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Learn. Mem. 2006 13: 441-450; originally published online Jul 17, 2006;
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Effects of stress and sex on acquisition and consolidation of human fear conditioning

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We examined the relationship between stress hormone (cortisol) release and acquisition and consolidation of conditioned fear learning in healthy adults. Participants underwent acquisition of differential fear conditioning, and consolidation was assessed in a 24-h delayed extinction test. The acquisition phase was immediately followed by an 11-min psychosocial stress period (arithmetic test combined with a public speech). Salivary cortisol was sampled at various time points before and after acquisition and retention of fear conditioning. Results showed two effects of endogenous cortisol. Post-acquisition cortisol correlated with fear acquisition in male but not female participants. In addition, post-acquisition cortisol correlated with consolidation of fear but only in those participants with high cortisol levels. We conclude that in the short term, a robust and sexually dimorphic relationship exists between fear learning and stress hormone levels. For those participants whose fear learning is accompanied by high stress hormone levels, a long-term relationship exists between cortisol release and memory consolidation. These short-term and long-term effects may relate to the differential involvement of mineralocorticoid and glucocorticoid receptor subtypes, respectively. The findings have implications for understanding the role of stress, sex, and hormones in different stages of fear learning and memory.

Fear conditioning constitutes an adaptive cognitive mechanism in most organisms, allowing them to effectively learn about danger-relevant relationships in the environment and thus enhancing their survival chances. In a typical fear conditioning procedure in the laboratory, a previously neutral conditioned stimulus (CS) predicts the occurrence of an aversive unconditioned stimulus (US) and, as a result, acquires emotional properties. Conditioning is established when the CS triggers a conditioned response in the form of arousal reactions. An increased release of adrenocortical stress hormones—glucocorticoids—through activation of the hypothalamic-pituitary-adrenal (HPA) axis represents an important physiologic component of such arousal responses to fearful stimuli. The principal glucocorticoid in humans is cortisol.

Basal and stress levels of glucocorticoids vary across individuals (Plazas et al. 1991; Meaney et al. 1993; Cools and Gingras 1998; Kirschbaum et al. 1999; Kabaj et al. 2000; Stone et al. 2001; Bartels et al. 2003; Rohleder et al. 2003; Steptoe et al. 2003) and play an important but complex role in learning and memory (McEwen and Sapolsky 1995; Lupien and McEwen 1997; Roozenaal 2003; Wolf 2003). While cortisol may be detrimental to memory function under some circumstances—i.e., when levels are elevated chronically (Starkman et al. 1992; Luine et al. 1993, 1994; Arbel et al. 1994; Bodnoff et al. 1995; Conrad et al. 1996; Newcomer et al. 1999; Park et al. 2001) or during memory retrieval (De Quervain et al. 1998, 2000; Buss et al. 2004)—they also have acute facilitative effects, presumably constituting part of the underlying neurobiological mechanism responsible for enhanced learning and memory of emotionally relevant material. Acute memory-enhancing effects of glucocorticoids may operate at two stages of memory processing, the initial acquisition (encoding) stage and the delayed consolidation stage. Positive relationships between acute cortisol levels and immediate effects on learning and memory are reflective of the former, whereas relationships with delayed retention imply either the former or the latter, although selective consolidation effects are inferred when cortisol is manipulated after the memory encoding task has terminated.

Studies in both humans and experimental animals have found that high endogenous baseline levels or exogenous administration of cortisol or stress prior to a memory task is associated with better immediate performance, implying beneficial effects on memory acquisition or encoding (Wilson et al. 1975; Beckwith et al. 1986; Shors et al. 1992; Fehm-Wolfsdorf et al. 1993; Bemelsmans et al. 2002; Domus et al. 2002; Lupien et al. 2002; McGowan et al. 2001; Abercrombie et al. 2003; Akirav et al. 2004; Putman et al. 2004; Maheu et al. 2005b; Zorawski et al. 2005). However, the issue is not unequivocal, and contradictory results have been reported (Wolkowitz et al. 1990; Kirschbaum et al. 1996; Lupien et al. 2005b; Zorawski et al. 2005). The interaction of several variables may be responsible for the discrepant findings, including the proposed inverted U-shaped dose-response function of glucocorticoids (Kovacs et al. 1977; Diamond et al. 1992; Lupien and McEwen 1997; Conrad et al. 1999), the distinct roles of different receptor types (Veldhuis et al. 1982; Oitzl and de Kloet 1992; Lupien and McEwen 1997; de Kloet 2004), the natural diurnal variation of endogenous cortisol levels (Fehm-Wolfsdorf et al. 1993; Lupien et al. 1999, 2002a; Maheu et al. 2005a), and procedural factors such as the timing of the retention test in relation to the learning phase. If the retention test is arranged temporally close to acquisition, possibly facilitative acquisition effects may be masked by the impairing effects on memory retrieval (De Quervain et al. 1998, 2000).

Hormonal manipulations affect individuals differently, and one source of across-subject variance is biological sex. (Shors et al. 1992) found that stress facilitated subsequent classical conditioning in male rats both 24 h later and immediately afterwards (Shors 2001), whereas detrimental effects were found in female
Glucocorticoids bind to two types of receptors: the low-threshold high-affinity mineralocorticoid receptors (MRs), which are largely occupied by endogenous glucocorticoids under basal conditions, and the high-threshold low-affinity glucocorticoid receptors (GRs), which primarily get activated under stress or during the circadian peak (Reul and de Kloet 1985; de Kloet et al. 1993; Reul et al. 2000). It has been suggested that the two receptor types subserving different cognitive functions (Lupien and McEwen 1997; Oitzl et al. 1997). MRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993). GRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993). GRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993). GRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993). GRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993). GRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993). GRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993). GRs are thought to mediate non-specific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1993; Sandi and Rose 1993).
habituation, late acquisition, extinction) as repeated measures. This analysis focused on the late block trials in the habituation and acquisition phases since we were interested in comparing performance on the retention test to that obtained at the end of habituation and acquisition training. A significant CS type by Phase interaction was found, $F_{(1,33)} = 4.77, P < 0.02$. Simple effects analysis revealed greater responding to the CS+ than to the CS− in late acquisition, $F_{(1,33)} = 6.87, P < 0.02$, and extinction, $F_{(1,33)} = 6.40, P < 0.02$, but not in habituation, $F < 1$. The factor Sex was not significant either as a main effect or as an interaction with the other variables ($Ps > 0.05$).

Unconditioned responding
SCRs to the US did not differ between female ($M = 1.02, SD = 0.33$) and male ($M = 1.06, SD = 0.51$) participants ($t < 1$), which shows equivalent unconditioned responding to the shock as a function of sex.

Subjective measures
While males selected a higher voltage level of the shock than females (females, $M = 64.67$ V, $SD = 19.50$; males, $M = 89.50$ V, $SD = 15.72; t_{(1,33)} = -4.17, P < 0.001$), they did not differ in the perceived subjective irritation of the shock (females, $M = 4.27, SD = 1.28$; males, $M = 3.75, SD = 1.29; t_{(1,33)} = 1.18, P = 0.25$). Females and males also did not differ in their perceived emotionality of the snake and spider images (females, $M = 2.20, SD = 1.32$; males, $M = 2.00, SD = 1.34; t < 1$) nor in the perceived stressfulness of the study overall (females, $M = 3.53, SD = 1.77$; males, $M = 2.95, SD = 1.50; t_{(1,33)} = 1.05, P = 0.30$). Female participants reported higher levels ($M = 2.83; SD = 0.80$) of perceived stress between sessions than males ($M = 2.26; SD = 0.76$) as measured by the Daily Stress Inventory (DSI; see Materials and Methods), $t_{(1,33)} = 2.17, P < 0.04$.

Conditioning, cortisol, and sex
Behaviorally, conditioning was best observed in the late phase of acquisition, as predicted. Given the temporal dynamics of saliva cortisol assessment (see Materials and Methods), the most appropriate time point to link endogenous cortisol to the late portion of acquisition training/stress induction was Sample 4, obtained about 45 min after the beginning of conditioning (and about 30–35 min after late acquisition). The relationship among sex, post-acquisition cortisol (Sample 4), and fear conditioning was analyzed in two ways—first, by median-split analysis (dividing participants into high vs. low stress groups according to cortisol values obtained from Sample 4) and second, by correlational analyses conducted on individual participant data. A three-way mixed ANOVA with Sex (female, male) and Cortisol group (high, low) as between-groups factors, and Phase (late acquisition, extinction) as a repeated measure revealed a significant three-way interaction, $F_{(1,40)} = 6.80, P < 0.02$ (see Fig. 2). Follow-up ANOVAs for each sex revealed a significant interaction of Cortisol group and Phase in males, $F_{(1,18)} = 15.11, P < 0.01$, but not in females. Simple-effects analysis revealed greater conditioned responding in high- than in low-cortisol males during late acquisition, $F_{(1,20)} = 17.83, P < 0.001$, but not in the 24-h delayed retention test, $F < 1$. In females, no differences were found between Cortisol groups in either phase of learning (late acquisition: $F < 1$; extinction: $F_{(1,12)} = 2.55, P = 0.14$). In line with these median-split results, a significant correlation between post-acquisition cortisol and differential conditioned responding during late acquisition was found in males, $r_{(18)} = 0.57, P < 0.01$, but not females, $r_{(13)} = -0.10, P = 0.74$ (Fig. 3). There were no significant correlations between post-acquisition cortisol levels and conditioned responding in the delayed retention test in males, $r_{(19)} = 0.19, P = 0.42$, or females, $r_{(13)} = 0.18, P = 0.53$. A 2 × 4 mixed ANOVA with Sex (female, male) as between-group factor and Cortisol sample (1–4) as repeated measure revealed no significant main effect of Sex ($F < 1$) and no significant interaction, $F_{(3,87)} = 2.06, P = 0.21$, on September 14, 2006.

Figure 1. (A) Mean skin conductance responses (SCR) to the reinforced CS+ and the non-reinforced CS− in females and males across different phases of conditioning and the 24-h delayed retention test. *$P < 0.02$ (CS+ vs. CS−). (B) Differential SCR expressed as the difference between responses to the CS+ and CS−. µS = microSiemens. Error bars reflect SEMs.
showing that there were no sex differences in baseline or post-acquisition cortisol levels.

In conclusion, these data constitute a successful replication of our previous report (Zorawski et al. 2005), revealing a robust relationship between post-acquisition cortisol levels and conditioned fear acquisition in males but not females, but no relationship between cortisol levels and consolidation of fear learning as assessed during the 24-h delayed retention test in either sex.

Verification of experimental stress manipulation on cortisol levels

To assess whether the psychosocial stress procedure increased post-acquisition cortisol levels, cortisol data from this experiment were compared with those of our previous study (Zorawski et al. 2005). The procedure of that study was identical to the current one except that no psychosocial stress induction procedure was carried out after the acquisition phase of conditioning. It therefore served as a control condition. A three-way mixed ANOVA with Group (previous study, current study) and Sex (female, male) as between-groups factors, and Cortisol sample (1–4) as a repeated measure, was carried out (Fig. 4). Results showed a significant Group by Cortisol sample interaction, $F_{(1,213)} = 3.86$, $P < 0.01$. Simple effects analysis revealed that post-acquisition cortisol levels were significantly higher in the current study for Sample 4, $F_{(1,73)} = 10.36$, $P < 0.01$, but not for the other samples (all $F$s < 1). It is important to note that there were no baseline cortisol differences between experimental groups. The selective increase in cortisol taken at time point 4 for participants in the current study demonstrates that the psychosocial stress procedure increased post-acquisition cortisol levels overall.

Cortisol, sex, and fear memory consolidation: Individual difference analysis of pooled data

Although the psychosocial stress procedure reliably increased post-acquisition cortisol levels in the current study, these participants on average did not exhibit better consolidation as measured by 24-h delayed retention. However, individual differences in endogenous cortisol levels may have interacted with the effects of the psychosocial stress procedure. To increase statistical power to observe a relationship between stress hormone levels and fear memory consolidation, we pooled data across our previous study (Zorawski et al. 2005) and the current study. Participants were divided into high- and low-cortisol groups by a median split of the pooled sample according to post-acquisition cortisol levels (Sample 4), and then Pearson correlation coefficients were conducted between post-acquisition cortisol levels and conditioned fear retention within each split group (Fig. 5). This procedure allows an analysis of the relationship between post-acquisition cortisol levels and consolidation of fear learning as a function of cortisol irrespective of the circumstances under which different cortisol levels occurred (e.g., endogenous cortisol levels for participants in Zorawski et al. [2005] vs. additional psychosocial stress induction in the current study). Fifty-six percent of participants in the high-cortisol group and 31% of participants in the low-cortisol group came from the current study. The analyses revealed a positive correlation between post-acquisition cortisol and conditioned fear retention in the high-cortisol group, $r_{(37)} = 0.35$, $P < 0.04$, but not in the low-cortisol group, $r_{(37)} = -0.09$, $P = 0.60$. In other words, we found a linear relationship between post-acquisition cortisol levels and 24-h delayed fear memory retention, but only for those participants exhibiting high stress levels at the end of acquisition training (irrespective of whether the cortisol levels were endogenously generated or experimentally induced). To determine whether this hormonal memory relationship was sexually dimorphic, we subdivided the high cortisol group by sex. Pearson correlation coefficients did not reveal a significant correlation between post-

Figure 2. Differential skin conductance responses (SCRs), expressed as the difference between CS+ and CS−, during late acquisition and the 24-h delayed retention test in females and males as a function of cortisol level. The participants were assigned to high- and low-cortisol groups according to a median split of post-acquisition salivary cortisol levels (+45 min). *$P < 0.001$; n.s. = not significant. Error bars represent SEMs.

Figure 3. Correlation between differential skin conductance responses (SCRs), expressed as the difference between CS+ and CS−, during late acquisition of conditioning and post-acquisition salivary cortisol levels (+45 min) in female (A) and male (B) participants.
acquisition cortisol and conditioned fear retention for females \( (P = 0.39) \), but there was a trend for significance in males \( (P = 0.06) \). However, the evidence is not sufficient to permit a sex-specific interpretation of this relationship, perhaps due to insufficient statistical power. The possibility that the pattern of results arose from carry-over effects from acquisition to retention in high-cortisol males was investigated by correlating acquisition and retention levels in these individuals, but no correlation was found \( (P = 0.19) \).

### Discussion

The current study investigated the role of sex, psychosocial stress, and cortisol in the acquisition and consolidation of fear conditioning. We found a positive relationship between post-acquisition salivary cortisol levels (reflecting levels present at the end of training) and acquisition of fear learning in male but not female participants. This relationship was not attributable to sex differences in US responsivity, baseline cortisol levels, or subjective measures of the study’s aversiveness. Furthermore, we observed a positive relationship between post-acquisition salivary cortisol levels and fear memory consolidation over a 24-h period but only in those participants whose fear learning was initially accompanied by high stress hormone levels.

The sexually dimorphic correlation between cortisol levels and acquisition of fear conditioning in the current study replicates our previously reported findings \( (\text{Zorawski et al. 2005}) \) and fits nicely with the existing animal literature. Wood et al. \( (2001) \) reported that corticosterone levels correlated with fear conditioning in male but not female rats, and Shors et al. \( (1992) \) found that stress enhanced subsequent fear conditioning in male rats, whereas the opposite held in female rats. The latter finding was reversed by treatments disabling the estrogen system, such as ovariectomy or administration of estrogen antagonists \( (\text{Wood and Shors 1998}) \). The beneficial effects of cortisol on fear learning may therefore be affected by estrogen, suggesting complex hormonal interactions in the modulation of emotional memory. Recent research in humans also supports the sex-specific results. Jackson et al. \( (2006) \) found that psychosocial stress induced prior to fear conditioning enhanced learning in males but impaired learning in females. Collectively, the fear conditioning data complement other research showing sex differences in amygdala activation and emotional memory modulation \( (\text{for review, see Cahill 2005}) \).

While our interpretation of the results fits well with the animal literature and theoretical models of the role of glucocorticoids in cognitive processes, putatively involving sexually dimorphic mechanisms \( (\text{e.g., Oitzl and de Kloet 1992; Shors et al. 1992; Sandi and Rose 1994; Lupien and Mclwenn 1997; Oitzl et al. 1997; Shors 2001; Wood et al. 2001; Lupien et al. 2002a}) \), we should nevertheless point out that correlational data do not permit conclusions regarding causality and directionality. For example, we cannot rule out the possibility that stronger aversive reactions to the conditioning procedure caused both better acquisition and higher cortisol levels after training. However, we note that individual differences in cortisol levels were relatively stable—the cortisol levels taken at baseline correlated positively with the post-acquisition levels within subjects in both the current study and Zorawski et al. \( (2005) \) \( (\text{both } P < 0.01) \). Furthermore, the reported subjective level of irritation from the shock did not correlate either with acquisition of conditioning or with post-acquisition cortisol levels in either males or females \( (\text{all } P > 0.17) \).

It may be surprising that overall cortisol levels were not elevated between baseline and post-acquisition time points. This result replicates previous studies of hormones and fear conditioning in humans \( (\text{Jackson et al. 2006; Zorawski et al. 2005}) \). The diurnal fluctuation in cortisol levels is relatively large and may mask task-induced effects, especially for experiments that are run in the afternoon when levels are naturally declining over time. We note that there was an increase from Sample 3 to Sample 4 in the present study \( (\text{Fig. 4}) \), which shows a relative increase in cortisol toward the end of training/stress induction. Because of the sluggishness of the hormonal response, it is difficult to specify a tighter coupling of cortisol levels across different points in training where individuals may vary with respect to peak learning or stress induction. Consequently, it was not possible to distinguish the relative contributions of the fear learning acquired late in acquisition training from the psychosocial stress induction in mediating the post-acquisition stress hormone levels. Nonetheless the sex-specific correlation with fear acquisition is similar to that found in Zorawski et al. \( (2005) \) where no psychosocial stress was induced. Finally, due to ethical constraints the amount of fear elicited in the laboratory is limited to a range where participants report feeling aversive but not painful reactions, which underestimates fear elicitation in real-world settings or some animal models.

The other key finding of the current study was the association between post-acquisition cortisol and fear memory consolidation. Numerous fear conditioning studies in animals and declarative memory studies in humans have proposed a role for glucocorticoids in the consolidation of emotional memory \( (\text{Flood et al. 1978; Oitzl and de Kloet 1992; Sandi and Rose 1994; Roozendaal and McGaugh 1996; Buchanan and Lavoie 2001; Zorawski and Killcross 2002, 2003; Abercrombie et al. 2003; Cahill 2005}) \).

Figure 4. Salivary cortisol levels of participants in Zorawski et al. \( (2005) \) and the current study at various time points on Day 1. Time points are expressed relative to the beginning of the conditioning session. \(* P < 0.01\). Error bars represent SEMs.
We believe that both of the principal findings can be accounted for by putative differential roles of MRs and GRs in cortisol effects on learning and memory (Lupien et al. 1997; Oitzl et al. 1997; Zorawski et al. 2005). MRs are largely occupied under low stress conditions whereas GRs typically become activated under more stressful situations (Reul and de Kloet 1985; de Kloet et al. 1993; Reul et al. 2000). The relative MR/GR occupation ratio of an individual participant would depend on basal cortisol levels and HPA reactivity on the one hand, and the salience of the conditioning and/or psychosocial stress procedures on the other. MRs have been suggested to mediate nonspecific aspects of memory acquisition, such as sensory integration and selective attention (Oitzl and de Kloet 1992; Sandi and Rose 1994; Lupien and McEwen 1997; Oitzl et al. 1997; Lupien et al. 2002a). Since the conditioning procedure itself did not produce any mean increase in cortisol levels (Fig. 4), and since all testing occurred in the afternoon when natural diurnal cortisol is typically low, it is possible that the observed relationship between post-acquisition cortisol levels and acquisition of fear learning was mainly mediated by MRs, with increased occupation leading to increased sensory integration or selective attention, and consequently better fear acquisition (Zorawski et al. 2005). The sex-specificity of this relationship, which occurred in males only, may be due to estrogen selectively affecting MR affinity and availability in females (Wood et al. 2001).

However, when post-acquisition cortisol levels reached a particular threshold, GRs may have become increasingly occupied. As GRs have been specifically implicated in memory consolidation processes (Oitzl and de Kloet 1992; Sandi and Rose 1994; Zorawski and Killcross 2002), high stress at the time of learning could have translated into a relationship between GR occupation levels and memory consolidation. We therefore hypothesize that only within the cortisol level range that involves GR activation would higher cortisol levels predict the amount of delayed retention. Future research should more thoroughly test whether estrogen mediates the relationship between GR activation and consolidation of fear memories.

Finally, the current results may have important clinical implications. Many neuropsychiatric syndromes have been linked to abnormal emotional learning and memory processes, including anxiety (Öhman 1979; Rosen and Schulkin 1998), post-traumatic stress disorder (Brewin 2001; Layton and Kriktorian 2002; Orr et al. 2002), and drug addiction (Everitt et al. 2001; Robinson and Berridge 2003). Abnormal stress hormone levels and stress reactivity may constitute a pathopsychological mechanism of maladaptive emotional learning and thereby contribute to the occurrence of such conditions. Interestingly, some of these conditions have different prevalence rates for men and women (Seedat and Stein 2000; Breslau 2002a). Since the conditioning procedure itself did not produce any sensory integration or selective attention, and consequently better fear acquisition (Zorawski et al. 2005). The sex-specificity of this relationship, which occurred in males only, may be due to estrogen selectively affecting MR affinity and availability in females (Wood et al. 2001).

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Conclusions

Fear conditioning constitutes a vital survival mechanism that allows organisms to rapidly learn about emotional events in their environment and adaptively organize their behavior accordingly. Maladaptive variation in fear learning abilities, however, may contribute to the development of psychopathological syndromes such as anxiety, post-traumatic stress disorder, and addiction. Stress hormones, such as cortisol, play an important role in modulating various aspects of fear conditioning, and some of
these relationships may be sexually dimorphic. A better understanding of the complex neurohormonal interactions among sex, stress, and fear may lead to better prevention and treatment of various forms of psychopathology. This paper contributes to this area of research by reporting a replicable, sexually dimorphic short-term relationship between cortisol levels and fear acquisition, and a long-term relationship between cortisol levels and memory consolidation for individuals whose learning is accompanied by high stress.

Materials and Methods

Participants

Fifty-six healthy adults were recruited via advertising at Duke University and were compensated for their participation. Of these, 35 participants (15 females) aged 18–38 years (males: M = 24.74, SD = 6.16; females: M = 22.87, SD = 3.89) were included in the study. Given the fear-relevant nature of the conditioned stimuli, participants completed a questionnaire assessing attitudes towards snakes and spiders (Klorman et al. 1974). Individuals scoring within one SD of the mean of phobics (Fredrickson 1983) were excluded from analysis, as in Zorawski et al. (2005). This exclusion criterion was established to provide a homogeneous, healthy sample that did not mix phobic and nonphobic individuals. Additional exclusion criteria included excessively low responsiveness to the US, or any self-reported history of psychiatric or neurological disorders, alcoholism, or substance abuse. In addition, participants were omitted due to technical failure or problems with the cortisol analysis. This exclusion criterion was established to provide a homogeneous, healthy sample that did not mix phobic and nonphobic individuals. Additional exclusion criteria included excessively low responsiveness to the US, or any self-reported history of psychiatric or neurological disorders, alcoholism, or substance abuse. In addition, participants were omitted due to technical failure or problems with the cortisol analysis. This exclusion criterion was established to provide a homogeneous, healthy sample that did not mix phobic and nonphobic individuals. Additional exclusion criteria included excessively low responsiveness to the US, or any self-reported history of psychiatric or neurological disorders, alcoholism, or substance abuse.

Procedure and fear conditioning protocol

Participants underwent a differential fear conditioning paradigm, as previously described by Zorawski et al. (2005). A picture of a snake and a picture of a spider obtained from the International Affective Picture System (Lang et al. 2001) served as the conditioned stimuli (CS). For each participant, one of the pictures served as the partially reinforced stimulus (CS+) while the other was not reinforced (CS−). Each stimulus was presented centrally on a 17-inch computer screen (22.5 cm × 21.5 cm; duration: 4 sec), in one of two pseudorandom sequences (inter-trial interval: 14 ± 2 sec). The designation of pictures as CS+ and CS− and the sequence presentation were randomized and counterbalanced across all participants.

An electric shock served as the US. The shock was delivered through an electrode (21 mm electrode spacing, Grass-Telefactor model F-E10S2) placed over the median nerve of the participants’ dominant wrist. The electrode leads were secured by a rubber strap attached to a Grass-Telefactor SD-9 stimulator by way of coaxial cable leads that were shielded and grounded through a radiofrequency filter. Saline-based gel was used as a conductive electrolyte (Parker Laboratories). The shock was 200 ms in duration and coterminated with the CS+. Prior to conditioning, shock level intensity was determined individually for each participant, being regarded as unpleasant and aversive but not painful.

The experiment consisted of two sessions separated by a 24-h period. On the first day, participants provided signed consent to participate in the study and were asked to fill out questionnaires which assessed their attitudes towards snakes and spiders as well as their medical history. Prior to conditioning and in order to put participants in a comparable, relaxed state, they were shown a neutral film clip of a scenic train ride through British Columbia (Highball Productions). Following the clip, participants underwent habituation and acquisition consecutively without a break. The habituation phase consisted of four nonreinforced trials of each CS type. The acquisition phase consisted of 16 trials of each CS type. Five of the 16 CS+ trials were reinforced by cotermination with the US, while CS trials were always unreinforced. Following this procedure, participants reported their subjective experience using 7-point scales, assessing the emotionality of the images presented in the study, the irritation caused by the shock, and the general stressfulness of the procedure. Participants then underwent a psychosocial stress procedure (see below).

On Day 2, participants returned to the lab during the same hours as on Day 1 (2 pm–6 pm) and filled out the Daily Stress Inventory (DSI) to assess the degree of stress they experienced in the last 24 h (Brantley et al. 1987). They then underwent an extinction test consisting of eight non-reinforced, pseudo-randomized presentations of each CS type. Participants were unaware that they would not receive shocks during this session and were connected to the stimulating electrode in the same way as on Day 1. At the end of the session, participants were fully debriefed about the purpose of the experiment and were compensated for their participation.

Task instructions for fear conditioning

Prior to conditioning and extinction, participants were informed that they would be shown pictures of a snake and a spider and that they might receive occasional shocks. In order to keep participants attentive, they were instructed to classify each picture as a snake or spider with their dominant hand, using the number pad of the keyboard. They were also asked to keep their nondominant hand still to avoid movement artifacts in the SCR recordings.

Physiologic measurements

SCR was assessed continuously throughout testing and constituted the dependent measure of learning. Ag-AgCl electrodes were filled with Sigma Gel as a conductive electrolyte and placed on the middle phalanges of the second and third digits of each participant’s nondominant hand (BIO PAC Systems). The responses were monitored at 250 Hz and stored offline using AcqKnowledge Software for subsequent analysis (BIO PAC Systems). The physiologic data were scored according to conventional methods, as previously described (LaBar et al. 1998, 2004).

Cortisol protocol

Cortisol was measured in saliva and collection procedures were identical to those reported in Zorawski et al. (2005). All sessions were conducted in the afternoon (approximately 2 p.m.–6 p.m.) when endogenous levels are typically low, to control for the natural diurnal rhythm of cortisol levels. Participants were asked to refrain from exercise, eating, drinking, chewing gum, and smoking for 30 min prior to the study, as these variables may obscure cortisol measurements. Cortisol samples were obtained at various time points throughout the study. Time points were chosen in consideration of the temporal dynamics of cortisol’s entry into saliva by passive diffusion and the fact that saliva cortisol levels typically peak ~30 min after a stressor (Kirschbaum and Hellhammer 1994). On Day 1, samples were taken ~15 min before (baseline) and 15, 30, and 45 min after the beginning of conditioning; on Day 2, samples were taken ~5 min prior to and 30 min after the onset of extinction.

For saliva collection, participants placed a cotton dental swab, a Salivette (Sarstedt), in their mouth for ~30 sec. Samples were labeled and refrigerated until assayed. It has been shown in numerous studies that saliva cortisol levels approximate free, nonprotein-bound cortisol, that the collection method (stimulated or nonstimulated) only affects the volume collected but not the cortisol concentration (Riad-Fahmy et al. 1982; Kirschbaum and Hellhammer 1994), and that cotton-swabbing constitutes a reliable and accurate measurement of cortisol (Shirtcliff et al. 2001). All of the cortisol samples were subjected to freeze thawing centrifugation and measured by ELISA (Oxford Biomedical Research). The sensitivity of the assay was 0.05 ng/mL. Standard, low, and high controls were run in every assay. Inter- and intra-assay coefficient of variation on the assay was <10% and 5%, respectively.
Psychosocial stress

An 11-min psychosocial stress procedure was employed directly following conditioning on Day 1. The procedure occurred in the same room from conditioning and the electrodes were not removed for this portion of the experiment. The procedure consisted of the paced auditory serial-addition task (PASAT; Gronwall 1977) followed by public speaking. Participants were told that it is important that they take the tasks seriously, do as well as possible, and that their performance would be compared against that of their peers. The PASAT consisted of 7-min arithmetic task in which single-digit numbers flashed on the screen. At each number presentation, participants were required to add the current number to its predecessor. Participants indicated these sums on a number pad on the screen using a computer mouse. A loud noise burst was delivered over headphones immediately after each incorrect response. The PASAT was designed in such a way that the frequency of number presentation increases rapidly, making it virtually impossible to give correct responses during the latter portion of the task. Upon conclusion of the PASAT, participants were provided with false feedback, indicating extremely poor performance relative to their peers.

Directly following the conclusion of the PASAT, participants completed a 4-min public speech task. Participants were knowingly video-taped for this portion of the task and were misinformed that their video would be sent to the principal investigator for evaluation. The content of their public speech consisted of answering questions regarding their performance on the PASAT, how their performance relates to their performance in other activities of their everyday lives, and whether their performance reflects their general intellectual ability. The timing of the psychosocial stress procedure allowed for saliva sample collections at time points comparable to those used by Zorawski et al. (2005). At the end of the session, participants were informed that the arithmetic and the public speech task were not true measures of their ability and were designed for the sole purpose of inducing stress.

Data analysis

Data were analyzed as described in Zorawski et al. (2005). Prior to data analysis, conditioned SCR s were square-root-transformed and range-corrected (LaBar et al. 1995), to normalize the distribution, and collapsed into “early” and “late” trial blocks for habituation and acquisition. Each block consisted of the mean of two trials per CS type for the habituation phase and the mean of eight trials per CS type for the acquisition phase. Extinction trials were not collapsed into early and late blocks, so these data reflected the mean of eight trials per CS type. Conditioning-related changes were hypothesized to occur in the late acquisition phase (LaBar et al. 1995, 2004) while retention performance in the 24-h delayed extinction test was taken as a measure of consolidation (LaBar et al. 1995, 2004) while retention performance in the 24-h delayed extinction test was taken as a measure of consolidation (LaBar et al. 1995). Consequently, data were not collapsed into early and late blocks, so these data reflected the mean of eight trials per CS type. Conditioning-related changes were hypothesized to occur in the late acquisition phase (LaBar et al. 1995, 2004) while retention performance in the 24-h delayed extinction test was taken as a measure of consolidation (LaBar et al. 1995). Consequently, data were not collapsed into early and late blocks, so these data reflected the mean of eight trials per CS type. Conditioning-related changes were hypothesized to occur in the late acquisition phase (LaBar et al. 1995, 2004) while retention performance in the 24-h delayed extinction test was taken as a measure of consolidation (LaBar et al. 1995).


Received January 16, 2006; accepted in revised form April 18, 2006.