After thawing, saliva samples were centrifuged at 2000 g for 10 minutes, which resulted in a clear supernatant of low viscosity. 100ul of saliva were used for duplicate analysis. Cortisol levels were determined employing a competitive solid phase time-resolved fluorescence immunoassay with fluoroimetric end point detection (DELFI). 96-well-Maxisorb microtiterplates were coated with polyclonal swine anti-rabbit immunoglobulin. After an incubation period of 48h at 4°C plates were washed three times with wash buffer (pH=7.4). In the next step the plates were coated with a rabbit anti-cortisol antibody and incubated for 48h at 4°C. Synthetic saliva mixed with cortisol in a range from 0-100nmol/l served as standards. Standards, controls (saliva pools) and samples were given

in duplicate wells. 50µl of biotin-conjugated cortisol was added and after 30min of incubation the non-binding cortisol / biotin-conjugated cortisol was removed by washing (3x). 200µl europium-streptavidin (Perkin Elmer, Lief science Turku, Finland) was added to each well and after 30 minutes and 6 times of washing 200µl enhancement solution was added (Pharmacia, Freiburg, Germany). Within 15 minutes on a shaker the enhancement solution induced the fluorescence which can be detected with a DELFIA-Fluorometer (Wallac, Turku, Finland). With a computer-controlled program a standard curve was generated and the cortisol concentration of the samples was calculated. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% -9.0%.

Results

Cortisol—The saliva samples of seven participants could not be assayed due to insufficient saliva. There was no difference between the ice water ($M = 4.34$ nmol/L, $SD = 2.40$) and warm water ($M = 4.73$ nmol/L, $SD = 3.29$) groups in baseline cortisol, but the increase in cortisol 15 minutes after the cold pressor task was significantly higher in the ice water group ($M = +2.95$ nmol/L, $SD = 4.48$) compared to the warm water group ($M = -.37$ nmol/L, $SD = 2.61$), $t(117) = 4.86$, $p < .001$. The ice ($M = +4.21$ nmol/L, $SD = 7.82$) and warm ($M = -.66$ nmol/L, $SD = 2.71$) water groups also showed large differences in cortisol change 25 minutes after the cold pressor, $t(118) = 4.48$, $p < .001$.

Picture Recall—Recall was analyzed with the factors of picture type (affect), gender, and stress in a normal identity generalized estimating equation.

Condition: Those who were not stressed ($M = 4.06$, $SE = .14$) recalled more pictures than those who had been stressed ($M = 3.64$, $SE = .14$), Wald $\chi^2(1, N = 254) = 4.04$, $p = .07$, QICC = 618.67, see Figure 3. A Pearson product correlation revealed that memory performance was not correlated with cortisol (see Table 2), all $ps > .40$.

Gender: Cortisol did not differ for men and women at baseline or after stress. As in Experiments 1 and 2, women ($M = 4.16$, $SE = .14$) recalled more pictures than men ($M = 3.55$, $SE = .15$). Wald $\chi^2(1, N = 254) = 7.01$, $p < .01$, QICC = 618.67 (see Figure 3). In Experiment 3, the question about average cycle length was explained in more detail to participants, and most participants gave answers (e.g., “28”, “33”) that indicated they understood the question. Thus, of those who reported cycle data, there were 11 women in menses (6 stress, 5 control), 18 women in the follicular stage (11 stress, 7 control), and 37 in the luteal stage (16 stress, 21 control). In addition, 27 women (13 stress, 14 control) were taking oral contraceptives, and 41 (21 stress, 20 control) were not. Analyses of menstrual cycle, oral contraceptives, cortisol changes, and their interactions showed no significant influences on recall and so are not further discussed.

Affect: As in Experiments 1 and 2, negative ($M = 5.18$, $SE = .14$) items were recalled more than neutral ($M = 2.53$, $SE = .14$), Wald $\chi^2(1, N = 254) = 247.12$, $p < .001$, QICC = 618.67.

Discussion

The results of Experiment 3 suggest that the consistent superiority in recall by the warm water group was not due to differences in the opportunity to rehearse the learned material during the arm immersion task. In this experiment, both groups were encouraged to think about the stimuli for 1 minute before the arousal manipulation, and both groups were mentally engaged in the counting task, and thus likely prevented from thinking about the pictures while their arms were immersed in water. Nevertheless, the warm water group continued to recall more pictures than the ice water group. As in previous studies, women also recalled more than men.

General Discussion

In each of three experiments, stress immediately after learning interfered with (rather than enhanced) long term memory for what had been learned. The control experience of holding one's arm in warm water led to significantly better recall two days later than did the stress experience of holding one's arm in ice water. This result is surprising because some previous studies using the same methodology have reported that stress improved memory after learning. The current results were robust, however, in that they survived variations in *what* material was learned (words vs. pictures), *how* stress was measured (self-report vs. cortisol), *when* the stress occurred (0 vs. 1-minute post-learning), *who* the participants were (males vs. females), and *whether* post-learning rehearsal was possible.

We did find a memory benefit for arousing stimuli (arousing vs. neutral words and pictures), replicating a well-established finding (see Buchanan & Adolphs, 2002 for a review). There was not, however, the previously found interaction of stress with arousing stimuli (e.g., Cahill et al., 2003). Our results indicate that arousing stimuli were better remembered than neutral stimuli, but that stress-induced arousal itself interfered with, rather than enhanced, memory.

Control participants consistently recalled more than stressed participants. These results, consistent as they are, do not necessarily indicate that prior studies were in error, of course. Three possible reasons for these effects are discussed below. Stress and memory have often been described using an inverted U-shaped function, wherein low and extreme levels of arousal are detrimental to memory performance, but moderate levels are beneficial. Indeed, Andreano and Cahill (2006) demonstrated a quadratic relationship between cortisol and memory in men. Perhaps our participants were not at a medium, optimal level of arousal? This explanation, however, is unlikely, as the changes in cortisol for the stress groups (+ 1.99 nmol/L in Experiment 2, +2.95 nmol/L in Experiment 3) were consistent with the idea that stress levels were in the moderate range. Furthermore, the changes in cortisol as a result of the stress manipulation in Experiments 2 and 3 were not correlated with memory performance; i.e., those with a relatively large or relatively small change in cortisol did not perform differently than those with a moderate change. Rather, memory performance differed as a function of undergoing the stress manipulation or not. There is no evidence in these or other studies using cold pressor stimulation that arm immersion in ice water results in extremely high stress levels.

The warm water was at a very pleasant temperature. Although the warm water condition was the same as in other studies (e.g., Cahill et al., 2003) finding stress-related memory enhancement, it is possible that the sensation of placing an arm in bath-water-like temperature is actually pleasurably arousing—it feels good. If this is the case, the warm water group might have experienced arousal without increases in cortisol so that instead of high vs. low arousal groups, we compared positive arousal versus negative arousal groups. To examine this possibility, we asked 15 additional participants to place their arm in warm water for 3 minutes, and then to rate their happiness, how pleasant the water was, how positive the water was, how stimulating/exciting, and how arousing the water was. Participants indicated that they were “neither happy nor unhappy” ($M=4.1$ on a 7-point scale), “neither pleasant nor unpleasant” ($M=4.1$), between “neither positive nor negative” and “a little positive” ($M=4.4$), “a little calm/relaxed” ($M=2.8$), and between “a little unaroused” and “neither aroused nor unaroused” ($M=3.5$). Thus, the warm water appears to be an ideal control condition, as it induces neither strong positive nor negative mood/affect, and is not arousing. As the warm water is not pleasurably arousing, heightened arousal cannot account for better memory in the control condition.

A second possibility involves suppression of stress. It could be that participants used their available resources to suppress or change their stress. Devoting resources to suppressing emotion has been found to negatively affect memory (Richards & Gross, 2000). However, such effects have been observed only in studies in which individuals were specifically instructed to suppress their emotion, which was not the case in the current studies.

A third possibility seems especially plausible. Studies of mood and cognition have shown that when people are made aware of the source of their mood, typical effects disappear or often reverse when affective reactions are not experienced as relevant to (attributed to) the task at hand (e.g., Schwarz & Clore, 1983). Similarly, anything that draws attention to the source of affect, arousal, or stress, or that psychologically separates the induction experience from the task experience, should eliminate the effects of affect, stress, or arousal.

This attribution account is a possible explanation for the surprising results of these studies. The most well-known studies demonstrating that arousal can be misattributed come from Zillman and colleagues (Cantor, Bryant, & Zillman, 1974), where arousal from irrelevant sources influenced performance on various tasks. In the current studies, while the stress manipulation and the learning procedure occurred very close in time, it is possible that participants attributed the stress they felt to the manipulation and not to the stimuli to be learned. This may have occurred because space limitations in all three experiments (i.e., very small testing rooms) dictated that all participants had to exit the small testing room after viewing the stimuli and enter a different room to immerse their arm in water, a procedure that, to the authors' knowledge, is unique to these studies. Standing up and moving to a different room may have made the water manipulation stand out as distinct and separate from the learning task. Indeed, merely walking through a doorway in between encoding and recall phases of a task has been shown to impair recall (Radvansky, Krawietz, & Tamplin, 2011). Thus, participants may have experienced their stress as associated with the ice water only rather than with the stimuli to be learned. If this were the case, then, as in the mood literature, we might expect to see a pattern like the one we observed—a reversal

of the typical findings. Future research could test this attribution account by having one group of participants in the stress condition remain in the testing room after viewing stimuli, and another group of participants in the stress condition leaving the room to undergo the stress manipulation.

Diamond and colleagues (see Diamond, Park, & Woodson, 2004; Diamond, Park, Campbell, & Woodsen, 2005, for reviews) have proposed an interesting mechanism related to this attribution possibility: that Long Term Potentiation induction from intense or extreme learning experiences, such as exposure to fear, can cause retrograde amnesia for previously learned, unrelated information. Although participants in our studies experienced moderate stress rather than the intense fear described by Diamond and colleagues, it is possible that similar mechanisms could explain our findings.

These results indicate that the requirements for stress to enhance memory are less clear and may be more restrictive than previously believed. The critical condition under which post learning arousal will enhance or interfere with memory is not yet clear, and poses a conceptual problem worthy of further attention. Whereas the neuro-chemical basis of arousal-produced memory consolidation appears to be understood, these findings suggest that the psychological conditions of this important phenomenon are still under-specified. Especially intriguing is the possibility that the key lies in the way in which participants punctuate their own experience: so that arousal is either linked with the to-be-remembered material, or is experienced as separate and distinct.

Overall, these results provide consistent evidence that stress does not uniformly enhance memory consolidation. Although prior research has shown that stress during recall can interfere with memory, the current experiments obtained evidence of interference when stress was introduced after learning and participants had returned to baseline levels of arousal before recall. This is the first evidence of which the authors are aware that stress can actually impair consolidation of declarative memories. We tested several hypotheses for these effects, including those concerned with stimulus type, rehearsal, gender, hormonal influences (from menstrual cycle and oral contraceptive use), and opportunity for post-encoding processing. Nevertheless, we continued to obtain the same robust finding that stress-induced arousal impairs long term memory.

Acknowledgments

Support is acknowledged from NIMH Research Grant R01 MH 50074 to the second author.

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Figure 1.
Word recall as a function of stress and gender.

Figure 2.
Picture recall as a function of stress and gender.

Figure 3.
Picture recall as a function of stress and gender.

Table 1

Correlation of Cortisol and Recall

	Correlation with Picture Recall		
	Overall	Stress	Control
Baseline	.04	.12	-.03
Post-Stress	-.02	.14	.10
Post-Stress - Baseline	-.07	-.03	.12

Table 2

Correlation of Cortisol and Recall

	Correlation with Picture Recall		
	Overall	Stress	Control
Baseline	-.04	-.07	-.02
15-min Post-Stress	-.04	.00	-.03
25-min Post-Stress	-.02	.03	-.02
15-min - Baseline	-.01	.05	.00
25-min - Baseline	.00	.05	.01