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Cortisol and DHEA-S are associated with startle potentiation during aversive conditioning in humans

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Abstract

Rationale—Fear conditioning reliably increases the startle reflex and stress hormones, yet very little is known about the effect of stress hormones on fear-potentiated startle. Cortisol and the sulfate ester of dehydroepiandrosterone (DHEA-S) are involved in stress and anxiety. Evidence suggests that low cortisol/DHEA-S ratio has a buffering effect on stress and anxiety in preclinical and clinical studies, suggesting that there may be a relationship between fear-potentiated startle and cortisol and DHEA-S activity.

Objective—The aim of the study was to examine whether there is a relationship between cortisol/DHEA-S ratio and fear-potentiated startle.

Methods—Thirty healthy subjects participated in a differential aversive conditioning experiment during which one of two stimuli (CS+) was paired with a shock, and the other was not (CS-). Conditioned responses were assessed with the startle reflex, defined as startle potentiation during CS+ compared to CS-. DHEA-S and cortisol levels were assayed from blood samples collected in both a baseline and an aversive conditioning session. Subjective state anxiety, arousal, and valence were assessed at various times during testing.

Results—Fear-potentiated startle was larger in individuals with high compared to low cortisol/DHEA-S ratio. Multiple regression analyses revealed that fear-potentiated startle was positively associated with cortisol and negatively associated with DHEA-S. There was no significant correlation between DHEA-S and cortisol levels.

Conclusion—These data suggest that cortisol and DHEA-S are involved in fear conditioning.

Keywords

Fear conditioning; Fear-potentiated startle; Cortisol; DHEA-S; Stress; HPA

Introduction

The startle reflex is a reflexive contraction of the skeletal and facial muscles in response to a sudden and intense stimulus (e.g., loud sound). Interest in the startle reflex has been motivated by several factors. Startle is a cross-species reflex, which enables an integration of preclinical and clinical research. It is mediated by a simple, well-defined pontine-based neural circuit (Davis 1997). Finally, startle shows several forms of plasticity, including fear-potentiated startle. Fear-potentiated startle refers to the increase in magnitude of the reflex during fear and anxiety states. For example, in both humans and animals, startle is potentiated in the presence of a conditioned cue (e.g., a light) that has been previously associated with an aversive stimulus (e.g., a shock) (Davis et al. 1993; Hamm et al. 1989). Studies in rodents have identified structures implicated in fear-potentiated startle. The central nucleus of the amygdala (CeA) is responsible for phasic fear-potentiated startle to predictable cues, whereas the bed nucleus of the stria terminalis (BNST) is involved in more sustained forms of startle potentiation (Davis 1998).

Although the neural structures that mediate fear-potentiated startle are well delineated in rodents, very little is known about the psychological and biological factors that influence the relationship between anxiety and startle in humans. One potential factor implicated in the anxiety/startle association is cortisol. Cortisol, a glucocorticoid secreted by the zona fasciculata of the adrenal cortex, is involved in stress, fear, arousal, and various cognitive functions (e.g., appraisal, attention), as well as in many regulatory physiological systems (reviewed in Dickerson and Kemeny 2004). Cortisol secretion is regulated by the hypothalamic—pituitary—adrenal (HPA) axis. Hypothalamic corticotrophin-releasing hormone (CRH) controls the release of adrenocorticotropin-releasing hormone (ACTH) from the pituitary, which in turn results in adrenal release of glucocorticoids. In rodents, corticosterone (the principal glucocorticoid in rats) has well-known inhibitory effects on subsequent release of CRH, but it also has excitatory effects outside the HPA axis, notably on the amygdala (Makino et al. 1994; Swanson and Simmons 1989).

The increase in glucocorticoid release by (conditioned) fear in rodents is well documented (Coover et al. 1973; Dunn et al. 1986). Glucocorticoids are involved in several aspects of fear and anxiety that are not restricted to acquisition and consolidation of fear conditioning. Several studies have demonstrated a role for glucocorticoids in the expression of fear responses (reviewed in Korte 2001), potentially by acting indirectly in the CeA or in the BNST outside the HPA axis (Schulkin et al. 1998). However, little is known about the association between startle and glucocorticoids. A recent study showed a positive correlation between morning cortisol and startle in a study of nonhuman primates who were reared in adverse circumstances (Sanchez et al. 2005). In addition, exposure to a conditioned stimulus that had been previously paired with a shock results in reliable fear-potentiated startle and corticosterone release in rodents (Campeau et al. 1997). In that study, a conditioned inhibitor (i.e., safety signal) paired with the conditioned stimulus reduced both fear-potentiated startle and corticosterone release. In humans, studies examining the acute effect of exogenous glucocorticoids on startle have provided mixed results. Oral hydrocortisone leads to a biphasic effect, with 5 mg increasing and 20 mg decreasing baseline startle (Buchanan et al. 2001). Hydrocortisone did not affect the affective modulation of startle by emotional pictures.

Other endogenous stress hormones, such as dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S), that have now become the focus of some attention, could affect stress reactivity and, consequently, fear-potentiated startle. DHEA and DHEA-S are produced by the zona reticularis of the adrenal cortex. DHEA-S circulates in an order of magnitude higher than that of cortisol, whereas DHEA is present in smaller quantities. Evidence suggests that DHEA and DHEA-S (DHEA(S)) promote well-being and may counteract the effect of stress. DHEA (S) enhance neuronal plasticity, increase neuronal excitability, and have neuroprotective effects (Wolf and Kirschbaum 1999). DHEA has antidepressant and anxiolytic effects in mice (Melchior and Ritzmann 1994). DHEA(S) facilitates cognitive processes (Cruess et al. 1999; Wolf et al. 1998) and reduces responses to stress (Morgan et al. 2004) in humans. Low levels of DHEA(S) have been reported in Alzheimer's disease (Sunderland et al. 1989), depression (Goodyer et al. 1996), anxiety disorders (Fava et al. 1989), anorexia nervosa (Zumoff et al. 1983), and major depressive disorder and chronic fatigue syndrome (Scott et al. 1999).

A potential mechanism for the antistress function of DHEA(S) is via its antiglucocorticoid effects (Blauer et al. 1991; Browne et al. 1992; Clerici et al. 1997; Fleshner et al. 1997). An imbalance between cortisol and DHEA(S) may be a key factor in physical and psychiatric diseases (Dubrovsky 1997; Hechter et al. 1997). The interplay between these two steroids can be quantified as a ratio of cortisol to DHEA(S) (cortisol/DHEA(S)) in plasma. Cortisol/ DHEA (S) ratios are associated with measures of global functioning (Goodyer et al. 1998) and response to clinical intervention (Cruess et al. 1999). Depressed patients exhibit higher cortisol/DHEA-S ratios than controls (Goodyer et al. 1996; Michael et al. 2000). Among psychiatrically healthy subjects, morning cortisol/DHEA ratio is significantly associated with higher anxiety (van Niekerk et al. 2001). Finally, it was recently reported that measures of military performance correlated positively with the DHEA-S/cortisol ratio during stress (Morgan et al. 2004).

Taken together, these results raise the possibility that DHEA(S) and the glucocorticoids have opposite associations with fear-potentiated startle, with the glucocorticoids being associated with an increased, and DHEA(S) with a decreased, magnitude of fear-potentiated startle. The purpose of this study was to assess the relationship between the magnitude of fear-potentiated startle during differential fear conditioning and plasma levels of cortisol and DHEA-S as well as the cortisol/DHEA-S ratio in healthy volunteers. It was hypothesized that a low cortisol/DHEA-S ratio would be associated with reduced fear-potentiated startle during fear conditioning.

Methods

Subjects

The participants were paid volunteers, healthy females ($N=18$) and males ($N=12$), recruited via advertisement. They all gave written informed consent that had been approved by the NIMH Human Investigation Review Board. Subjects were excluded from participation if they met criteria for any psychiatric diagnosis using structured diagnostic criteria (First et al. 1995), were taking medication, suffered from any clinically significant abnormality of the 12-lead EKG, showed any clinically significant abnormality on chemistry or hematology tests, or during a medical examination. Subjects were also excluded if they had any history of alcoholism or substance abuse/dependence, if they smoked, or if they screened positive for an illicit drug on urine toxicology.

Procedure

The study was approved by the NIMH Institutional Review Board. To reduce variability in steroid levels due to circadian rhythm, screening and testing started at 9 a.m. for each subject. The study was part of a larger psychopharmacological investigation that examined acquisition,

extinction, and retention of fear conditioning during separate sessions on different days. The present report is concerned only with the data from the acquisition session, when all subjects were treated equivalently (they received a placebo pill). All subjects participated in a screening session prior to inclusion in the fear conditioning experiment. The screening session consisted of a medical and a psychiatric examination, blood and urine collection, an assessment of baseline startle (nine acoustic startle stimuli), the completion of questionnaires, and a shock work-up procedure. The work-up procedure was implemented to establish the intensity of the shock at a level that was “uncomfortable, but not painful.”

On the aversive conditioning day, an intravenous (i.v.) line was placed shortly after participants' arrival. Approximately 80 min later, electrodes to record eyeblink, skin conductance, and heart rate were attached. Results from the latter two physiological measures will not be discussed in this paper. Subjects' baseline startle reflex was then assessed with nine acoustic startle stimuli presented every 17 to 23 s. The shock electrodes were placed on the subjects' left forearm, and a reminder shock was administered. Instructions concerning the experiment were given prior to the start of the aversive conditioning experiment. Subjects were told that they would receive shocks and startling sounds, and that pictures of a snake and a spider would be shown on a computer monitor. They were also informed that paying attention to the pictures might help them predict the shock.

Aversive conditioning design

Eight-second duration pictures of a snake and of a spider served as conditioned stimuli. One was reinforced with a shock (CS+), whereas the other was not (CS-). The pictures which served as CS+ were counterbalanced across subjects. During acquisition, a shock was administered at CS+ offset. The acquisition of aversive conditioning comprised two acquisition phases separated by a 5-min break period. The first phase started with (1) the delivery of four startle stimuli followed by (2) two preacquisition blocks of two unreinforced CS+ and two CS- presentations per block, and (3) four acquisition blocks of two reinforced CS+ and two CS- per block. The second phase consisted of another four blocks of two reinforced CS+ and two CS- per block. Hence, a total of 16 CS+ were presented and reinforced with a shock, and 16 CS- were presented. Acoustic startle stimuli were delivered during each CS (5–7 s following CS onset) and during intertrial intervals (ITI).

Apparatus and material

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments). The eyeblink component of the startle reflex was assessed with two electrodes placed under the left eye. The acoustic startle stimulus was a 40-ms duration, 103 dB(A) burst of white noise with a near instantaneous rise time presented binaurally through headphones. Amplifier bandwidth was set to 30–500 Hz. The electric shock (100-ms duration, 3–5 mA) was produced by a constant current stimulator and administered on the wrist.

Questionnaires

During screening, participants completed the state and trait portion of Spielberger's State-Trait Anxiety Inventory (STAI), the Beck Depression Inventory (BDI), the Beck Anxiety Inventory (BAI), and the NEO personality inventory (NEO-PI, form S; Costa and McCrae 1985). The NEO is a self-report personality inventory that comprises five factors—neuroticism, extraversion, openness, agreeableness, and conscientiousness (Costa and McCrae 1992). The following subjective reports were collected during the aversive conditioning experiment. State anxiety was assessed with the state portion of the STAI twice, just before and just after the first acquisition phase. Ratings of arousal and valence were obtained using the 9-point Self-Assessment Manikin (SAM) (Bradley and Lang 1994). The SAM was also administered twice, just before and just after the first acquisition phase. For the ratings after the first acquisition,

subjects were asked to retrospectively rate how they felt during acquisition. In the present analysis, SAM ratings were scored so that higher ratings were associated with increased negative valence and with increased arousal.

Cortisol/DHEA-S collection

Plasma samples of cortisol and DHEA-S were collected during the screening day, and three times during the testing day: (1) 30 min following the placement of the i.v. line (baseline), (2) 1 h later, just before the administration of the shock reminder (preshock), and (3) 15 min following the second acquisition phase (conditioning). Blood samples were collected using an i.v. on the testing day, and using venipuncture during the screening day.

Plasma cortisol and DHEA-S determination

Serum cortisol and DHEA-S were analyzed by the Clinical Chemistry Service at the National Institute of Health, Bethesda, MD, using polyclonal rabbit anticortisol and anti-DHEA-S antibody coated tubes. Cortisol and DHEA-S were measured using chemiluminescent immunoassay kits (Diagnostic Products Corporation, Los Angeles, CA, USA). The intraassay coefficient of variation was 7.5% for cortisol and 9.5% for DHEA-S.

Data analysis

Cortisol/DHEA-S ratios were calculated based on averages of the cortisol and DHEA-S levels during the aversive conditioning session. A median-split of these ratios within each gender was used to divide subjects into a low and a high cortisol/DHEA-S ratio group. Peak amplitude of the blink reflex was determined in the 20–100 ms time frame following stimulus onset relative to baseline (average baseline EMG level for the 50 ms immediately preceding stimulus onset). The eyeblink magnitude data were averaged for each condition within each phase, and the resulting scores were analyzed with analyses of variance (ANOVA) with repeated measures. Greenhouse—Geisser corrections were used for main effects and interactions involving factors with more than two levels. Linear regression analyses were conducted to examine whether and to what extent the variables of age, gender, trait anxiety, neuroticism, subjective reports of anxiety, arousal, valence, and hormone levels could explain the variance in fear-potentiated startle. DHEA-S and cortisol levels were log-transformed to reach normality. The p value was set at ≤ 0.05 for all statistical tests.

Results

Cortisol and DHEA-S

Table 1 shows the cortisol and DHEA-S data. Screening day cortisol could not be assayed for four subjects because of errors in manipulating the blood samples. Cortisol levels increased and DHEA-S remained unchanged from the screening day to the testing day. A Group (high cortisol/ DHEA-S ratio, low cortisol/DHEA-S ratio) $\times$ Sex (male, female) $\times$ Day (screen, test) ANOVA revealed a significant main effect of day for cortisol [$F_{(1,22)}=5.8, p<0.02$], but not for DHEA-S. During the testing day, cortisol levels decreased from baseline to the preshock and conditioning periods, whereas DHEA-S levels remain the same during these two periods. The cortisol and DHEA-S data were analyzed with separate Group (high cortisol/DHEA-S ratio, low cortisol/DHEA-S ratio) $\times$ Sex (males, females) $\times$ Time (baseline, preshock, acquisition) ANOVA. There was a reduction in cortisol levels from the baseline period to the preshock and acquisition periods [Time linear trend, $F_{(1,26)}=6.0, p<0.02$]. DHEA-S levels remained constant throughout the testing procedure.

Subjective reports

Subjective reports of anxiety, valence, and arousal are shown in Table 2. Subjective ratings were analyzed with a Group (high cortisol/DHEA-S ratio, low cortisol/DHEA-S ratio)×Sex (male, female)×Time ANOVA. The factor Time had three levels (screening, preacquisition, acquisition) for state anxiety and two levels (preacquisition, acquisition) for arousal and valence. State anxiety increased linearly over time [linear trend, $F(1,26)=13.3$, $p<0.001$], indicating enhanced anxiety from the screening day to the aversive conditioning testing day, further increasing from before to after the first acquisition phase. The increase in state anxiety was greater in the high ratio compared to the low ratio group, but this effect did not reach significance. Arousal levels were higher in the high cortisol/DHEA-S ratio group compared to the low cortisol/DHEA-S ratio Group [$F(1,26)=7.4$, $p<0.01$]. Ratings of negative valence increased from before acquisition to after the first acquisition phase in the high cortisol/DHEA-S ratio group [$F(1,26)=25.2$, $p<0.0009$], but not in the low cortisol/DHEA-S ratio group [$F(1,26)=0.5$], resulting in a Group×Time interaction [$F(1,26)=9.2$, $p<0.005$].

Conditioning, startle reflex

Figure 1 shows the startle data during conditioning separately for the two acquisition phases. As a result of conditioning, startle was potentiated during the CS+ compared to the CS- and the ITI. In accordance with our hypothesis, startle potentiation was larger in the high ratio compared to the low ratio group, which was significant only in phase 2. The data were analyzed with a Group (high ratio, low ratio)×Sex (male, female)×Phase (1,2)×Stimulus Type (ITI, CS-, CS+) ANOVA. There was a significant reduction in startle magnitude from phases 1 to 2 [$F(1,26)=6.4$, $p<0.02$], due to startle habituation. There was also a significant main effect of Stimulus Type [$F(2,52)=16.6$, $p<0.0009$], a Group×Stimulus Type interaction effect [$F(2,52)=4.5$, $p<0.03$], and a Group×Stimulus Type×Phase interaction effect [$F(2,52)=4.9$, $p<0.01$]. There was no significant gender effect. To interpret the interactions, separate ANOVAs were conducted within each phase. There was a significant Stimulus Type main effect [$F(2,56)=11.8$, $p<0.0009$] in phase 1, which was due to greater startle magnitude during CS+ compared to CS- [$F(1,28)=7.1$, $p<0.01$] and to ITI [$F(1,18)=14.5$, $p<0.001$]. The Group×Stimulus Type interaction was not significant ($p>0.1$), suggesting similar conditioning in the two groups. In contrast, there was a significant Group×Stimulus Type interaction in phase 2 [$F(2,56)=7.1$, $p<0.007$]. Between-group analyses contrasting CS+ to ITI, CS+ to CS-, and CS- to ITI (Group×Stimulus Type ANOVAs) revealed that startle potentiation was greater in phase 2 in the high cortisol/DHEA-S ratio group compared to the low cortisol/DHEA-S ratio group during CS+ relative to ITI [$F(1,28)=6.9$, $p<0.01$] and CS+ relative to CS- [$F(1,28)=8.5$, $p<0.01$]. There was no between groups difference in the comparison of CS- relative to ITI [$F(1,28)=1.0$]. Because of the differences between phases 1 and 2 in conditioning between the two cortisol/DHEA-S ratio groups, it is unclear whether the low ratio group exhibits significant conditioning performance in phase 1. Therefore, we conducted an additional within-group analysis of conditioning performance during phase 1 to assess conditioning separately in each group. In both the low and high cortisol/DHEA-S ratio groups, startle was higher during CS+ compared to ITI [$F(1,14)=6.9$, $p<0.02$; $F(1,14)=8.2$, $p<0.01$, respectively], but there was only a trend for startle to be higher during CS+ compared to CS- [$F(1,14)=3.6$, $p<0.08$; $F(1,14)=3.4$, $p<0.08$, respectively]. These results suggest very similar conditioning in the two groups in phase 1.

Regression analyses

Regression analyses were conducted using all subjects. A backward regression variable selection procedure was used to select the relevant variables among possible predictors age, gender, trait anxiety, NEO-neuroticism, subjective reports of anxiety, arousal, valence, and the cortisol/DHEA-S to explain between subjects variance in fear-potentiated startle. Fear-potentiated startle was defined as the difference between CS+ and CS- in the second phase of

acquisition, because this was the phase in which individual differences emerged. The cortisol/DHEA-S ratio was the only variable selected. The linear regression analysis with fear-potentiated startle as dependent variable and cortisol/DHEA-S ratio as independent variable showed that the model was significant, $F_{(1,28)}=13.0$, $p<0.001$. The adjusted coefficient of determination (R^2) for the model was 0.29, and the regression coefficient was 0.82. To examine whether only one or the two-hormone variables contributed to the model, another backward regression analysis was conducted using plasma cortisol and DHEA-S separately instead of their ratio. Again, the model was significant with the two variables [$F_{(2,27)}=6.8$, $p<0.01$]. R^2 for the model was 0.29. The regression coefficients were 16.8 ($p<0.05$) and -19.4 ($p<0.01$) for cortisol and DHEA-S, respectively. A Pearson correlation analysis revealed no significant correlation between cortisol and DHEA-S ($p>0.3$).

Discussion

The main results of this study were that higher cortisol/DHEA-S ratios were associated with increased fear responses, based on startle and subjective measures of valence and arousal. Subjects in the high cortisol/DHEA-S ratio group exhibited increased fear-potentiated startle to the CS+ compared to the CS- and increased ratings of arousal and negative valence, compared to subjects with low cortisol/DHEA-S ratios. Further, levels of cortisol and DHEA-S separately were associated with the magnitude of fear-potentiated startle to the CS+. The regression analysis showed significant regression coefficients that were positive for cortisol and negative for DHEA-S. This is consistent with the view that the two steroids affect responses to stress in an opposite direction (Kalimi et al. 1994).

To the best of our knowledge, this is the first report of an association between fear-potentiated startle and cortisol and DHEA-S. It is well established that fear-potentiated startle during aversive conditioning reflects fear elicited by the CS+ (see Grillon and Baas 2003). Smaller fear-potentiated startle in the low cortisol/DHEA-S ratio group compared to the high cortisol/DHEA-S ratio group was also accompanied by lower levels of arousal and negative valence. These results suggest that the low cortisol/DHEA-S ratio group was less fearful of the CS+ in the second phase compared to the high cortisol/DHEA-S ratio group. Such a fear reduction could be due to habituation to the shocks, which may have become less aversive following repeated presentations in this group. An alternative interpretation is that the small differentiation between CS+ and CS- in the low cortisol/DHEA-S ratio group reflected reduced or unsuccessful fear conditioning. This is an unlikely explanation because the low and high cortisol/DHEA-S ratio groups conditioned similarly in phase 1. It is more likely that the low cortisol/DHEA-S ratio group learned the appropriate CS+/-shock contingency, but that the shock was no longer as anxiogenic in the second phase as it was in the first phase.

The positive association between cortisol and fear-potentiated startle is consistent with preclinical data. A positive correlation between freezing (a measure of fear) and corticosterone has been reported in young monkeys (Kalin 1993). Similarly, corticosterone potentiates conditioned fear in rodents (Corodimas et al. 1994). A possible interpretation of these effects is that corticosterone increases fear by acting on CRH outside the HPA axis. Several studies have demonstrated that CRH is anxiogenic in several tests in rodents (Berridge and Dunn 1986; Cole and Koob 1988; Dunn and File 1987; Takahashi et al. 1989). CRH also increased markedly the magnitude of the startle reflex when given intraventricularly (Liang et al. 1992; Swerdlow et al. 1986). The mechanism by which fear potentiation occurs remains to be elucidated. The amygdala is involved in the potentiation of startle to an explicit cue (i.e., CS+) in rodents (Davis 1998). In addition, although corticosterone is known to reduce CRH mRNA in the paraventricular nucleus of the hypothalamus, it can increase CRH mRNA in the amygdala (Schulkin et al. 1998). On the other hand, the amygdala does not seem to mediate CRH-enhanced startle. Neurotoxic lesions of the amygdala do not block CRH-enhanced startle

(Lee and Davis 1997), and CRH infused into the amygdala do not increase baseline startle (Liang et al. 1992). It is more likely that CRH potentiates startle via action at the level of the BNST. Intra-BNST infusion of CRH increases startle amplitude (Liang et al. 1992), and injection of a CRH antagonist into the BNST, but not into the CeA, blocks CRH-enhanced startle (Lee and Davis 1997).

There was a significant increase in state anxiety from the screening session to the preconditioning session, suggesting an increase in anticipatory anxiety about the upcoming fear conditioning experiment and a further increase during acquisition. Plasma cortisol increased initially (baseline), but decreased subsequently. The cortisol data are difficult to interpret because placement of the intravenous catheters is likely to be responsible for the initial increase in cortisol and may have subsequently masked any effect due to anticipatory anxiety.

DHEA-S showed no changes from the screening day to the testing day and no changes during the testing day, suggesting that DHEA-S is not released under mildly stressful conditions such as those in the present experiment. Nevertheless, DHEA-S was negatively associated with the magnitude of fear-potentiated startle. One potential interpretation of this finding is that DHEA-S exerts an antianxiety effect. Antianxiety or antistress role for DHEAS has been reported for physical and mental diseases (Dubrovsky 1997; Hechter et al. 1997), as well as during strenuous and dangerous military trainings (Bernton et al. 1995; Morgan et al. 2004). DHEA release following ACTH injection is also associated with decreased avoidance and decreased negative mood symptoms in women with PTSD (Rasmusson et al. 2004). Based on these results, it has been suggested that DHEA/DHEA-S is involved in the termination of a stress response and/or in reducing anxiety (Majewska 1992). Anxiolytic and antidepressant effects of DHEA-S have been demonstrated in preclinical studies (Frye and Lacey 1999; Melchior and Ritzmann 1994; Prasad et al. 1997). Anxiolytic effects can be found with small amount of DHEA and DHEA-S (Melchior and Ritzmann 1994). Consistent with these findings, DHEA-S reduces contextual fear conditioning in rodents (Fleshner et al. 1997).

Human studies using cortisol measures suffer from several factors that complicate interpretation. One such limitation comes from diurnal variations in cortisol. Possible changes in cortisol over several hours induced by an experimental manipulation, such as a possible increase in cortisol during conditioning, could be masked by the normal reduction in morning cortisol level. If the aversive conditioning experiment per se (as opposed to anticipation of the procedure) produced HPA activation, this activation probably was relatively small. Other stressors such as social challenges produce a reliable increase in cortisol throughout the day (Kudielka et al. 2004). Another limitation is that we did not have a true measure of baseline cortisol during the screening day because of the use of venipuncture, which may have engendered anticipatory anxiety and discomfort. Finally, we did not collect information from females on the phase in the menstrual cycle during which they were tested. Human studies have produced inconsistent results with respect to potential changes in HPA reactivity over the menstrual cycle (Abplanalp et al. 1977; Kirschbaum et al. 1999; Marinari et al. 1976; Tersman et al. 1991). Neither DHEA nor DHEA-S appears to vary systematically with menstrual phase (Vermeulen 1980). The effects of ovarian hormonal fluctuations on fear-potentiated startle to threat of shock are also unknown. Baseline startle is unaffected by such fluctuations, but some forms of startle plasticity such as prepulse inhibition are influenced by ovarian hormones (Jovanovic et al. 2004). Similarly, it is possible that ovarian hormones affect fear-potentiated startle given the interaction between the HPA axis and reproductive hormones (Kalantardidou et al. 2004). However, because both groups consisted of males as well as females, this is unlikely to explain all of our effects. Indeed, a reanalysis of the data using only males confirmed the main findings. There was a greater fear-potentiated startle to the CS+ compared to the CS- in the second conditioning phase in the high (CS- = 33.9, CS+ = 54.9, $p = 0.06$) compared to the low cortisol/DHEA-S (CS- = 28.0, CS+ = 23.7, $p > 0.1$) group [$F_{(1,10)} = 7.0$, $p < 0.02$].

The current study was an attempt at understanding hormonal mechanisms modulating fear-potentiated startle during aversive conditioning. We found that cortisol was positively associated, and DHEA-S was negatively associated with the magnitude of fear-potentiated startle. The cortisol data extend findings from preclinical research on fear conditioning. The DHEA-S data are consistent with emerging literature, suggesting a stress-buffering effect of DHEA-S. Future studies should further examine the role of DHEA and DHEA-S in fear and fear learning as they may help identify mechanisms involved in the resilience to stress.

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Fig. 1.

Eyeblick/startle magnitude during intertrial interval (ITI), CS+, and CS- in the low and high cortisol/DHEA-S ratio group during acquisition of aversive conditioning. The results are averaged for each stimulus type and group within the first and the second phase of acquisition. There was no significant group difference in phase 1, but startle potentiation (CS+ minus CS- and CS+ minus ITI) was significantly greater in the high cortisol/DHEA-S ratio group compared to the low cortisol/DHEA-S group in phase 2 (see text). * Indicates a significant group difference in startle magnitude. *Error bars* are standard errors

Table 1
Demographic information and cortisol and DHEA-S results

	All subjects (N=30)	Low cortisol/DHEA-S ratio (N=15)	High cortisol/DHEA-S ratio (N=15)
Age (years)	27 (7.5)	28.0 (8.1)	25.0 (4.6)
Ratio female/male	18/12	9/6	9/6
Cortisol (µg/dl) screen	11.2 (4.7)	10.9 (4.0)	12.0 (5.8)
Baseline	14.9 (8.4)	12.4 (2.7)	16.7 (10.9)
Preshock	12.3 (6.1)	10.4 (3.5)	14.6 (7.7)
Postacquisition	12.3 (7.9)	10.0 (3.3)	14.3 (10.0)
DHEA(µg/ml) screen	2.0 (1.1)	2.7 (0.8)	1.3 (0.6)*
Baseline	2.3 (1.2)	2.8 (1.0)	1.2 (0.7)*
Preshock	2.3 (1.2)	2.9 (1.0)	1.2 (0.6)*
Postacquisition	2.3 (1.3)	2.9 (1.0)	1.2 (0.6)*

* Indicates significant group differences at the $p<0.001$ level

Table 2

Mean (SD) subjective measures of fear, arousal, and valence

	All subjects (N=30)	Low cortisol/ DHEA-S ratio (N=15)	High cortisol/ DHEA-S ratio (N=15)
BDI	1.3 (2.1)	1.4 (2.0)	1.0 (1.2)
BAI	1.2 (1.8)	1.3 (1.8)	1.6 (2.1)
STAI-trait anxiety	28.0 (7.8)	28.9 (6.1)	27.7 (4.4)
STAI-state anxiety screen	25.8 (7.0)	25.9 (7.2)	25.6 (4.4)
Precquisition	30.0 (7.4)	29.2 (7.0)	30.8 (7.8)
Acquisition ^a	35.0 (12.1)	32.2 (9.1)	37.8 (14.8)
SAM arousal ^b pre-acquisition	1.5 (1.7)	0.8 (0.8)	2.2 (1.9) *
Acquisition ^a	1.8 (2.1)	0.8 (0.9)	2.7 (2.3) *
SAM valence ^b pre-acquisition	3.2 (1.5)	3.1 (1.4)	3.3 (1.6)
Acquisition ^a	4.0 (1.5)	3.2 (1.0)	4.7 (1.6) **

BDI Beck Depression Inventory, *BAI* Beck Anxiety Inventory, *STAI-trait* trait portion of the Spielberger' State-Trait Anxiety Inventory, *STAI-state* state portion of the Spielberger' State-Trait Anxiety Inventory

* Indicates significant group differences at the $p < 0.001$ level

** Indicates significant within-group difference (post minus pre) at $p < 0.001$ level

^a Ratings were taken during the rest period between acquisition 1 and acquisition 2

^b Note that the arousal and valence ratings were calculated so that increased scores reflect higher arousal and higher negative valence